NSAIDs and Cardiovascular Drugs in Neurodegenerative and Cerebrovascular Diseases

Mendel Haag

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NSAIDs and Cardiovascular Drugs in Neurodegenerative and Cerebrovascular Diseases

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Introduction

Neurodegenerative and cerebrovascular diseases are frequent in elderly populations and comprise primarily of dementia (mainly Alzheimer disease (AD)), Parkinson disease (PD) and stroke. The prevalence of these neurological disorders rises with older age. From 55 years to 90 years and above, the prevalence of dementia increases from less than 1% to over 40%.[1-3] For PD, the prevalence increases over the same age range from less than 0.5% to more than 4%,[4,5] and for stroke from approximately 1% to nearly 10%.[6,7] Similar age-related patterns are observed for incidence figures.[8-11] In the Netherlands, the population of persons of 65 years and older is expected to increase from 2.4 million in 2007 to 3.9 million in 2050.[12] At a global level, 2 billion persons above 65 years are expected by 2050.[13] As a consequence of the aging population the incidence and prevalence of age-related neurological diseases will increase accordingly. Moreover, these neurological disorders all constitute highly disabling diseases, with appreciable impact on quality-of-life at the patient level, but also on society, both economically and socially.[12,14-17] Currently, there is no effective cure for AD[18], PD[19] or the consequences of stroke.[20] Hence, identification of determinants of these neurological diseases and development of preventive strategies is of paramount importance. This search is, in part, directed at currently available drugs which target established risk factors of neurological disease, or that have, in *in vivo* or *in vitro* studies, shown to interfere with more specific elements of the supposed pathogenic pathway of disease. Two drug groups commonly used by elderly are of interest, namely nonsteroidal anti-inflammatory drugs (NSAIDs) and cardiovascular medication. The general objective of this thesis was to study the role of these drugs as determinants of neurodegenerative and cerebrovascular diseases.

NONSTEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS)

NSAIDs are among the most widely prescribed drugs worldwide owing to their anti-inflammatory, antipyretic and analgesic properties.[21] Registered indications relevant to the elderly population include mild to moderate pain and symptomatic relief in musculoskeletal and joint diseases. In the early 1970's, a decade after their market introduction, it was discovered that NSAIDs mediate their anti-inflammatory effects through inhibition of the cyclooxygenase (COX) enzyme.[21] COX is the enzyme required for the conversion of arachidonic acid to prostaglandins, a group of compounds with extensive functions in human physiology. At least two isoforms of COX to date have been identified, COX1 and COX2.[22-24] NSAIDs differ with respect to relative selectivity

for either of the COX-enzymes.[25] The 'traditional' NSAIDs are mostly non-selective and inhibit both COX-enzymes concurrently. Due to their inhibition of COX1, which is involved in gastric mucosal defence, the 'traditional' NSAIDs are known for their gastrointestinal adverse effects.[26-28] In order to reduce these adverse events, the more recently developed compounds selectively inhibit COX2. However, in September 2004, a COX2-selective NSAID was voluntarily withdrawn from the market as a result of concerns regarding its cardiovascular safety based on clinical trial data.[29-31] It was thought that selective COX2-inhibition, without concomitant inhibition of COX1, causes platelet aggregation and thereby induces a prothrombotic state.[32,33] Retrospective analyses of observational series and non-cardiovascular clinical trials suggested, however, that cardiovascular events may also occur with non-selective NSAIDs. Hence, it was debated whether the cardiovascular risk is restricted to the COX2-selective compounds.[28,34-36] Furthermore, it is unclear whether NSAID use poses similar risks for different cardiovascular and cerebrovascular events. We investigated the association between NSAID use and risk of stroke in **Chapter 1.1** and transient ischemic attack (TIA) in **Chapter 1.2** and determined whether associations differed for the different NSAID groups, based on their COX-selectivity.

NSAIDs have been a focus of pharmacoepidemiological research in neurodegenerative disease.[37,38] Inflammation is a process that has been related to the onset of numerous neurodegenerative disorders.[39,40] Findings from epidemiological studies have repeatedly suggested that anti-inflammatory drugs, particularly NSAIDs, could protect against AD and possibly against PD.[37,41,42] Initially, it was thought that the anti-inflammatory properties of NSAIDs explain the protective effect of NSAIDs on AD and PD. However, NSAIDs also exhibit other effects potentially relevant to neurodegenerative disease. Of particular interest to researchers in the field of AD is the effect of NSAIDs on amyloid-β42-protein processing.[43] *In vitro* and *in vivo* studies have shown consistently that a certain subgroup of NSAIDs lowers amyloid-β42 levels.[44,45] Amyloid-β42 protein is the major constituent of the senile plaques, a hallmark of Alzheimer pathology.[46,47] If amyloid-β42 level lowering NSAIDs would protect against AD this would support a role of amyloid-β42 in the pathogenesis of AD. Moreover, it would provide a potential precursor for future drug development.[48] In **Chapter 1.3** we examined the association between NSAID use and PD. Next, in **Chapter 1.4**, we investigated whether NSAIDs that lower amyloid-β42 peptide protect against AD.

CARDIOVASCULAR MEDICATION

The presence of vascular risk factors, such as hypertension, hypercholesterolemia and diabetes mellitus places a patient at risk for cerebrovascular events. Hence, vascular risk management is a well-established strategy in the prevention of cerebrovascular events.[49] In neurodegenerative disease the involvement of vascular pathology has long been recognized for vascular dementia, though this view is relatively new for AD.[50,51] Despite the accumulating evidence that the elderly population may benefit from vascular risk management to prevent dementia, no such strategy currently exists. Two of these major vascular risk factors, of interest in Alzheimer research and for which pharmacological treatments are available, are hypertension and hypercholesterolemia.[52] Cholesterol-lowering drug therapy nowadays consists mostly of statins.[53] Antihypertensives are a group of diverse compounds that lower blood pressure though different mechanisms.[54] The main pharmacological effects of these two drug groups on cholesterol levels and blood pressure were considered to be of potential value to AD.[55] Alternatively, evidence from several lines of research suggests that other mechanisms of action or differences among the compounds within these drugs groups should not be precluded.[56,57] A particular issue was raised for statins, since some statins are able to cross the blood brain barriers more easily than others, and the latter might therefore lack the ability to affect actual brain cholesterol homeostasis.[58] In **Chapter 2.1** we studied the association of statins and AD and evaluated whether there is a difference in the risk of AD for lipophilic statins and hydrophilic statins. In **Chapter 2.2** we investigated whether use of antihypertensives is associated with a reduced risk of dementia.

In neurodegenerative and cerebrovascular disorders clinical symptoms typically become manifest late in the disease course. The occurrence of clinical disease may therefore not reflect the underlying spectrum of disease-related pathology. Nowadays, non-invasive imaging techniques, such as computed tomography and magnetic resonance imaging (MRI) allow for the investigation of pre-symptomatic brain pathology.[59] Well-studied markers of brain pathology are white matter lesions (WML).[60,61] Cerebral white matter is important for transmission of impulses between different brain regions. WML are frequently observed on MRI scans in elderly persons and thought to result from vascular brain pathology.[62] They have been associated with the risk of stroke, cognitive impairment and dementia.[63-65] Besides their correlation with advanced age, several

cerebrovascular risk factors have been associated with the prevalence and progression of cerebral WML.[66] Of these, increased blood pressure is the most widely recognized.[67] Consequently, control of blood pressure with antihypertensive agents was put forward as a possible strategy for the prevention of WML progression. Thus far, the evidence from two clinical trials and a study in healthy volunteers suggest that antihypertensive treatment protects against WML progression.[68,69] In the general population, however, evidence on the relationship between antihypertensive use and progression of WML later in life is sparse. In **Chapter 2.3** we studied whether antihypertensives could reduce the risk of WML progression.

MRI technology has advanced significantly over the years, contributing to its speed, sensitivity and spatial resolution. One area of research in neuroimaging that has benefited from these technical improvements is the research on cerebral microbleeds, another potential marker of vascular brain pathology.[70,71] Cerebral microbleeds are hemosiderin deposits from red blood cells that presumably have leaked out of small brain vessels. The presence of cerebral microbleeds is associated with an increased risk of adverse neurological events in stroke patients.[72,73] The etiology of cerebral microbleeds is largely unknown but is thought to resemble that of intracerebral hemorrhage. The clinical relevance of microbleeds has received particular attention in cardiovascular and cerebrovascular disease risk management, which often includes the decision to prescribe antithrombotic drugs to patients at risk for ischemic events. However, the use of antithrombotics was found to increase the risk of symptomatic hemorrhage[74] and concerns are raised as to whether this also applies to microbleeds. A better understanding of the effect of antithrombotics on microbleeds is needed to aid in these clinical decisions. In the final chapter, **Chapter 2.4**, we thus explore the association between the use of antithrombotic medication and the presence of cerebral microbleeds.

The studies presented in this thesis are based on the Rotterdam Study, an ongoing population-based study into causes and consequences of age-related diseases with detailed information on drug exposure from pharmacy prescription data during follow-up. For our final study on antithrombotics and presence of microbleeds we included a randomly selected sample from the first expansion of the Rotterdam Study cohort of persons 60 years and older.[75]

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Chapter 1

Nonsteroidal anti-inflammatory drugs (NSAIDs)

Chapter 1.1

NSAIDs and risk of Stroke

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ABSTRACT

Background: In clinical trials, cyclooxygenase (COX)-2-selective nonsteroidal anti-inflammatory drugs (NSAIDs) were associated with an increased risk of thromboembolic events. We studied the association between NSAID use and risk of stroke in the prospective, population-based Rotterdam Study.

Methods: We followed 7,636 persons free of stroke at baseline (1991-1993) for incident stroke until September 2004. Data on all filled prescriptions came from pharmacy records. With Cox regression models, we calculated crude and adjusted hazard ratios (HR) of stroke for time-dependent current use, compared with never use, of NSAIDs grouped according to COX selectivity (COX1-selective, nonselective, and COX2 selective) and individual NSAIDs.

Results: At baseline, the mean age of the study sample was 70.2 years, and 61.3% were female. During 70,063 person-years of follow-up (mean, 9.2 years), 807 persons developed a stroke (460 ischemic, 74 hemorrhagic, and 273 unspecified). Current users of nonselective (HR 1.72; 95% confidence interval [CI], 1.22-2.44) and COX2-selective (HR 2.75; 95% CI, 1.28-5.95) NSAIDs had a greater risk of stroke, but not users of COX1–selective NSAIDs (HR1.10; 95% CI, 0.41-2.97). Hazard ratios (95% CIs) for ischemic stroke were 1.68 (1.05-2.69) for nonselective and 4.54 (2.06-9.98) for COX2-selective NSAIDs. For individual NSAIDs, current use of the nonselective naproxen (HR 2.63; 95% CI, 1.47-4.72) and the COX2-selective rofecoxib (HR 3.38; 95% CI, 1.48-7.74) was associated with a greater risk of stroke. Hazard ratios (95% CIs) for diclofenac (1.60 [1.00-2.57]), ibuprofen (1.47 [0.73-3.00]), and celecoxib (3.79 [0.52-27.6]) were greater than 1.00 but were not statistically significant.

Conclusions: In the general population, we found a greater risk of stroke with current use of nonselective and COX2-selective NSAIDs. The risk of stroke was not limited to the use of COX2-selective NSAIDs.

INTRODUCTION

In clinical trials the use of cyclooxygenase (COX)-2-selective nonsteroidal anti-inflammatory drugs (NSAIDs) has been associated with an increased risk of cardiovascular events and death.[1-5] Most of the post hoc analyses of the trials focused on combined cardiovascular or cerebrovascular events as the end point, without further specification of the risk of stroke.

Moreover, few observational studies have investigated the association between NSAID use and the risk of stroke.[6-11] Of these, one nested case-control study showed that current NSAID use was associated with a greater risk of ischemic stroke.[7] In another nested case-control study, a greater risk of ischemic stroke was found for some COX2-selective NSAIDs, but this was also found for the nonselective NSAID diclofenac.[11] Predominantly, null findings were reported in 4 studies that investigated the outcome of hemorrhagic stroke.[6,8-10] It is supposed that selective inhibition of the COX2 enzymes by the COX2-selective NSAIDs induces a prothrombotic state, unlike NSAIDs that inhibit COX2 to a lesser degree and additionally inhibit COX1.[12-14] However, the cardiovascular risk observed for the various COX2-selective NSAIDs has not been consistent in all clinical trials.[15] In a recent placebo-controlled trial, the non-COX2-selective NSAID naproxen sodium was associated with an increased cardiovascular risk.[4] Evidence from observational studies has also shown that NSAIDs differ in their potential to cause cardiovascular events.[16-18] In all, it remains inconclusive if the greater risk of cardiovascular events is specific for COX2-selective NSAIDs or whether other pharmacological properties of NSAIDs could cause these detrimental effects. We investigated the association between NSAID use and the risk of incident stroke in a large, prospective, population-based cohort study and whether any observed association was restricted to COX2-selective NSAIDs.

METHODS

Study population

The Rotterdam Study is a prospective, population-based cohort study of age-related disorders.[19] The medical ethics committee of the Erasmus Medical Center, Rotterdam, the Netherlands, approved the study. Between 1990 and 1993, all persons 55 years or older living in Ommoord, a district of Rotterdam, were invited to participate. Of the

10,275 eligible persons, 7,983 (77.7%) signed informed consent. Of these individuals, 7,722 were free of stroke at baseline. Follow-up examinations were conducted in 1993 to 1994, 1997 to 1999, and 2000 to 2004. In addition, the cohort was continuously monitored for major disease outcomes and death through linkage with records from general practitioners and bimonthly updates from the municipality records. This resulted in a virtually complete follow-up for stroke. Nearly all persons (99.7%) were registered at 1 or more of 7 automated pharmacies serving the Ommoord area. Of these pharmacies, records of all filled prescriptions were available as of January 1st, 1991. To ensure at least a 6-month history of medication use, we excluded 86 persons for whom follow-up ended before July 1st, 1991. Consequently, the study population included 7,636 persons. The end of the study period for this analysis was September 30th, 2004, on which date rofecoxib was withdrawn from the market after clinical trial data had shown an increased risk of thromboembolic events in the rofecoxib treatment arm.

Drug exposure

Complete information on all filled prescriptions for all persons was obtained in automated format from the pharmacies. This included the product name, international nonproprietary name, Anatomical Therapeutic Chemical code, total number of delivered units (e.g., tablets or capsules), prescribed daily number of units, date of delivery, and drug dosage. The duration of a prescription was calculated as the total number of delivered units divided by the prescribed daily number of units. Drug dosage was defined by the defined daily dose (DDD), the recommended daily dosage of a drug taken by adults for the main indication of the drug. Based on data from in vitro and clinical studies, NSAIDs were classified as COX1 selective, nonselective, and COX2 selective according to their relative selectivity for the COX1 and COX2 enzymes at therapeutic dosages (Table 1).[20-26] For some NSAIDs, COX selectivity is unknown or equivocal (ie, benzydamine hydrochloride, tiaprofenic acid, tolfenamic acid, phenylbutazone, tenoxicam, and aceclofenac).

Table 1. Classification of NSAIDs according to COX-selective properties and total person-months exposure during follow-up

Type of NSAID	Person-months exposure
COX1-selective NSAIDs	
Indometacine	2,181.0
Piroxicam	3,108.9
Ketoprofen	1,306.3
Flurbiprofen	222.4
Azapropazon	272.5
Non-selective NSAIDs	
Diclofenac*	15,126.7
Naproxen	6,776.9
Ibuprofen	8,208.6
Nabumeton	1,008.2
Sulindac	467.6
COX2-selective NSAIDs	
Rofecoxib	1,882.2
Celecoxib	342.6
Meloxicam	767.0
Etoricoxib	49.8
Valdecoxib	12.6

*Includes combination products of diclofenac

Salicylates (ie, acetylsalicylic acid and carbasalate calcium) are pharmacologically related to NSAIDs and inhibit platelet aggregation via COX1; although, contrary to NSAIDs, their effects are irreversible.[27,28] On these grounds, salicylates could be regarded as COX1-selective NSAIDs; however, they are mostly prescribed at a low dose as platelet inhibitors for the prevention of cardiovascular disease and stroke. We did not include salicylates in the COX1-selective NSAID group for the following reasons: (1) they are indicated for stroke prevention; (2) NSAID use is cautioned in persons already using salicylates because of the increased risk of gastrointestinal tract bleeding; and (3) some NSAIDs possibly antagonize the platelet inhibition induced by salicylates.[27,28] This might obscure the protective effect of salicylates. Because of these potential sources of confounding by salicylates, all analyses were adjusted for the current use of salicylates, and the effect of salicylates on the association between NSAID use and stroke was studied through stratification.

Diagnosis of stroke

A history of stroke at the time of enrollment into the Rotterdam Study was assessed by asking “did you ever suffer from a stroke, diagnosed by a physician?” Positive answers to this question were verified by reviewing the medical records. A history of transient ischemic attack was also assessed during the baseline interview. After enrollment into the Rotterdam Study, participants were continuously monitored for all major events, including cerebrovascular disease, through automated linkage of the study database with files from general practitioners. Information on vital status was obtained at regular intervals from the municipal authorities in Rotterdam. When an event or death had been reported, additional information was obtained from the general practitioner and from information in the hospital records (including brain imaging) and discharge letters in the case of admittance or referral. In addition, nursing home physicians’ files and files from general practitioners of participants who moved out of the district were scrutinized. Research physicians discussed information on all potential strokes and transient ischemic attacks with an experienced neurologist to verify all diagnoses while blinded to drug exposure. Subarachnoid hemorrhages were excluded. Subtyping of strokes in the Rotterdam Study has been extensively described previously.[29] In brief, a stroke was subclassified as ischemic when a computed tomographic (CT) scan or magnetic resonance image (MRI) that was made within 4 weeks after the stroke occurred ruled out other diagnoses or when indirect evidence (eg, deficit limited to 1 limb or completely resolved within 72 hours and atrial fibrillation in absence of anticoagulants) indicated the ischemic nature of the stroke. Hemorrhagic stroke was diagnosed when a relevant hemorrhage was shown on a CT scan or MRI or when the patient permanently lost consciousness or died within hours after onset of focal signs. If a stroke could not be subclassified as ischemic or hemorrhagic as a consequence of a lack of the aforementioned data, it was classified as “unspecified”.[29]

Other covariates

Potential confounders were chosen a priori. Baseline covariates included age, sex, systolic blood pressure, body mass index, total serum cholesterol level, and smoking status. Time-dependent covariates included myocardial infarction; atrial fibrillation; heart failure; transient ischemic attacks (TIA); coronary artery bypass graft (CABG); percutaneous transluminal coronary angioplasty (PTCA); diabetes mellitus; and use of antihypertensives, salicylates, and antithrombotics. Cardiovascular conditions were assessed at the baseline interview and follow-up examinations, together with a review of medical records. In addition, electrocardiography was performed to determine

myocardial infarction and atrial fibrillation.[30] Sitting blood pressure was measured on the right upper arm using a random-zero sphygmomanometer. In the analyses, the mean of 2 measurements, measured at 1 occasion, was used. Diabetes mellitus was defined as non-fasting serum glucose level exceeding 200 mg/dL (to convert to millimoles per liter, multiply by 0.0555) or the use of oral blood glucose-lowering drugs or insulin. Exposure to antihypertensives, salicylates, and antithrombotics was obtained from pharmacy records.

Statistical analysis

For all subjects, we calculated the duration of follow-up between the start of the study and the date of death, diagnosis of stroke, or end of the study period, whichever came first. We calculated the hazard ratio (HR) (and 95% confidence interval [CI]) of stroke with a Cox proportional hazards model (SPSS 11.01 software; SPSS Inc, Chicago, Illinois), in which calendar time was used as the time axis. Separate analyses were performed for all strokes, ischemic strokes, and hemorrhagic strokes. All analyses were adjusted for age and sex (crude model). In a second model, we adjusted for other potential confounders as previously described (adjusted model). During follow-up, at each time an event occurred, we determined the exposure to NSAIDs. Use of NSAIDs was classified as never, current, or past use of an NSAID and subsequently categorized into groups according to COX selectivity and as individual NSAIDs. Persons who had not used an NSAID before the date of an event were categorized as “never user”. Persons were considered current users of an NSAID if an event date fell between the start date and end date of a prescription. If a person had previously used an NSAID but no longer used the drug on an event date, they were considered a past user. Due to the initiation and cessation of prescribed drug use, persons can switch from the never to the current exposure category and from the current to the past exposure category or vice versa. Never use was the reference for all analyses. Simultaneous current use of 2 or more NSAIDs was rare (0.1%) and was excluded from the analyses. For the individual NSAIDs, we investigated a dose-effect relationship by dichotomizing the mean dose of current use as 1 DDD or less and greater than 1 DDD. We performed several sub-analyses. First, we considered that noncompliance or a pharmacological “carryover” could affect the observed associations. Hence, in a sensitivity analysis we extended the risk window by 14 days after the end date of the prescription to see whether this altered our result. Second, the analysis was performed in a subcohort with a history of at least 1 NSAID prescription during follow-up to study the role of potential confounding by indication or contraindication. Third, previous studies have shown that COX2-

selective NSAIDs are preferentially prescribed to persons with substantial comorbidity.[31] To investigate whether this played a role in our study population, we determined for all COX-selective NSAID groups whether a history of use was related to the risk of stroke. Finally, the effect of concomitant use of salicylates could affect our results for reasons described previously. We thus performed an analysis stratified by current use of salicylates.

RESULTS

Baseline characteristics of the cohort at risk are given in Table 2. At baseline, the mean age of the participants was 70.2 years, and the majority was female (61.3%). During 70,063 person-years of follow-up, 807 persons developed a stroke. Of these individuals, 460 were diagnosed as having an ischemic stroke and 74 as having a hemorrhagic stroke, while for 273 individuals the type of stroke could not be specified. The mean follow-up was 9.2 person-years. In our study population, 61 persons who experienced a stroke were current users of any NSAID at the time of the event, whereas 290 persons with a stroke had never used an NSAID during the study period.

Table 2. Demographic and clinical characteristics of the study cohort at baseline

Characteristic	
Age, y*	70.2 (± 9.6)
Female sex	4,684 (61.3%)
Smoking, ever	4,659 (60.9%)
Osteoarthritis in hand, knee or hip	595 (7.8%)
Diabetes mellitus	794 (10.4%)
Myocardial infarction	862 (10.8%)
Atrial fibrillation	339 (4.4%)
Transient ischemic attacks	207 (2.7%)
Percutaneous Transluminal Coronary Angioplasty	55 (0.7%)
Coronary artery bypass graft	164 (2.2%)
Heart failure	232 (3.0%)
Body Mass Index, kg/m^2*	26.3 (± 3.7)
Total serum cholesterol, mmol/l*	6.6 (± 1.2)
Systolic blood pressure, mm Hg*	139 (± 22)

*Data are given as mean, ± SD

As shown in Table 3, current use of any NSAID was associated with a greater risk of stroke compared with never use. Adjustment for confounders resulted in higher estimates. Associations were stronger if only ischemic strokes were considered. Use of any NSAID was related to the risk of hemorrhagic stroke (HR, 2.03; 95% CI, 0.81-5.11), albeit nonsignificant. Table 3 also shows that current users of nonselective NSAIDs and of COX2-selective NSAIDs had a higher risk of stroke compared with never users. We found no association for COX1-selective NSAID use with the risk of stroke. Current use of NSAIDs with unknown COX-selectivity on the index date was infrequent (0.02%) and thus not studied further. All associations were stronger if only ischemic strokes were considered (Table 3). There were no exposed cases in the class of COX2-selective NSAIDs for hemorrhagic stroke precluding comparison of the effect of different NSAIDs on the risk of hemorrhagic stroke.

As shown in Table 4, for individual NSAIDs, current use of the non-selective NSAID naproxen and the COX2-selective rofecoxib were associated with a greater risk of stroke. All but 1 person among the COX2-selective users, who experienced an event, used rofecoxib. Although HRs for current use of diclofenac (HR, 1.60; 95% CI, 1.00-2.57), ibuprofen (adjusted HR, 1.47; 95% CI, 0.73-3.00), and celecoxib (adjusted HR, 3.79; 95% CI, 0.52-27.6) were greater than 1.00, none of them reached the level of conventional statistical significance. The low number of events for celecoxib (n=1), as well as for all other individual NSAIDs, prohibited further investigation of these exposure categories. There was no clear dose-response effect, since doses of 1 DDD or less and greater than 1 DDD were both associated with a greater risk of stroke. However, analyses were compromised by low case numbers and unequal distribution of exposed cases across dosage categories. As for our subanalyses, the extension of the risk window by 14 days after the cessation of drug use attenuated the risk estimates (adjusted HRs [95% CIs] of stroke for current use of any current NSAID, 1.61 [1.20-2.16]; for COX1-selective NSAIDs, 1.01 [0.37-2.72]; for nonselective NSAIDs, 1.55 [1.11-2.16]; and for COX2-selective NSAIDs, 2.71 [1.26-5.86]). If we performed the analysis in a subcohort with at least 1 NSAID prescription during follow-up (517 events), we observed little change in HRs compared with the analyses in which never use was defined as the reference (data not shown). Past use of COX2-selective NSAIDs compared with never use was associated with a greater risk of stroke (adjusted HR, 2.04; 95% CI, 1.34-3.09); no such association was observed for a history of any NSAID

Table 3. Hazard ratios for all stroke and ischemic stroke with current use of any NSAID and NSAIDs grouped according to COX-selectivity

	All stroke				Ischemic stroke			
NSAID use	**Cases**	**HR (95% CI), crude***	**Cases**	**HR (95% CI), adjusted†**	**Cases**	**HR (95% CI), crude**	**Cases**	**HR (95% CI), adjusted**
Never use	290	1.00 (ref.)	222	1.00 (ref.)	156	1.00 (ref.)	128	1.00 (ref.)
Any NSAID	61	1.58 (1.19-2.08)	51	1.77 (1.29-2.41)	34	1.74 (1.20-2.53)	31	1.92 (1.29-2.87)
Non-selective NSAID	48	1.58 (1.16-2.15)	39	1.72 (1.22 -2.44)	24	1.58 (1.02-2.44)	21	1.68 (1.05-2.69)
COX1-selective NSAID	5	0.95 (0.39-2.31)	4	1.10 (0.41-2.97)	2	0.78 (0.19-3.16)	2	0.99 (0.24-4.01)
COX2-selective NSAID	7	2.40 (1.12-5.14)	7	2.75 (1.28-5.95)	7	4.20 (1.93-9.13)	7	4.54 (2.06-9.98)

Abbreviations: CI - confidence interval, HR - hazard ratio

* Sex, age adjusted

† Additionally adjusted for systolic blood pressure, BMI, total serum cholesterol and smoking. Time-dependent covariates included myocardial infarction, atrial fibrillation, heart failure, TIA, CABG, PTCA, diabetes mellitus and use of antihypertensives, salicylates and anti-thrombotics.

Table 4. Hazard ratios of all stroke and ischemic stroke with current use of individual NSAIDs

	All stroke				Ischemic stroke			
NSAID use	**Cases***	**HR (95% CI), crude†**	**Cases**	**HR (95% CI), adjusted‡**	**Cases**	**HR (95% CI), crude†**	**Cases**	**HR (95% CI), adjusted‡**
Never use	290	1.00 (ref.)	222	1.00 (ref.)	156	1.00 (ref.)	128	1.00 (ref.)
Non-selective NSAIDs								
Diclofenac	20	1.35 (0.86-2.13)	19	1.60 (1.00-2.57)	12	1.61 (0.89-2.90)	11	1.70 (0.91-3.17)
Ibuprofen	11	1.32 (0.72-2.42)	8	1.47 (0.73-3.00)	3	0.76 (0.24-2.40)	3	1.02 (0.32-3.32)
Naproxen	15	2.67 (1.58-4.48)	11	2.63 (1.47-4.72)	7	2.37 (1.11-5.06)	6	2.65 (1.23-5.69)
COX2-selective NSAIDs								
Rofecoxib	6	3.24 (1.42-7.37)	6	3.38 (1.48-7.74)	6	5.73 (2.48-13.2)	6	5.56 (2.38-12.9)

Abbreviations: CI - confidence interval, HR - hazard ratio

* Results are shown for analyses with ≥5 current exposed cases for the outcome of all stroke.

† Sex, age adjusted

‡ Additionally adjusted for systolic blood pressure, BMI, total serum cholesterol and smoking. Time-dependent covariates included myocardial infarction, atrial fibrillation, heart failure, TIA, CABG, PTCA, diabetes mellitus and use of antihypertensives, salicylates and anti-thrombotics.

use (HR, 1.13; 95% CI, 0.95-1.35) or for the other COX-selective groups (COX1-selective NSAIDs, [HR, 1.13; 95% CI, 0.94-1.35]; and nonselective NSAIDs, [HR, 1.16; 95% CI, 0.91-1.48]). Notably, almost all users of COX2-selective NSAIDs had used other types of NSAIDs earlier during follow-up and were, in general, long-term users of NSAIDs. Only 2 cases were current concomitant users of salicylates and NSAIDs; hence, stratification on concomitant use of salicylates could not be performed.

DISCUSSION

In the general population, we found an overall greater risk of stroke with use of NSAIDs, especially in the categories of nonselective NSAIDs and COX2-selective NSAIDs. The risk of stroke was most pronounced with COX2-selective NSAID use.

Strengths of our study design included its prospective design, large number of participants, long follow-up period, and a general population-based setting, which makes selection bias unlikely. Information bias was prevented by prospectively collected and complete automated pharmacy records of all filled prescriptions and blinded adjudication of cerebrovascular events. Certain limitations of our study, however, deserve comment. First, as with most of the clinical trials and observational studies performed to date, inferences must be interpreted in the context of small numbers despite a mean follow-up of more than 9 years. Second, although in the Netherlands long-term use of NSAIDs was fully reimbursed until the beginning of 2004, some misclassification might have occurred owing to intermittent use of “over-the-counter” NSAIDs. If this biased our results, however, it will have led us to underestimate an effect rather than overestimate the risk of stroke. Finally, “preferential prescribing” might have played a role in the COX2-selective NSAIDs group, since we observed a higher risk of stroke for past users of COX2-selective NSAIDs. This channeling bias with COX2-selective NSAIDs has been described previously in another Dutch patient population setting.[31] However, since the risk estimates were higher for current use of COX2-selective NSAIDs than for past use, this type of confounding cannot fully explain the greater risk of stroke.

Our results are largely in agreement with the currently available data from randomized clinical trials. In the Alzheimer Disease Anti-inflammatory Prevention Trial (ADAPT), use of naproxen was associated with a similar 2-fold increased risk of stroke compared with placebo, which is in striking accordance with the results of the present study.[4]

This same study did not report an effect of celecoxib on the risk of stroke. In our study, no statistically significant effect was found for celecoxib either, although the effect size was similar to that of rofecoxib. Because of low numbers, however, an effect of celecoxib on the risk of stroke cannot be excluded. The similar occurrence of ischemic cerebrovascular events for the rofecoxib and the naproxen treatment arms of the Vioxx GI Outcomes Research (VIGOR) study corresponds with our finding of a greater risk of stroke for both these NSAIDs.[1] However, because VIGOR did not include a placebo treatment, it does not provide evidence for the direction of the association.[1] More compelling data consistent with an increase in the risk of stroke with rofecoxib use are provided by the results of the "Adenomatous Polyp Prevention on Vioxx trial," in which a 2-fold increased risk for cerebrovascular events was observed compared with placebo after 36 months of follow-up.[2]

Several other observational studies also investigated the use of NSAIDs on the risk of ischemic stroke. Our finding of an overall greater risk of ischemic stroke with current use of NSAIDs corresponds with the case-control study by Bak *et al*.[7] However, direct information on potential confounders was not available in this study, and only concomitant drug use could be used as proxy for the presence of confounding conditions. Andersohn *et al.*[11] found a greater risk of ischemic stroke for rofecoxib, etoricoxib, and diclofenac but not for celecoxib. The higher risk was most pronounced with COX2-selective NSAID use, which corresponds with our observations. In our study we observed similar odds ratios for celecoxib and rofecoxib. However, celecoxib was not introduced in the Netherlands until 2001, and the use among persons in our cohort was limited, precluding definite conclusions. Like Andersohn *et al*.[11] our results also put forward a higher risk of stroke with the use of diclofenac, although statistical significance was not reached. These observational studies, including our own, suggest that an effect of NSAIDs on the risk of ischemic stroke is not restricted to the COX2-selective compounds.

The classification of COX selectivity used in the present study complies with the generally accepted labeling of NSAID selectivity. Nevertheless, some debate exists regarding the COX-selective properties of, mainly, diclofenac, celecoxib, and naproxen. Some have argued that diclofenac is COX2 selective, since diclofenac would not differ much from celecoxib in terms of its ability to inhibit COX2.[32] However, therapeutically relevant COX2 selectivity will be difficult to attain, since the concentration of diclofenac necessary to achieve 80% inhibition of COX2 is expected to cause a similar inhibition

of COX1.[22] For naproxen, relative selectivity for COX1, and hence a cardioprotective effect, has been suggested, since naproxen causes near-maximal inhibition of platelet aggregation similar to aspirin.[26,33] However, clinical evidence does not suggest that relative COX1 selectivity is achieved.[4,34] Despite the alleged differences in COX selectivity of these compounds, we found higher risks of stroke for both diclofenac and naproxen and also for celecoxib and rofecoxib. These findings do not necessarily exclude the possibility of an effect through a COX-related mechanism. Because selective inhibition of COX2 causes platelet aggregation, use of COX2-selective NSAIDs could, as suggested previously, cause a prothrombotic state.[12-14] However, since both COX1 and COX2 are involved in vascular homeostasis, any pharmacological inhibition of the COX enzymes could be expected to disturb the thrombotic equilibrium, which would explain our observations. In addition, other COX mediated processes relevant to the pathophysiologic mechanisms of cerebrovascular events might be involved, such as inflammatory response and renovascular physiology.[35,36] This could provide an alternative explanation for the cerebrovascular risk not being restricted to COX2-selective NSAIDs.

In conclusion, our study suggests that the greater risk of stroke is not limited to the use of COX2-selective NSAIDs. Our risk estimates are in line with estimates from the literature. The existing knowledge regarding the effects of pharmacological interference of COX is currently incomplete. Evaluation of COX activity *in vivo*, together with post-marketing surveillance and observational studies, will be essential to elucidate the potential mechanisms underlying the cerebrovascular effects associated with these drugs.

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Chapter 1.2

NSAIDs and risk of Transient Ischemic Attack

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ABSTRACT

Background: In clinical trials, cyclo-oxygenase (COX)-2-selective nonsteroidal anti-inflammatory drugs (NSAIDs) were associated with an increased risk of thrombotic events. We studied the association between use of NSAIDs and risk of transient ischemic attack (TIA) in the prospective, population-based Rotterdam Study.
Methods: We followed 7,392 persons free of TIA or stroke at baseline (1991-1993) for first incident TIA until September 2004. Data on all filled prescriptions came from pharmacy records. Persons were considered current user of a NSAIDs if the period of NSAID use overlapped the date of the event. With Cox-regression models, we calculated crude and adjusted hazard ratios (HR) of TIA for time-dependent current use, compared to never use, of any NSAID and of non-COX2-selective NSAIDs and COX2-selective NSAIDs.
Results: At baseline, the mean age of the participants was 70.1 years and the majority was female (61.4%). Persons were followed up to 15.1 years (average 9.4 years (± SD 4.0)), 330 persons developed a TIA. There were 22 current users of NSAIDs at the time of incident TIA: 19 non-COX2-selective NSAID users and 3 COX2 selective NSAID users. Current use of any NSAID was associated with an increased risk of TIA (sex, age adjusted HR 1.61 (1.02-2.54)). The HR for current use of non-COX2-selective NSAID use was HR 1.51 (0.93-2.45) and HR 2.85 (0.89-9.17) for COX2-selective NSAID use. Additional adjustments for potential confounders did not change the association by more than 5%.
Conclusion: In the general population, we found a greater risk of TIA with current use of NSAIDs.

INTRODUCTION

In clinical trials, the use of cyclooxygenase-(COX)-2 selective NSAIDs has been associated with an increased risk of cardiovascular and cerebrovascular events and death.[1-5] Subsequent analyses of observational series and non-cardiovascular clinical trials suggested that cardiovascular events may also occur with non-selective NSAIDs.[6-9] Hence, it was debated whether the cardiovascular risk was restricted to the COX2-selective NSAIDs. Furthermore, there is limited knowledge on the risk of NSAID use for individual cardiovascular, but particularly, cerebrovascular events. Previously, we reported from the Rotterdam Study that the use of both non-selective and COX2-selective NSAIDs was associated with a greater risk of ischemic stroke.[11] An outcome which is strongly related to ischemic stroke is transient ischemic attack (TIA), which is essentially an ischemic attack of 'transient' nature.[12] Like ischemic strokes, TIAs are caused by focal cerebral or retinal ischemia. Persons who suffered from a TIA are at increased risk of stroke, cardiovascular events and death.[13-15] We studied the association between use of NSAIDs and risk of TIA in the Rotterdam Study, a large observational prospective population based study.[16]

METHODS

Study population

The Rotterdam Study is a prospective, population-based cohort study of age related disorders.[18] The medical ethics committee of the Erasmus Medical Center approved the study.[16] Between 1990 and 1993 all persons aged 55 years or older living in Ommoord, a district of Rotterdam, were invited to participate. Of the 10,275 eligible persons, 7,983 (78%) signed informed consent. We excluded 455 persons with either prevalent TIA or stroke, as we considered that persons with a history of TIA or stroke have a different risk of incident TIA. Follow-up examinations were conducted in 1993 to 1994, 1997 to 1999 and 2000 to 2004. In addition, the cohort was continuously monitored for major disease outcomes and death through linkage with records from general practitioners and bimonthly updates from the municipality records. Nearly all persons (99.7%) were registered at one or more of seven automated pharmacies serving the Ommoord area. Of these pharmacies, records of all filled prescriptions were available as of January 1st 1991. To ensure at least six months of medication history, we excluded 86 persons for whom follow-up ended before July 1st 1991. Consequently, the study population

included 7,392 persons. The end of the study period for this analysis was September 30th 2004, the date on which rofecoxib was withdrawn from the market after clinical trial data showed an increased risk of thrombotic events with rofecoxib treatment.

Drug exposure

Complete information on all filled prescriptions for all persons was obtained in automated format from the pharmacies. This included the product name; international non-proprietary name; Anatomical Therapeutic Chemical code[17]; total number of delivered units (e.g. tablets/capsules); prescribed daily number of units; date of delivery and drug dosage. Drug dosage was defined by the defined daily dose (DDD), the recommended daily dosage of a drug taken by adults for the main indication of the drug. NSAIDs were classified as non-COX2-selective NSAIDs and COX2-selective NSAIDs according to their selectivity for the COX2 enzyme at therapeutic dosages, based on data from *in vitro* and clinical studies (Table 1).[18-24]

Table 1. Classification of NSAIDs

Non-COX2-selective NSAIDs	COX2-selective NSAIDs
Diclofenac*	Rofecoxib
Naproxen	Celecoxib
Ibuprofen	Meloxicam
Nabumeton	Etoricoxib
Sulindac	Valdecoxib
Indometacine	
Piroxicam	
Ketoprofen	
Flurbiprofen	
Azapropazon	

*Includes combination products of diclofenac

Diagnosis of TIA

During the visit at the research center, a trained Rotterdam Study physician asked all participants, “Did you experience a short period with disturbances of sensibility in your face, arms, or legs, which lasted less than 24 hours over the last 3 years?” Similar questions were asked for disturbances in strength, speech, and vision. When answers were positive, time of onset, duration, and disappearance of symptoms and whether a general practitioner had been consulted were recorded. Additionally, a

detailed description of the symptoms in ordinary language was obtained.[25] On the basis of this information, one of the investigators, a neurologist, classified events as TIA or no TIA.[26] An attack was regarded as being a TIA according to the guidelines of the Ad Hoc Committee for the Classification and Outline of Cerebrovascular Disease[12] : i.e., (1) weakness, clumsiness, or sensory alteration in one or both limbs on the same side, speech or language disturbance, loss of vision in one eye or part of the eye, or homonymous hemianopsia for symptoms that pertain to the carotid territory; (2) weakness or clumsiness (sometimes changing from one side to another), sensory alteration, complete blindness or homonymous hemianopsia, ataxia, imbalance, or unsteadiness not associated with vertigo; and/or (3) two or more of the following: diplopia, dysphagia, dysarthria, or vertigo for symptoms that pertain to the vertebrobasilar territory.[27] Symptoms completely resolved within 24 hours. No clear evidence for the diagnosis of migraine, epilepsy, Ménière's disease, hyperventilation, cardiac syncope, hypoglycaemia, or orthostatic hypotension was to be present.
A reproducibility study in which 121 case histories were reclassified by the same neurologist, blinded for the initial diagnosis, revealed a weighted of κ0.77 ($p<0.05$), indicating a good reproducibility. Follow-up for all events was completed until January 1st, 2005 for 96.2 % of potential person years. A second source of information on TIA came from those subjects who had replied affirmatively to the question "Did you ever suffer from a stroke?" Of these subjects, supplementary medical information, such as a copy of the hospital discharge records, was obtained from the general practitioner. On the basis of the available information, these subjects were also classified by the same neurologist as having had a stroke, a TIA but not a stroke, or neither a TIA nor a stroke. Diagnosis of strokes in the Rotterdam Study has been extensively described earlier.[28]

Other covariates

Potential confounders were chosen a priori. Baseline covariates included age, sex, cardiovascular disease (myocardial infarction, atrial fibrillation, heart failure, coronary artery bypass graft, percutaneous transluminal coronary angioplasty) and the following cardiovascular risk factors; systolic blood pressure, total serum cholesterol, diabetes mellitus, current smoking, diabetes mellitus and use of salicylates. Sitting blood pressure was measured on the right upper arm using a random-zero sphygmomanometer. The average of two measurements, measured at one occasion, was used. Total serum cholesterol was measured in non-fasting blood drawn at baseline. Cardiovascular conditions were assessed at the baseline interview and follow-up examinations, together with a review of medical records. In addition,

an electrocardiogram was performed to determine myocardial infarction and atrial fibrillation. Diabetes mellitus was defined as non-fasting serum glucose level exceeding 11.1mmol/l or the use of oral blood glucose lowering drugs or insulin. Exposure to salicylates was obtained from pharmacy records.

Statistical analysis

During follow-up, at each date an event occurred, we determined the status of NSAID use for the person who suffered the event and for all persons in the remainder of the cohort and categorized NSAID use as never use, current use or past use. Persons were considered 'current' user of a NSAID if an event-date fell between the start-date and end-date of a prescription. If a person previously used a NSAID, but no longer used the drug on an event date, they were considered as 'past' users. The duration of a prescription was calculated as the total number of delivered units divided by the prescribed daily number of units. We also investigated a dose-effect relationship by dichotomizing the average dose of current use as ≤1DDD and >1 DDD. Never use was the reference for these analyses. However, since 'never' users of NSAIDs do not have an indication or may have a contraindication for NSAID use this could lead to bias due to confounding-by-indication or counfounding-by-contraindication.[29] Therefore, we also performed an analysis in which we compared 'current' users to 'past' NSAID users.

For all subjects, we calculated the duration of follow-up between start of the study and date of death, diagnosis of first incident TIA, or end of the study period, whichever came first. If a person suffered from a stroke during follow-up, persons were censored from the date of the stroke onwards. We calculated the hazard ratios (HR) (and 95% confidence intervals (CI)) of TIA with a Cox proportional hazards model[30] (SPSS 11.01 software) in which calendar-time was used as the time-axis. All analyses were adjusted for age and sex (Model I). In a second model, we adjusted for other potential confounders being cardiovascular disease, blood pressure, total serum cholesterol, current smoking, diabetes mellitus and salicylates (Model II).

RESULTS

Baseline characteristics of the study population are shown in Table 2. At baseline, the mean age of the participants was 70.1 years and the majority was female (61.4%).

Table 2. Demographic and clinical characteristics of the study cohort at baseline **(**n=7,392)

Characteristic	
Age, y (± SD)	70.1 (± 9.6)
Female sex (%)	4,540 (61.4%)
Smoking, current (%)	1,601 (21.7%)
Diabetes mellitus (%)	756 (10.2%)
Cardiovascular disease (%)	1,107 (15.0%)
Myocardial infarction (%)	817 (11.1%)
Atrial fibrillation (%)	322 (4.4%)
Heart failure (%)	213 (2.9%)
Percutaneous transluminal coronary angioplasty (%)	53 (0.7%)
Coronary artery bypass graft (%)	153 (2.1%)
Total serum cholesterol, mmol/l (± SD)	6.6 (± 1.2)
Systolic blood pressure, mmHg (± SD)	139.1 (± 22.3)

Persons were followed up to 15.1 years (average 9.4 years (± SD 4.0)). During a total of 64,435 person years of follow-up, 365 persons developed a TIA. Of these, 35 persons were censored based on incident stroke, leaving 330 TIA events in the analysis. There were 122 persons who experienced a TIA who had 'never' used an NSAID during follow-up, whereas 22 persons who experienced a TIA were current user of a NSAID at the time of the event.

Current use of NSAIDs was associated with a greater risk of TIA compared to never use (Table 3). Current users of both non-selective NSAIDs and of COX2-selective NSAIDs had a higher risk of TIA than never users, but statistical significance was not reached. There was no clear dose-response effect as doses ≤1DDD and >1DDD were both associated with a greater risk of TIA. Neither of the individual covariates changed the risk estimates by more than 5% in sex-age adjusted models. In Model II concurrent adjustment for all covariates did not materially change the associations.

Table 3. Hazard ratios for TIA with current use of any NSAID and NSAIDs grouped according to COX2-selectivity

		Risk of TIA	
NSAID use	**N_{cases}**	**HR (95% CI), Model I***	**HR (95% CI), Model II†**
Never use		1.00 (ref.)	1.00 (ref.)
Current use of:			
Any NSAID	22	1.61 (1.02-2.54)	1.59 (1.01-2.52)
≤1 DDD	11	1.68 (0.90-3.11)	1.67 (0.90-3.10)
>1 DDD	11	1.54 (0.83-2.87)	1.52 (0.82-2.84)
Non-COX2-selective NSAID	19	1.51 (0.93-2.45)	1.49 (0.92-2.21)
COX2-selective NSAID	3	2.85 (0.89-9.17)	2.80 (0.87-9.02)

Abbreviations; HR - hazard ratio, TIA - transient ischemic attack, NSAIDs - nonsteroidal anti-inflammatory drugs, COX - cyclooxygenase, CI - confidence interval
* Sex, age adjusted.
† Additionally adjusted for blood pressure, total serum cholesterol, current smoking, diabetes mellitus cardiovascular disease (myocardial infarction, atrial fibrillation, heart failure, coronary artery bypass graft, percutaneous transluminal coronary angioplasty systolic) and use of salicylates.

If, instead of never use, past use of NSAIDs served as reference, HRs attenuated by less than 10% (adjusted HR with current use of any NSAID 1.47; 95% CI 0.94-2.29, non-COX2-selective NSAIDs 1.39 95% CI; 0.83-2.23 and COX2-selective NSAIDs 2.58 (0.81-8.20). Past use of NSAIDs compared to never use was not associated with the risk of TIA (adjusted HR with any past use of any NSAID 1.08 (95% CI 0.85-1.38), non-COX2-selective NSAIDs 1.13 (95% CI 0.89-1.44), and COX2-selective NSAIDs 1.12 (95% CI 0.88-1.38)).

DISCUSSION

In the general population, we found an overall greater risk of TIA with use of NSAIDs. Risk estimates with current use of non-COX2-selective NSAIDs and COX2-selective NSAIDs were both increased, though neither reached statistical significance.

Strengths of our study design included its prospective design, large number of participants, a general population-based setting and the long follow-up period. The availability of pharmacy records throughout follow-up allowed for an accurate estimation of NSAID exposure on the date of the event as opposed to baseline or periodic assessment of drug use. Also, compared to prescription records, pharmacy records provide certainty as to whether prescriptions are filled and when they are filled. One important limitation of our study is the small number of events. This prohibited extensive subanalyses and resulted in lack of statistical power, particularly in the analyses across subgroups of NSAIDs. Furthermore, the number of cases limits the number of covariates that can be adjusted for in a multivariate model. We showed that adjustment for each individual covariate did not materially change the risk estimates. Also in the full model, risk estimates were essentially unchanged. We also investigated the potential influence of confounding-by-indication or contra-indication by using past-use as a reference and found that this did not change our observations. Another limitation is that misclassification may have occurred in the identification of TIA during follow-up. Though we obtained information about TIA both by self-report and from medical records, people may underreport symptoms of TIA. This could have resulted in an underestimation of the true number of events. However, we have no reason to believe that misclassification of the outcome, if any, was different between users and non-users of NSAIDs and thus biased our observations. Finally, although in the Netherlands chronic use of NSAIDs was fully reimbursed until the beginning of 2004, some misclassification might have occurred due to intermittent use of 'over the counter' (OTC) NSAIDs. If this biased our results, however, it leads to underestimating an effect rather than overestimating the risk of TIA.

Our findings fit our previous observation[11] and those of others of an increased risk of cerebral events with NSAID use. Few studies have, however, reported on the association between NSAID use and risk of TIA in specific. In the Alzheimer's Disease Anti-Inflammatory Prevention Trial (ADAPT)[31] the use of the non-COX2-selective naproxen or the COX2-selective celecoxib was not associated with an increased risk of TIA compared to placebo based on 10 or less cases in exposure and reference group. In an analysis across 8 phase IIb/III osteoarthritis clinical trials, a higher rate of TIA was observed in the rofecoxib arm than in the placebo arm.[32] No risk estimates were given. In a Taiwanese cohort study of long-term (≥180 days) NSAIDs users, patients with preexisting medical conditions had a 6-fold higher risk of TIA associated with the use of NSAIDs compared to patients without these conditions.[33] Other studies included TIA

in a combined cerebrovascular endpoint.[2,34] However, since this combined outcome is generally dominated by stroke events, these data are difficult to compare to our current findings.

There is considerable overlap in underlying etiology of ischemic stroke and TIA events and in their risk factors.[27,35,36] Our previous observation of an increased risk of stroke with NSAID together with our current findings strongly suggest that NSAIDs act on a common pathway leading to either of these events. Several hypotheses regarding the mechanism of the increased thrombotic risk of NSAIDs have been proposed. It was generally thought that selective inhibition of COX2 causes a prothrombotic state due to a disturbance in the COX2 and COX1 mediated balance of prostacyclin, which inhibits platelet aggregation, and respectively thromboxane, which facilitates platelet aggregation. More recent studies have suggested that prostacyclin may not only oppose prothrombotic effects of thromboxane but also acts as a general constraint on multiple thrombosis stimuli via different mechanisms of action. Additionally, other processes affected by NSAIDs relevant to the pathophysiology of cerebrovascular events might be involved, such as inflammatory response and renovascular physiology. Howevere these effects are less likely to lead to acute cerebrovascular events.

In conclusion, in the general population we found a greater risk of TIA with current use of NSAIDs.

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Chapter 1.3

NSAIDs and risk of Parkinson disease

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ABSTRACT

Background: Several lines of evidence suggest a role of inflammatory processes in Parkinson disease, although it is still unclear whether inflammation is a cause or rather a consequence of neurodegeneration.

Methods: In a prospective population-based cohort study among 6,512 participants aged ≥55 years, with repeated in-person examination, we evaluated the association between cumulative use of nonsteroidal anti-inflammatory drugs (NSAIDs) and the risk of Parkinson disease. Complete information on filled prescriptions was available from automated pharmacy records. Data were analyzed by means of Cox proportional hazards regression analysis, adjusted for age, sex, smoking habits and coffee consumption.

Results: After an average 9.4 years of follow-up, 88 incident cases of Parkinson disease were detected. No association was found between use of NSAIDs and the risk of Parkinson disease (adjusted hazard ratio for any NSAID use, 1.50; 95% confidence interval, 0.95-2.37).

Conclusion: Our findings do not support the hypothesis that NSAIDs might decrease the risk of Parkinson disease.

INTRODUCTION

The causes of the excessive dopaminergic cell death in Parkinson disease (PD) are still poorly understood. Activated glial cells and increased levels of proinflammatory cytokines have been observed in brains and cerebrospinal fluid of PD patients.[1,2] Microglial activation has also been observed in association with the loss of dopaminergic neurons in animal models of PD.[3] However, the exact pathogenetic role of this immune response and whether it is a cause or rather a consequence of neurodegeneration is as yet unclear. Prospective studies on inflammatory markers in relation to PD have not yet been reported.

A causal role of inflammation in PD has been suggested by 2 large cohort studies in which self-reported baseline use of nonsteroidal anti-inflammatory drugs (NSAIDs) was associated with a decreased risk of incident PD.[4,5] In these studies duration and cumulative dosage of NSAID use were also estimated based on data from repeated questionnaires. However, the main exposure being studied was self-reported baseline use, which is a crude measure that does not account for changes in use of NSAIDs during the follow-up period.[6] We prospectively evaluated the association between NSAID use and incident PD taking into account cumulative use, in a large prospective population-based cohort study.

METHODS

The Rotterdam Study is a prospective population-based cohort study among 7,983 persons aged ≥55 years in a district of Rotterdam, the Netherlands.[7] Both at baseline (1990-1993) and 3 follow-up rounds (1993-1994, 1997-1999 and 2002-2004), all participants were interviewed and underwent extensive physical examination. We used a 2-phase design to identify subjects with PD.[8] In the first phase, all participants were screened for parkinsonian signs (resting tremor, rigidity, bradykinesia or impaired postural reflexes) according to a standardized protocol. Those who screened positive received a structured clinical workup, comprising the motor examination of the Unified Parkinson's Disease Rating Scale and neurological examination and history taking by a general physician specialized in research on neurological disorders or a resident in neurology, to establish parkinsonism and to classify subtypes. When PD was suspected or in case of doubt about the diagnosis, participants were invited for a further evaluation

by a neurologist, who decided on the final diagnosis. In addition to the in-person follow-up, the cohort was continuously monitored by computer linkage with the general practitioners' automated medical record systems. Through this surveillance system, we were notified of incident cases of parkinsonism and had access to the subjects' medical records. Medical records of all cases of incident parkinsonism detected through the computerized surveillance system were reviewed by a neurologist. The information obtained from the continuous monitoring system was also used for subjects who could not be reexamined in person at follow-up (due to death, migration, disease, logistic reasons or refusal). PD was diagnosed if ≥2 parkinsonian signs were present in a person not taking antiparkinsonian drugs, or if at least 2 signs had been present before starting medication and at least 1 sign improved after medication had been started, and when all causes of secondary parkinsonism could be excluded.

Data on use of NSAIDs [including use of salicylates (i.e. acetylsalicylic acid and carbasalate calcium)] were derived from 7 fully automated pharmacies in which nearly all participants (99.7%) were registered. These pharmacies continuously provided details on all filled prescriptions from January 1st, 1991, including the product name, generic name, number of tablets, date of delivery, prescribed number of tablets and daily drug dosage. The duration of a prescription was calculated as the total number of delivered units divided by the prescribed daily number of units. Smoking status [classified as ever (current or former) or never smoking], self-reported use of NSAIDs at time of enrollment, coffee intake, and a diagnosis of osteoarthritis, rheumatoid arthritis or gout were assessed during the baseline interview. Baseline neurological screening was performed in 6,969 participants. We excluded participants diagnosed as having any parkinsonism (n=130) or dementia (n=273) at baseline and 54 persons for whom <6 months of data on their history of medication use was available. This resulted in a study population of 6,512 participants at risk to develop incident PD. Data were analyzed by means of Cox proportional hazards regression, with calendar time as the time axis to account for differences in usage patterns and availability of NSAIDs during the study period. Analyses were initially adjusted for age and sex, and additionally for smoking habits and coffee consumption, as these were considered potential confounders.[4,9] We also adjusted the analyses for self-reported diagnoses of osteoarthritis, rheumatoid arthritis or gout at baseline, being the main indications for NSAID treatment. First, we evaluated the association between self-reported NSAID use at baseline and the risk of incident PD. Subsequently, we investigated total use of NSAIDs during the study period as registered in the pharmacy records. Any use was defined as at least 1 filled

prescription of NSAIDs during the study period. Total cumulative use was calculated from the sum of all prescription durations filled during follow-up. We created 3 mutually exclusive time-dependent variables of cumulative use: no use, use ≤1 year (defined as total cumulative use ≤365 days) and use >1 year (total cumulative use >365 days). To examine potential bias due to changes in NSAID use in preclinical PD, we repeated the analyses after excluding the last 2 years before diagnosis. Participants who developed PD within the first 2 years after baseline were excluded from these analyses.

RESULTS

After a total of 61,449 person-years of follow-up (mean= 9.4), we identified 88 participants with incident PD. Of those, 50 cases were detected through the structured workup at the research center and 38 through the computerized surveillance system.

Table 1 shows the baseline characteristics of the study population. NSAID use was reported by 415 participants (6.4%) during the baseline interview. In total 4,654 participants (71.5%) had used an NSAID during the study period, of whom 590 (9.1% of the study population) had a total cumulative use of >1 year. The median duration of filled prescriptions of NSAIDs was 22 days (interquartile range: 0-93).

Table 1. Baseline characteristics of the study population (n=6,512)

Characteristic	
Age (yr), mean (SD)	68.7(8.6)
Women, no (%)	3,845 (59.0)
Ever smokers, no (%)	4,252 (65.3)
Coffee drinkers, no (%)	5,528 (84.9)
Osteoarthritis, no (%)*	1,149 (17.6)
Rheumatoid arthritis, no (%)*	165 (2.5)
Gout, no (%)*	28 (0.4)
Self-reported NSAID use at baseline, no (%)	415 (6.4)

* Self-reported diagnosis at baseline interview

We found no association between self-reported NSAID use at baseline and risk of incident PD (age- and sex-adjusted hazard ratio= 1.04, 95% confidence interval= 0.45–2.43). No significant association was seen between cumulative NSAID use and risk of PD, neither for any use nor for use <1 year or use >1 year. The results were unaltered after additional adjustments for smoking and coffee consumption (Table 2). Taking into account a 2-year lag time (including 74 incident PD cases in the analyses) even resulted in an association in the opposite direction of what had been expected, possibly as a result of small numbers. Adjustment for self-reported osteoarthritis, rheumatoid arthritis or gout at baseline did not substantially change the results (data not shown).

Table 2. Cumulative duration of NSAID use and the risk of Parkinson disease

		HR (95% CI)		
NSAID use	**PD cases**	**Sex, age adjusted**	**Full model***	**With 2-year lag time**
Never use	34	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Any use	54	1.47 (0.93-2.30)	1.50 (0.95-2.37)	1.70 (0.97-2.99)
≤1 year	48	1.44 (0.91-2.29)	1.48 (0.93-2.35)	1.66 (0.94-2.95)
>1 year	6	1.71 (0.70-4.20)	1.77 (0.72-4.35)	2.15 (0.77-5.96)

* Adjusted for age, sex, smoking, and coffee consumption

DISCUSSION

In this prospective, population-based study we found no significant beneficial effect of NSAID use on the risk of PD. In fact, the risk of PD associated with use of NSAIDs – if anything – appeared increased. Among the strengths of our study are the almost complete follow-up and intensive case finding methods using in-person screening, which minimizes the possibility of misclassification of disease status. Besides, instead of relying on questionnaires or doctor prescriptions, we drew data on filled prescriptions from pharmacy records, which is a more precise method of exposure assessment and reduces misclassification of drug use. A potential drawback of using computerized pharmacy data is the lack of information on use of over-the-counter medication. Theoretically, use of over-the-counter medication may differ between participants

with and without PD, due to socioeconomic or other differences. During the 1990s preparations of low dosages of ibuprofen and naproxen became available in the Netherlands as over-the-counter medication. However, until 1999 these drugs were fully covered by insurance companies, and after 1999 chronic use (i.e. >3 months) of these drugs remained reimbursed, if given on prescription. Only in 2004 did reimbursement of these drugs become restricted to higher-dosage forms. Regular users of NSAIDs are thus unlikely to have obtained their drugs over the counter during the study period, and misclassification because of occasional use of over-the-counter NSAIDs would only concern the lower-exposure categories. Therefore, we think that the effect of over-the-counter medication on our findings is limited. Another potential limitation might be the discrepancy between prescribed medication and actual intake by the study participants. Nevertheless, in the current study filled prescriptions, i.e. medications that were actually collected at the pharmacy were taken into account, making this kind of misclassification less likely. We did not have data on use of NSAIDs before the start of the study period. We thus could not evaluate the effect of longer-term past exposure on the risk of PD. A final drawback of the current study is the limited power due to low numbers. The small number of participants who used NSAIDs for >1 year resulted in unstable estimates of the long-term effect of NSAID use. The relatively low number of 88 incident PD cases also precluded subanalyses, e.g. to compare the effects of non-COX2 selective NSAIDs versus COX2-selective NSAIDs, or to examine aspirin and non-aspirin NSAIDs separately. These kinds of subanalyses are potentially interesting, as recent evidence suggests that the hypothesized neuroprotective effects of NSAIDs might differ across subtypes.[10]

There is growing evidence for a role of inflammation in PD pathogenesis.[1,2,11] A profusion of microglia, the hallmark of inflammation in the brain, is present in the basal ganglia in PD and is associated with the loss of dopaminergic neurons.[1] A beneficial effect of NSAIDs on PD risk has been hypothesized, given that NSAIDs inhibit the enzyme cyclooxygenase, which plays an important role in inflammation.[4] In animal models, NSAIDs were found to attenuate 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced dopamine depletion and degeneration of dopaminergic neurons.[1,4] Thus far, no studies have been reported that prospectively evaluated the association between direct markers of inflammation and the risk of PD. However, in a large prospective cohort study among 142,902 health professionals, regular use of non-aspirin NSAIDs was associated with a 50% lower risk of PD.[4] A second large US-based prospective study demonstrated a significantly reduced risk of PD in users of ibuprofen.[5] In both studies,

however, case ascertainment relied on self-reported diagnoses of PD (confirmed by review of medical records) and not in-person screening methods. More importantly, continuous automated pharmacy data were not available in these studies, and NSAID exposure was defined as self-reported use assessed through questionnaires. In a recent population based case-control study with recording of NSAID use through a computerized pharmacy database, no association was found between ever or cumulative use and the risk of PD, which fits our present findings.[12] This study included 206 cases and 383 controls, identified through registers of a health maintenance organization in the USA. A very large register-based nested case-control study from the UK, which included 1,258 PD patients, showed no significant association either. However, in this study, which was also based on computerized prescription data, stratified analyses indicated a higher risk of PD in women and a lower risk in men.[13]

In conclusion, although an inverse association between NSAID use and the risk of PD has been suggested by 2 large cohort studies, the hypothesis that NSAIDs may lower the risk of PD could not be confirmed in our study, or in other studies based on automated pharmacy data.

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Chapter 1.4

NSAIDs and risk of Alzheimer disease

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ABSTRACT

Background: Observational studies suggested nonsteroidal anti-inflammatory drugs (NSAID) use might reduce the risk of Alzheimer disease (AD). Since then, *in vitro* and *in vivo* studies put forward that this protective effect may result from amyloid-peptide-β42 (Aβ42)-level lowering properties of certain NSAIDs, rather than from a general anti-inflammatory effect.

Methods: We investigated the association between use of NSAIDs that lower Aβ42-levels *in vitro* and *in vivo* and non-Aβ42-lowering NSAIDs, with the risk of AD in the prospective, population-based Rotterdam Study. We followed 6,992 participants from baseline (1990 -1993) until January 2005 for incident AD. Data on all filled prescriptions were obtained from pharmacy records. At each date of AD diagnosis, cumulative duration of NSAID use was calculated for both the person who was diagnosed with AD and the remainder of the cohort. Duration of use was categorized as: short- (≤1 month), intermediate- (>1 and <24 months) and long-term use (≥24 months) for Aβ42-lowering and non-Aβ42-lowering NSAIDs. Never-use of any NSAID was the reference in all analyses. Hazard ratios (HR) of AD were calculated with Cox-regression analyses, adjusting for age, sex and potential confounders.

Results: During 62,883 person years of follow-up, 582 persons developed AD. Long-term use of Aβ42-lowering NSAIDs was associated with a decreased risk of AD compared to never-use (adjusted HR 0.42; 95%CI 0.21-0.83). Long-term use of non-Aβ42-lowering NSAIDs was not associated with a reduced risk of AD (adjusted HR 1.15; 95%CI 0.47-2.82).

Conclusion: The reduced risk of AD associated with long-term use of NSAIDs is confined to NSAIDs with Aβ42-lowering properties. Our findings corroborate *in vitro* and animal studies which suggested that NSAIDs influence AD pathogenesis through an amyloid-processing related mechanism.

INTRODUCTION

Alzheimer disease (AD) is the most common cause of dementia in the elderly.[1] Neuropathologically, AD is characterized by the abundant presence of senile plaques and neurofibrillary tangles in specific brain regions.[2] A leading hypothesis regarding AD pathogenesis postulates a central role for amyloid peptide-β42 (Aβ42), the major constituent of senile plaques.[3] Aβ42 can accumulate in the brain either as a result of increased production, aberrant processing of the amyloid precursor protein, or because of decreased clearance.[4] Furthermore, brains of AD patients show signs of chronic inflammation, typically co-localized with the plaques. It has been suggested that a neuroinflammatory response may play a crucial role in AD pathogenesis.[5]

Epidemiologic studies have indicated that the use of nonsteroidal anti-inflammatory drugs (NSAIDs) might protect against AD.[6-12] We previously reported from a follow-up of the prospective cohort of the Rotterdam study until 1998, that long-term use of NSAIDs was associated with a reduced risk of AD.[10] Initially, the assumed explanatory mechanism underlying the protective effect of NSAIDs was that this class of drugs would reduce the inflammatory response observed in AD.[5] However, results from *in vitro* and *in vivo* mice model studies, that were published since, suggest that certain NSAIDs may have a more specific effect on Alzheimer pathology namely through interference with amyloid-β processing.[13-16] If true, this would shed new light on the interpretation of trials that found no beneficial effect on AD.[17,18] Thus far only one observational study distinguished between Aβ42- and non-Aβ42-lowering NSAIDs in relation to the risk of AD. The investigators found that the use of both Aβ42- and non-Aβ42-lowering NSAID, as obtained from annual interviews, was associated with a reduced risk of AD.[11]

In the Rotterdam Study we have detailed information on drug exposure from pharmacy dispensing records as well as on dementia outcomes.[19] This allowed us to investigate the current hypothesis that the use of NSAIDs that lower Aβ42-levels *in vitro* and *in vivo* is associated with a reduced risk of AD whereas no such effect exists for use of non-Aβ42-lowering NSAIDs.

METHODS

Study population

The Rotterdam Study is a prospective, population-based cohort study of age related disorders.[19] The medical ethics committee of the Erasmus Medical Center, Rotterdam, the Netherlands, approved the study. Between 1990 and 1993 all persons aged 55 years or older living in Ommoord, a district of Rotterdam, were invited to participate. Of the 10,275 eligible persons, 7,983 (77.7%) signed informed consent. Of these, 7,528 (94.3%) were screened for dementia and 7,046 were found to be free of dementia at baseline.[20] Follow-up examinations, including screening and clinical workup for dementia, were conducted in 1993 to 1994, 1997 to 1999 and 2000 to 2004. In addition, the cohort was continuously monitored for major disease outcomes and death through linkage with records of general practitioners, the Regional Institute for Outpatient Mental Health Care and bimonthly updates from the municipality records. This resulted in a virtually complete follow-up for dementia until January 1st, 2005. Nearly all persons (99.7%) were registered at one or more of seven automated pharmacies serving the Ommoord area. Of these pharmacies, records of all filled prescriptions were available as of January 1st, 1991. To ensure at least six months medication history, we excluded persons for whom follow-up ended before July 1st, 1991. Consequently, the study population consisted of 6,992 persons.

Drug exposure

Complete information on all filled prescriptions for all persons was obtained in automated format from the pharmacies. This included the product name; international non-proprietary name; Anatomical Therapeutic Chemical code;[21] total number of delivered units (e.g. tablets/capsules); prescribed daily number of units; date of delivery and drug dosage. The duration of a prescription is calculated as the total number of delivered units divided by the prescribed daily number of units.

NSAIDs were classified a priori as Aβ42-lowering or non-Aβ42 lowering NSAID based on their reported effects on Aβ42-levels in *in vitro* studies and *in vivo* mice model studies (Table 1).[13-16,22] A separate category was made for NSAIDs with an unknown effect on Aβ42-levels; these drugs were infrequently prescribed (1%) and not further investigated.

Table 1. Classification of the nonsteroidal anti-inflammatory drugs (NSAIDs) according to Aβ42-lowering properties, prescription frequencies and cumulative duration of use during follow-up

Type of NSAID	Number of prescriptions (% of total NSAID prescriptions)	Cumulative duration of use in days * (% of total days of NSAID use)
Aβ42-lowering NSAIDs	33,171 (73.2%)	795,437 (71.3%)
Diclofenac	16,771 (37.0%)	347,722 (31.2%)
Ibuprofen	9,209 (20.4%)	219,559 (19.7%)
Diclofenac comb.	2,460 (5.4%)	60,029 (5.4%)
Piroxicam	2,437 (5.3%)	86,542 (7.7%)
Indometacin	1,771 (3.9%)	62,840 (5.6%)
Sulindac	403 (0.9%)	12,518 (1.1%)
Flurbiprofen	120 (0.3%)	6,227 (0.6%)
Non-Aβ42-lowering NSAIDs	11,638 (25.8%)	304,888 (27.4%)
Naproxen	7,765 (17.1%)	177,317 (15.9%)
Rofecoxib	1,446 (3.2%)	41,457 (3.7%)
Nabumeton	874 (1.9%)	30,914 (2.7%)
Ketoprofen	863 (1.9%)	32,805 (2.8%)
Meloxicam	574 (1.2%)	18,901 (1.7%)
Celecoxib	88 (0.2%)	2,872 (0.3%)
Phenylbutazone	13 (<0.1%)	337 (<0.1%)
Etoricoxib	12 (<0.1%)	234 (<0.1%)
Valdecoxib	3 (<0.1%)	52 (<0.1%)
Unknown effect on Aβ42†	441 (1.0%)	14,245 (1.3%)

* Since in the analysis exposure is time-dependent, the prescription overlap which is taken into account in the analysis, cannot be depicted in these descriptives

† No further specification because of small prescription frequency (includes azapropazon, tiaprofeic acid, aceclofenac, tolfenamic acid, tenoxicam, tolmetin, benzydamine).

Salicylates (i.e. acetylsalicylic acid and carbasalate calcium) are pharmacologically related to NSAIDs. However, they are typically prescribed as platelet inhibitors in low dosages in which they have a negligible anti-inflammatory effect. Moreover, these drugs do not have Aβ42-lowering properties.[13,14] Therefore, we did not categorize salicylates with NSAIDs but studied them separately in relation to the risk of AD.

Diagnosis of dementia

The diagnosis of dementia was made following a three-step protocol.[20] Screening was done with the Mini Mental State Examination (MMSE) and Geriatric Mental State schedule (GMS) organic level for all persons.[23,24] Screen-positives (MMSE score <26 or GMS organic level >0) underwent the Cambridge examination for mental disorders of the elderly.[25] Persons who were suspected of having dementia underwent more extensive neuropsychological testing. When available, imaging data were used. In addition, the total cohort was continuously monitored for incident dementia through computerized linkage between the study database and digitalized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. The diagnosis of dementia and subtype of dementia was made in accordance with internationally accepted criteria for dementia (DSM-III-R), AD (NINCDS-ADRDA), and vascular dementia (NINDS-AIREN) by a panel of a neurologist, neuropsychologist and research physician, blinded for drug exposure of the study population.[26-28]

Other covariates

Baseline covariates included age, sex, and level of education, diabetes mellitus and cardiovascular disease. Incident covariates included cumulative use of antihypertensives, cumulative use of salicylates, newly diagnosed diabetes mellitus and cardiovascular disease. Education was assessed at the baseline interview and dichotomized into low education (including primary education only or low vocational training) and high education (including intermediate-level vocational training, secondary education or university). Diabetes mellitus was defined as antidiabetic medication use or a non-fasting or 2-hour post load glucose level of ≥200 mg/dL or fasting glucose of ≥126 mg/dL. Cardiovascular disease included myocardial infarction, coronary artery bypass graft, percutaneous transluminal coronary angioplasty, TIA, atrial fibrillation and heart failure. Data on antihypertensive and salicylate use was obtained from pharmacy records. Apolipoprotein E (APOE) genotyping was performed on coded DNA samples, and participants were classified according to presence or absence of at least one APOE-ε4 allele.[29]

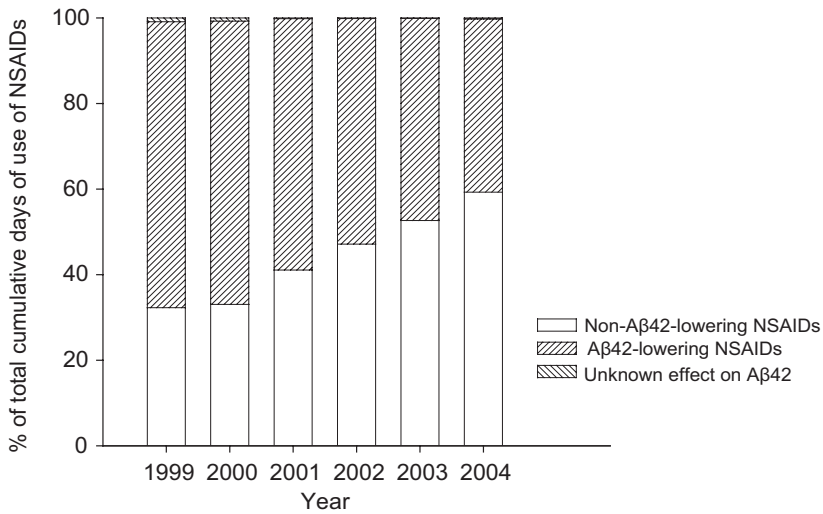
Statistical analyses

At each date of AD diagnosis, cumulative duration of use was calculated for both the person who was diagnosed with AD and the remainder of the cohort. Conform our earlier study duration we subsequently categorized duration of use as: short- (≤1 month), intermediate- (>1 and <24 months) and long-term use (≥24 months). This

was done for both Aβ42-lowering NSAIDs and non-Aβ42-lowering NSAID subtypes separately, and -to allow for comparison with earlier studies- also for use of any NSAID. A cohort member could contribute person-time to more than one category of duration of use with increasing NSAID use. Never use of any NSAID was the reference category for all analyses. For all subjects, we determined the duration of follow-up between start of the study and death, diagnosis of dementia, or end of the study period, whichever came first. We calculated the hazard ratio (HR) of AD (and 95% confidence intervals (CI)) with a Cox- proportional-hazards model (SPSS 11.01 software; SPSS Inc, Chicago, Illinois) in which calendar-time was used as the time-axis.[30] All analyses were adjusted for age, sex and, in the analyses of Aβ42-lowering NSAIDs, for cumulative use of non-Aβ42-lowering NSAIDs and vice versa (Model I). To adjust for potential confounders we additionally included level of education, cumulative use of antihypertensives, cumulative use of salicylates, diabetes mellitus and cardiovascular disease (Model II).

We performed several additional analyses. First, patterns of NSAID use have changed over the years in that the use of non-Aβ42-lowering NSAIDs has increased relative to that of Aβ42-lowering NSAIDS after the year 2000 (Figure 1). We considered that if Aβ42-lowering NSAIDs decrease AD risk, but not non-Aβ42-lowering NSAIDs, the effect estimates for the risk of AD associated with the use of Aβ42-lowering and non-Aβ42-lowering NSAIDs would remain stable over time, but the effect on AD risk associated with the use of any NSAIDs would decrease with increasing length of follow-up. We investigated this by calculation of the HR for any NSAID, and Aβ42-lowering and non-Aβ42-lowering NSAIDs separately for each year after 1999. Second, we considered that persons in the prodromal phase of AD might change their drug use. To evaluate whether this biased our results we performed an additional analysis where we excluded the last two years before diagnosis.[8] Third, we investigated the association between the use of salicylates and AD using the same methods, except that we here adjusted for cumulative use of NSAIDs. Finally, we investigated whether the association with AD was different for carriers and non-carriers of an APOE-ε4 allele by stratified analyses.

Figure 1. Proportions of cumulative use of NSAID subtypes calculated as the percentage of the total cumulative days of use of any NSAID per year from 1999 to 2004



RESULTS

During 62,883 person years of follow-up (mean 9.0 years), 739 persons developed dementia, of whom 582 were diagnosed with AD, 81 with vascular dementia and 76 with other types of dementia. The total number of NSAID prescriptions during the complete follow-up was 45,250 representing a total of 1,114,569 cumulative days of use. Prescription frequencies and total cumulative duration of NSAID use are given in Table 1. Baseline characteristics of the study population are shown in Table 2. At baseline, the mean age of the participants 69.4 years and the majority was female (60%).

Compared to never use, long-term use of Aβ42-lowering NSAIDs was associated with a decreased risk of AD (HR adjusted for age, sex and use of other NSAIDs: 0.41 95%CI 0.21-0.82). There was no difference in risk of AD with long-term use of non-Aβ42-lowering NSAIDs compared to never use (HR adjusted for age, sex and use of other NSAIDs 1.19: 95%CI 0.48-2.90). Additional adjustment for other potential confounders did not change any of the estimates (Table 3).

Table 2. Baseline characteristics of the study population (n=6,992)

Characteristic	
Age in years (mean, ± SD)	69.4 ± 9.1
Female (%)	4,195 (60.0%)
Primary education, low vocational training or less (%)	2,697 (38.6%)
Cardiovascular disease (%)	1,469 (21.0%)
Diabetes mellitus (%)	722 (10.3%)
APOE-ε4 allele present (%)	1,793 (25.6%)
Use of any NSAID at baseline (%)*	441 (6.3%)

Abbreviations: NSAIDs - nonsteroidal anti-inflammatory drugs
* Defined as any prescription filled within 90 days before or after start of study period

Table 3. Hazard ratios of Alzheimer disease associated with the use of subtypes of nonsteroidal anti-inflammatory drugs (NSAIDs) according to Aβ42-lowering properties

	Risk of Alzheimer disease		
NSAID use	**Number of cases**	**Hazard ratio (95% CI), Model I ***	**Hazard ratio (95% CI), Model II †**
No use	218	1.00 (ref.)	1.00 (ref.)
Aβ42-lowering NSAID use			
≤1 month	141	0.92 (0.74-1.15)	0.92 (0.74-1.15)
>1 and <24 months	180	1.00 (0.81-1.23)	1.01 (0.82-1.24)
≥24 months	9	0.41 (0.21- 0.82)	0.42 (0.21-0.83)
Non-Aβ42-lowering NSAID use			
≤1 month	84	0.89 (0.69-1.16)	0.89 (0.68-1.15)
>1 and <24 months	77	1.15 (0.87-1.52)	1.16 (0.88-1.53)
≥24 months	5	1.19 (0.48-2.90)	1.15 (0.47-2.82)

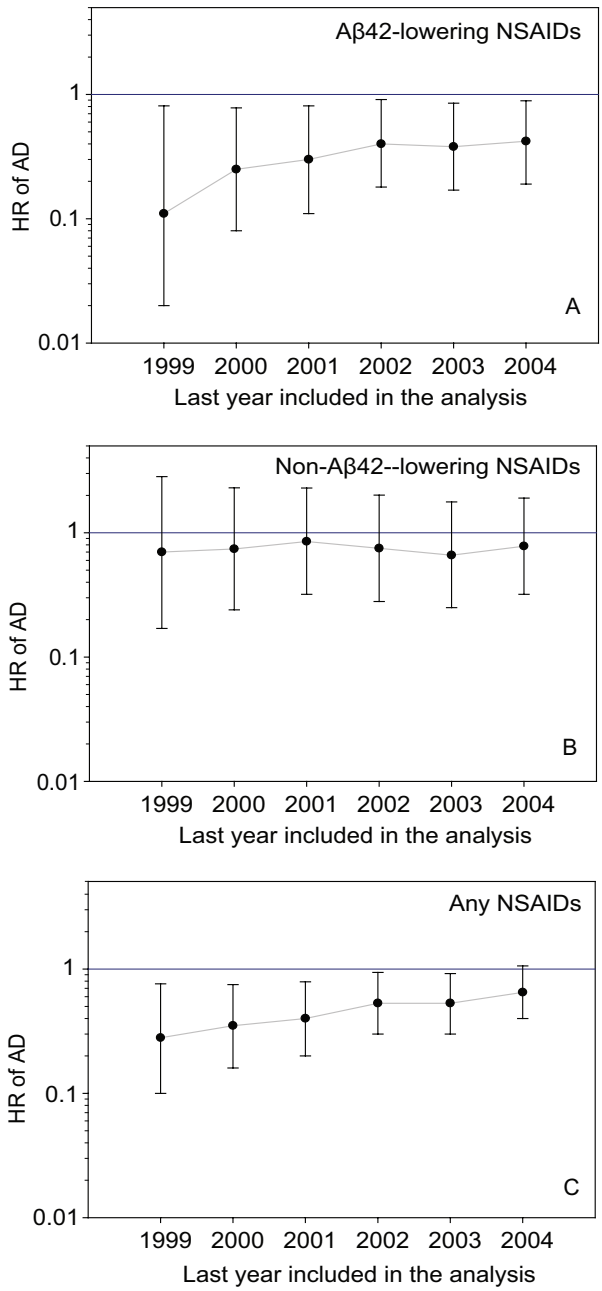
* Model I: adjusted for age, sex and cumulative use of other NSAIDs
† Model II: as model I and additionally adjusted for level of education, cumulative use of antihypertensives and salicylates, diabetes mellitus, cardiovascular disease

The utilization of NSAIDs changed during follow-up, in that there was a gradual shift towards more use of non-Aβ42-lowering NSAIDs relative to Aβ42-lowering NSAIDs after the year 2000 (Figure 1). The HR for long-term use of Aβ42-lowering NSAIDs and non-Aβ42-lowering NSAIDs remained stable over the years (Figure 2). However, concurrent with the increasing proportion of use of non-Aβ42-lowering NSAIDs, the HR of AD for

long-term use of any NSAID gradually attenuated with increasing follow-up (Figure 2), becoming non-significant at maximum follow-up (adjusted HR of AD for long-term use of any NSAID 0.64 (95%CI 0.39-1.05), Figure 2).

The exclusion of 2 years before diagnosis did not alter our findings. The HR for long term-use of Aβ42-lowering NSAIDs was 0.52 (95%CI 0.25-1.06) and for non-Aβ42-lowering NSAIDs 1.41 (95%CI 0.52-3.84). In the categories of long-term use for Aβ42-lowering as well as for non-Aβ42-lowering NSAIDs, only one case with an APOE-ε4 allele was available, precluding additional analyses. Use of salicylates was not associated with risk of AD, regardless of duration of use (HR for short-term use 0.97: 95%CI 0.61-1.54, intermediate-term use 1.10: 95%CI 0.87-1.39, and long-term use 0.81: 95%CI 0.51-1.29).

Figure 2. Hazard ratios of Alzheimer Disease (AD) from 1999 to 2004 for long-term use of Aβ42-lowering NSAIDs (A), non-Aβ42-lowering NSAIDs (B) and any NSAIDs (C), before 1999 no cases were available in Aβ42-lowering NSAID exposure categories



DISCUSSION

In this large prospective population-based study, we found that long-term use of Aβ42-lowering NSAIDs was associated with a significantly decreased risk of AD. Use of non-Aβ42-lowering NSAIDs was not associated with the risk of AD.

Strengths of our study include its prospective design, large number of participants, long follow-up period and population-based setting. The high response rate and virtually complete follow-up for dementia make selection bias very unlikely. A particular asset of our study is that we had complete pharmacy records of all filled prescriptions, providing us with very specific and detailed information on drug use. However, we cannot exclude some misclassification of drug exposure. First, we may have misclassified some NSAIDs with regard to their Aβ42-lowering properties. In determining the Aβ42-level modulating properties of NSAIDs much depends on the assay, cell culture and mice model being used. However, we based our classification mainly on a study by Eriksen *et al* who determined the Aβ42-modulating properties of almost all NSAIDs in a single assay.[14] Where necessary, we added data from additional correlating assays.[13,15,16] If misclassification has occurred, this would have lead to a dilution of the observed protective effect. Second, we had no information on over-the-counter (OTC) drug use. During the 1990's few preparations of low dosages of ibuprofen and naproxen became available OTC. However, in the Netherlands until 1999 these drugs were fully reimbursed, and after 1999 chronic use (>3 months) of these drugs remained reimbursed, if given on prescription. It was not until 2004 when reimbursement of these drugs was restricted to higher dosage forms. Therefore, it is unlikely that during the follow-up period of our cohort regular users of NSAIDs obtained their drugs OTC. Some misclassification may have occurred because of occasional use of OTC NSAIDs, but this was most likely non-differential as both Aβ42-lowering NSAIDs and non-Aβ42-lowering NSAIDs were available OTC. To the extent that this has biased our results, it will have led us to underestimate an effect. Finally, prodromal AD might lead to changes in drug use. To examine whether the inclusion of persons with prodromal AD had biased our results, we performed an analysis where we excluded the last two years before diagnosis. Since this did not alter our results we do not think that this has played a major role, if any. Long-term use of Aβ42-lowering NSAIDs was associated with a decreased risk of AD, whereas no effect was observed for non-Aβ42-lowering NSAIDs. In an earlier analysis of our cohort after shorter follow-up we found that long-term use of any NSAIDs was associated with a reduced risk of AD.[10] By analyzing

our data with different lengths of follow-up we were now able to demonstrate that that could be attributed to the effect of Aβ42-lowering NSAIDs, which were the more frequently prescribed type of NSAIDs in the earlier years of follow-up of our cohort. The observation that the HRs for both Aβ42-lowering NSAIDs and non-Aβ42-lowering NSAIDs were stable over follow-up provides strong support for the protective effect that we observed for Aβ42-lowering NSAIDs being real.

Numerous observational studies investigated the association between NSAIDs and risk of AD with conflicting results.[6-12,31] An explanation for the inconsistencies between these previous studies can be the variation in the types of NSAIDs that were used, but this was not investigated. Whilst two more recent observational studies and a pooled cohort study again confirmed the protective effect of NSAIDs in AD, they did not provide support for a different effect of Aβ42-lowering and non-Aβ42-lowering NSAIDs in AD.[18-20] The classification of NSAIDs was the same across studies and identical to our classification. However, there were considerable differences in study design, particularly in terms of source of information on drug exposure and exposure definition, which could explain the discrepancies. For example, the study by Szekely *et al.* relied on annual assessment of drug exposure which makes misclassification of drug exposure likely. Also, no consideration was given to the duration of use for the subtypes of NSAIDs.[11] Recent large randomized controlled trials did not provide evidence for a protective effect of long-term therapy of NSAIDs.[17,18] Of note, the treatment regimens in neither of these trials included Aβ42-lowering NSAIDs.

Our study is the first observational report to suggest a differential effect of subtypes of NSAIDs on AD risk based on their ability to modulate Aβ42-levels. Although we are unable to test whether these Aβ42-lowering NSAIDs actually lowered brain Aβ42-levels, our results are in accordance with *in vitro* and *in vivo* mice model studies that consistently show an Aβ42-lowering effect for this specific subgroup of NSAIDs.[13-16] To our best knowledge the effect of the currently available NSAIDs on Aβ42-load of the brain has not yet been studied in humans under experimental conditions.

By which mechanism NSAIDs can affect amyloid-processing and ultimately modulate Aβ42-levels in the brain remains to be elucidated. NSAIDs are pleiotropic drugs and numerous mechanisms have been suggested, such as those involving nuclear factor κB[32], inhibition of Rho activity[33], repression of β-secretase possibly via activation of proliferator-activated receptor-γ[34], or modification of the activity or specificity of

γ-secretase.[35,36] NSAIDs may also act on the level of Aβ42-aggregation in plaques.[37] Importantly, this Aβ42-lowering effect appears independent of inhibition of cyclooxygenase, the key target for the anti-inflammatory activity of NSAIDs.[13]

In conclusion, long-term use of NSAIDs which lower Aβ42-levels *in vitro* and *in vivo* in mice models is associated with a decreased risk of AD. The protective effect was observed solely for this type of NSAID. Our findings suggest that these NSAIDs do not influence risk of AD through their anti-inflammatory effect, but rather through an amyloid-processing related mechanism.

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Chapter 2

Cardiovascular drugs

Chapter 2.1

Statins and risk of Alzheimer disease

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ABSTRACT

Background: Cross-sectional reports suggest that statin-users are less likely to have Alzheimer disease (AD). Prospective studies have provided inconsistent evidence. Moreover, it is unclear whether the association differs for lipohilic statins, those that could more easily pass the blood-brain-barrier, and hydrophilic statins.
Objectives: To prospectively evaluate whether use of statins is associated with the risk of AD, and to determine whether associations differ for lipophilic and hydrophilic statins.
Method: We followed 6,992 participants of the prospective, population-based Rotterdam Study, from baseline (1990-1993) until January 2005 for incident AD. Data on all filled prescriptions came from pharmacy records. At each date an event occurred, cholesterol-lowering drug use for the person who experienced the event and all remaining persons in the cohort was categorized as 'any' or 'never' use. We distinguished between statin, lipophilic and hydrophilic statins, and non-statin cholesterol-lowering drugs. Data were analyzed with Cox-regression analysis, adjusting for sex, age and potential confounders.
Results: During follow-up (mean 9 years), 582 persons developed AD. Compared to never use of cholesterol-lowering drugs, statin use was associated with a decreased risk of AD (HR 0.57; 95%CI 0.37-0.90), but non-statin cholesterol-lowering drug use was not (HR 1.05; 95%CI 0.45-2.44). HRs were equal for lipophilic (HR 0.54; 95%CI 0.32-0.89) and hydrophilic statins (HR 0.54; 95%CI 0.26-1.11).
Conclusion: In the general population, use of statins, but not of non-statin cholesterol-lowering drugs, was associated with a lower risk of AD compared to never use of cholesterol-lowering drugs. The protective effect of statins was independent of the lipophilicity of statins.

INTRODUCTION

Cross sectional observational studies have suggested an association between the use of cholesterol lowering drugs, particularly 3-hydroxy-3-methylglutaryl-coenzyme-A-reductase inhibitors (statins), and the risk of Alzheimer disease.[1-6] Thus far, the evidence from prospective studies for a protective effect of statins on the risk of Alzheimer disease is less clear and inconsistent.[3,7-13] Important limitations of these studies, however, included use of interview data on drug exposure or cross-sectional exposure assessment, and short duration of follow-up. No clinical trial has yet been reported that investigated the efficacy of statin therapy in preventing Alzheimer disease in persons without cognitive impairment.[14-16]

The mechanism by which cholesterol-lowering drugs may affect Alzheimer pathogenesis is unclear. Since cholesterol is essential for normal function of the brain, the effect of these drugs on serum cholesterol was considered a potential underlying mechanism of action. However, brain cholesterol is synthesized *in situ* and exchange with the periphery is prevented by the blood-brain-barrier.[17] Hence, it was debated whether lowering of serum cholesterol levels by these drugs could actually affect brain cholesterol homeostasis.[17] This led to the hypothesis that only the lipophilic statins, those that could cross the blood-brain-barrier more easily, would affect brain cholesterol metabolism.

The Rotterdam Study is a large prospective population-based cohort study with detailed information on drug exposure from pharmacy dispensing records, data on potential confounders and systematic assessment of dementia outcomes.[18] We investigated whether the use of statins or other, non-statin, cholesterol-lowering agents, was associated with the risk of Alzheimer disease. In addition, we determined whether any observed associations differed between lipophilic and hydrophilic statins.

METHODS

Study population

The Rotterdam Study is a prospective, population-based cohort study of age related disorders.[18] The medical ethics committee of the Erasmus Medical Center, Rotterdam, the Netherlands, approved the study. Between 1990 and 1993 all persons aged 55

years or older living in Ommoord, a district of Rotterdam, were invited to participate. Of the 10,275 eligible persons, 7,983 (78%) signed informed consent. Of these, 7,528 (94%) were screened for dementia and 7046 were found to be free of dementia at baseline.[19] Follow-up examinations, including screening and clinical workup for dementia, were conducted in 1993 to 1994, 1997 to 1999 and 2000 to 2004. In addition, the cohort was continuously monitored for major disease outcomes and death through linkage with records of general practitioners, the Regional Institute for Outpatient Mental Health Care and bimonthly updates from the municipality records. This resulted in a virtually complete follow-up for dementia until January 1st, 2005. Nearly all persons (99.7%) were registered at one or more of seven automated pharmacies serving the Ommoord area. Of these pharmacies, records of all filled prescriptions were available as of January 1st, 1991. To ensure at least six months medication history, we excluded persons for whom follow-up ended before July 1st, 1991. Consequently, the study population consisted of 6,992 persons.

Drug exposure

Complete information on all filled prescriptions for all persons was obtained in automated format from the pharmacies. This included the product name; international non-proprietary name; Anatomical Therapeutic Chemical (ATC) code; total number of delivered units (e.g. tablets/capsules); prescribed daily number of units; date of delivery and drug dosage. The duration of a prescription is calculated as the total number of delivered units divided by the prescribed daily number of units.

Cholesterol-lowering drugs were classified as statins (simvastatin, pravastatin, fluvastatin, atorvastatin, cerivastatin, rosuvastatin) or non-statin cholesterol lowering drugs (fibrates, bile acid binding resins or nicotinic acid and derivatives). Statins were further subdivided into lipophilic (simvastatin, atorvastatin, cerivastatin) and hydrophilic statins (pravastatin, fluvastatin, rosuvastatin) based on their relative lipid-solubility.[20-23] Lipophilic statins are thought to pass the blood-brain barrier more efficiently than hydrophilic statins, which could be relevant to their effect on Alzheimer pathology.[24-26] All drugs under study are available in the Netherlands only on prescription. In the 1998 Dutch guidelines on the prevention of cardiovascular disease, simvastatin is the statin of first choice for treatment of hypercholesterolemia, followed by pravastatin.[27]

Diagnosis of Alzheimer disease

The diagnosis of dementia was made following a three-step protocol. Screening was done with the Mini-Mental State Examination (MMSE) and Geriatric Mental State schedule (GMS) organic level for all persons. Screen-positives (MMSE score <26 or GMS organic level >0) underwent the Cambridge examination for mental disorders of the elderly.[28,29] Persons who were suspected of having dementia underwent more extensive neuropsychological testing. When available, imaging data were used. In addition, the total cohort was continuously monitored for incident dementia through computerized linkage between the study database and digitalized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. The diagnosis of dementia and subtype of dementia was made in accordance with internationally accepted criteria for dementia (DSM-III-R), AD (NINCDS-ADRDA), and vascular dementia (NINDS-AIREN) by a panel of a neurologist, neurophysiologist and research physician, blinded to drug exposure of the study population.[30-32]

Other covariates

Covariates included age, sex, education level, smoking, total serum cholesterol, body mass index, systolic blood pressure, diabetes mellitus and cardiovascular and cerebrovascular disease. Education was assessed at the baseline interview and dichotomized into low education (including primary education only or low vocational training) and high education (including intermediate-level vocational training, secondary education or university). Smoking status was also self-reported and categorized as ever or never smoker. Total serum cholesterol was measured in non-fasting blood drawn at baseline. Sitting blood pressure was measured on the right upper arm using a random-zero sphygmomanometer. Diabetes mellitus was defined as a non-fasting or 2-hour post load glucose level of ≥11.1 mmol/l or antidiabetic medication use at baseline. Cardiovascular disease included myocardial infarction, coronary artery bypass graft, percutaneous transluminal coronary angioplasty, atrial fibrillation and heart failure. Cerebrovascular disease included transient ischemic attacks and stroke. APOE genotyping was performed on coded DNA samples, and participants were classified according to presence or absence of at least one APOE-ε4 allele.

Statistical analyses

For all subjects, we calculated the duration of follow-up between start of the study and death, diagnosis of dementia, or end of the study period, whichever came first. Prescription drug use was available on a day-to-day basis from the pharmacy prescription database. At each event-date, we were therefore able to determined cholesterol-lowering drug use for the person who suffered the event and for all persons in the remainder of the cohort. Subsequently, cholesterol-lowering drug use was categorized as either 'any' or 'never' use, depending on whether a person had used cholesterol-lowering drugs prior to or on that event-date, or not.[33] Hence, if for a person cholesterol-lowering drug therapy was initiated at some point during follow-up, that person would then switch from the exposure category of 'never' use to 'any' use. In case two prescriptions overlapped, the use of the first drug was assumed to have been discontinued and treatment to have proceeded with the second drug. A cohort member could contribute person-time to more than one category of cholesterol-lowering drug use if a person had used more than one cholesterol-lowering drug during follow-up. Accordingly, the numbers of cases in the analyses of the statin subtypes do not add up to the numbers of cases in the analyses of statins as a group. 'Never' use of any cholesterol-lowering drug was the reference for all analyses. We distinguished between statin and non-statin cholesterol lowering drugs and between lipophilic and hydrophilic statins. In addition, we separately investigated the use of simvastatin, the most lipophilic statin prescribed and pravastatin, the most hydrophilic statin prescribed. We calculated the hazard ratio (HR and 95% confidence intervals (CI)) of the risk of Alzheimer disease associated with cholesterol-lowering drug use using a Cox-proportional-hazards model with time-dependent covariates (SPSS 11.01 software). Calendar-time was used as the time-axis in the model to account for changes in prescription guidelines and availability of cholesterol-lowering drug use over time. All analyses were adjusted for age and sex, and, if applicable, use of other cholesterol lipid-lowering drugs (model I). To adjust for potential confounders we additionally included baseline education, smoking, total serum cholesterol, body mass index, systolic blood pressure and diabetes mellitus and cardiovascular disease. In addition, we included cardiovascular disease as time-dependent covariate in the model to adjust for cardiovascular disease diagnosed during follow-up.

For statins, dose- and duration relationships were studied by dichotomizing statin use around the median of the cumulative duration of use (≤ and >2.9 years) and around the median of the defined daily dose (DDD), averaged over the total period of use used during follow-up (≤ and >0.89 DDD).

We performed several additional subanalyses. First, we acknowledge that, even though cholesterol-lowering drug therapy is generally used chronically, persons could have stopped therapy, for example as a result of adverse events, non-compliance or a change in diet or life-style which rendered cholesterol-lowering therapy no longer indicated. To take this into account we performed an analysis where we restricted the exposure category of 'any' use to at least one prescription of cholesterol-lowering drugs filled in the year prior to the date of an event. Second, most of the preceding cohort studies enrolled persons of 65 years and older in the early 1990's. Because statins were not widely used before the mid-1990s, it is likely that these persons did not use statins before the age of 65 contrary to our own study population. Therefore, we repeated the analysis for persons ≥65 years of age at baseline. Third, we considered that physicians might be less motivated to prescribe chronic preventive therapies to persons with mild cognitive impairment. To evaluate whether this biased our results we performed an analysis where we excluded the last two years before diagnosis. Fourth, in the main analyses we used calendar-time as time-axis in the COX-model. In an additional model we used age as time-axis, as was done in an earlier study on cholesterol-lowering drug use and Alzheimer disease.[8] By using age as time-axis, no assumption is made on the functional form for the age-specific incidence rates. Finally, apolipoprotein E (APOE) is responsible for lipid metabolism and is the primary transport protein of cholesterol in the brain.[34,35] Furthermore, it has been suggested that the response to statins is dependent on APOE-genotype.[9,36] Therefore, for statins we determined whether any effect modification was observed due to the presence of an APOE-ε4 allele.

RESULTS

In total 6,992 persons, free of dementia at baseline and with at least 6 months medication history, were included in the analysis. In Table 1 baseline characteristics of the study population are given.

Table 1. Baseline characteristics of the study population (n=6,992)

Characteristic	
Age (± SD) in years	69.4 ± 9.1
Sex (% female)	4,195 (60.0%)
Smoking, ever (%)	4,386 (62.7%)
Diabetes mellitus (%)	722 (10.3%)
Systolic blood pressure (mmHg)	139.3 ± 22.3
Cardiovascular disease (%)*	1,877 (26.8%)
Cerebrovascular disease†	262 (3.7%)
Total cholesterol (mmol/L)	6.6 ± 1.2
Body-mass index, mean ±SD (kg/m^2)	26.3 ± 3.7
Primary education, low vocational training or less (%)	2,697 (38.6%)
APOE-ε4 allele present (%)	1,793 (25.6%)

* Myocardial infarction, coronary artery bypass graft, percutaneous transluminal coronary angioplasty, atrial fibrillation and heart failure
† Stroke and transient ischemic attack

Persons were followed up to 15.3 years (average 9.2 years), with a total of 62,883 person years of follow up. During follow-up 739 persons developed dementia, of whom 582 were diagnosed with Alzheimer disease, 81 with vascular dementia and 76 with other types of dementia. The total number of filled prescriptions during follow-up was 30,241. Prescription frequencies of statins and non-statin lipid lowering drugs and for individual statins are shown in Table 2. Simvastatin was the most frequently used statin, followed by pravastatin.

Table 2. Prescription frequencies of cholesterol-lowering drug during follow-up

Cholesterol-lowering drug	Total number of filled prescriptions (% of total number of filled prescriptions, n=30,241)
Statins	27,713 (91.6%)
Lipophilic statins	21,855 (72.3%)
Simvastatin	17,742 (58.7%)
Atorvastatin	3,949 (13.0%)
Cerivastatin	164 (0.5%)
Hydrophilic statins	5,858 (19.4%)
Pravastatin	4,079 (13.5%)
Fluvastatin	1,735 (5.7%)
Rosuvastatin	44 (0.2%)
Non-statin-cholesterol-lowering drugs*	2,528 (8.4%)

* Fibrates, bile-acid binding resins or nicotinic acid and derivatives

We observed that use of statins was associated with a lower risk of Alzheimer disease than never use of cholesterol-lowering drugs. No protective effect was observed with use of non-statin cholesterol lowering drugs. Additional adjustment for other potential confounders did not change the estimates (Table 3).

The effect-sizes were equivalent for use of lipophilic statins and hydrophilic statins versus never-use (Table 3). Compared to non-use, we did not find evidence for a stronger protective effect for the lipophilic simvastatin than with the hydrophilic pravastatin (adjusted ORs: 0.61; 95% CI 0.36-1.03 and 0.38; 95% 0.15-0.99, respectively).

Table 3. Hazard ratios (HR) of Alzheimer disease with use of statins, lipophilic and hydrophilic statins separately, and non-statin cholesterol-lowering drugs

	Alzheimer disease			
	Cases*	HR (95% CI)	Cases	HR (95% CI)
Cholesterol lowering drug use		**Model I†**		**Model II‡**
Never use	546	1.00 (ref.)	438	1.00 (ref.)
Statins	30	0.58 (0.38-0.88)	28	0.57 (0.37-0.90)
Lipophilic statins	25	0.55 (0.34-0.89)	23	0.54 (0.32-0.89)
Hydrophilic statins	9	0.52 (0.26-1.05)	9	0.54 (0.26-1.11)
Non-statin cholesterol lowering drugs§	12	1.04 (0.45-2.40)	12	1.05 (0.45-2.44)

* The number of exposed cases for any statin differs from the sum of exposed cases for statin subtypes. This is because persons can contribute exposed person-time to more than one type of statin.
† Model I: Age, sex adjusted and use of other lipid-lowering drugs, if applicable.
‡ Model II: as Model I additionally adjusted education, systolic blood pressure, smoking, total serum cholesterol, body mass index, diabetes mellitus and cardiovascular and cerebrovascular disease.
§ Fibrates, bile acid binding resins or nicotinic acid and derivatives.

No dose-response relationship for statin use was detected, as dosages below and above the median were both associated with a decreased risk of Alzheimer disease (adjusted HR 0.57; 95% CI 0.32-1.00 for ≤0.89 DDD and HR 0.58; 95% CI 0.32-1.04 for >0.89 DDD). Likewise, the protective effect was observed irrespective of duration of statin use (adjusted HR 0.44; 95% CI 0.25-0.80) for ≤2.9 years and adjusted HR 0.78; 95% CI 0.44-1.32 for >2.9 years).

Our findings were robust in all planned subanalyses. Restricting the exposure definition of 'any use' to at least one prescription in the year prior to the event date did not alter our findings: adjusted HR 0.51; 95% CI 0.31-0.83 for statin use and HR 1.55; 95% CI 0.54-4.44 for non-statin cholesterol lowering drug use. Of the 30 persons who had used statins at time of Alzheimer diagnosis, 22 persons had filled at least 1 statin prescription in the year prior to diagnosis. For non-statin cholesterol-lowering drugs this was 5 out of 12.

When we restricted our analyses to participants of 65 years and older at baseline, the HRs for Alzheimer disease remained similar (adjusted HR for statin use 0.61; 95% CI 0.38-0.98, adjusted HR for non-statin cholesterol lowering drugs 1.14; 95% CI 0.49-2.68). The exclusion of 2 years before diagnosis did not change the magnitude of the estimates either. The adjusted HRs were 0.60 (95% CI 0.39-0.92) for statin-use and 1.10 (95% CI 0.48-2.52) for non-statin cholesterol-lowering drug use versus never-use. Likewise, if we used age as time-axis rather than calendar-time, the associations remained unchanged (adjusted HR for statin use 0.55; 95% CI 0.36-0.86, adjusted HR for non-statin cholesterol lowering drugs 1.17; 95% CI 0.52-2.64). Finally, in the analyses where we stratified on the presence or absence of an APOE-ε4 allele, the protective effect of statin use was similar for persons with an APOE-ε4 allele (adjusted HR 0.50; 95% CI 0.26-0.94) and for persons without an APOE-ε4 allele (adjusted HR 0.61; 95% CI 0.32-1.18).

DISCUSSION

In the prospective population-based cohort of the Rotterdam Study, we found that use of statins, but not of non-statin cholesterol lowering drugs was associated with a lower risk of Alzheimer disease compared to never use of cholesterol-lowering drugs. No difference was observed between the effects of hydrophilic and lipophilic statins.

Strengths of our study include the active surveillance of incident dementia, including in person screening, together with the high response rate and virtually complete follow-up, which limited chances of selection or information bias. An important asset of our study is the availability of continuous pharmacy dispensing data on all members of the cohort. In terms of exposure misclassification this constitutes a major advantage compared to studies that have to rely on self-reported drug use and drug exposure obtained at baseline.[11,12] Nevertheless, contrary to a clinical trial setting, treatment was not randomly assigned in our study and confounding by indication should be considered. In the case of lipid-lowering drugs, physicians might less readily prescribe these agents to individuals with early signs of cognitive impairment because of concerns regarding adherence, treatment complications and priorities in health-care resource allocation. However, the results did not change when we included a 2-year lag-period before diagnosis of Alzheimer disease, which suggested that this confounding by indication did not play a major role, if any, in our study. Finally, the number of exposed cases available

in the analyses limited extensive analyses of duration of use and in various patient groups.

We found that statin use was associated with a reduced risk of Alzheimer disease. Our findings are in line with the cross-sectional reports, which showed a lower prevalence of dementia amongst statin users.[1-3] Most of the prospective studies did not find a protective effect of statins or only for persons younger than 80 years of age.[3,7-10,12,13] Important limitations of these studies included the limited durations of follow-up, as short as 3 years[12], and low number of incident Alzheimer cases.[11,12] Moreover, several studies relied, in part, on (self) reported drug use at baseline, with persons who started statin therapy during follow-up consequently being classified as non-users.[3,11,12] This may have led to misclassification of drug exposure and, hence, may have biased the risk estimates towards one. In the very large population database of United States Veterans Affairs (VA) medical system, a protective effect was specifically observed with the use of simvastatin, but not for other statins.[37] The latter study is different from previous observational studies in that persons taking cardiovascular medication, excluding statins, were used as the comparator group. In our analyses adjustment for cardiovascular disease did not change the observed associations. Some clinical studies have studied the effect of statins on cognitive decline, but thus far none have reported on the prevention of Alzheimer disease with statins or other lipid-lowering drugs.[14-16] We did not find a duration-response relationship. Only few other observational studies investigating the use of statins and incident Alzheimer disease report on the duration-of statin use. Zamrini *et al.*[7] reported a weakened protective effect among those with 12 months of more use, whilst a post-hoc analysis of Zandi *et al.*[12] observed a reduced risk for Alzheimer disease with more than 3 years of statin use. Further research would be required to investigate these aspects of statin use.

In our study both hydrophilic and lipophilic statins were associated with a reduced risk of Alzheimer disease. Two other prospective studies specifically investigated lipophilic and hydrophilic statins separately but found neither associated with a reduced risk of Alzheimer disease.[10,13] As mentioned above, results from the VA database showed a protective effect for the lipophilic simvastatin, but not for other statins.[37] Our observation of a protective effect regardless of the lipophilicity of statins challenges the hypothesis that only lipophilic statins would reduce the risk of Alzheimer disease. Various explanations could be given as to why no difference in effect for lipophilic and hydrophilic statins was observed. One, though many studies show that the ability of

statins to permeate the blood-brain barrier depends on their lipophilic or hydrophilic character [23,24,26], many other factors besides lipid solubility also determine a drug's distribution in different tissues. These include pharmacokinetic properties and the affinity to specialized transport mechanisms.[20,21,23,24] For example, though atorvastatin is lipophilic of nature, it is suggested that little of the drug is distributed beyond the liver, as had been expected.[22] Two, if changes in endogenous cholesterol synthesis cause alterations of brain cholesterol metabolism the ability of statins to cross the blood-brain-barrier might be irrelevant. This would also hold if statins act through a mechanism for which brain penetration might not be important at all. Besides inhibition of cholesterol synthesis, statins namely also affect physiological processes such as endothelial functioning, atherosclerosis and oxidative stress reactions.[38] Many of these processes have also been associated with Alzheimer disease. Other effects, more typical to Alzheimer pathology, could include inhibition of amyloid synthesis and reduction of neurofibrillary tangle burden.[13,39] At least for some of these mechanisms it is likely that brain penetration of statins is extraneous.

The effect of statins on Alzheimer risk was not modified by APOE genotype. APOE is essential in cholesterol metabolism and it is suggested that the response to statin treatment varies with APOE genotype, though agreement in the literature is not complete.[36] Our results comply with two other observational studies that did not find evidence for effect modification by APOE genotype.[6,12] A recent report by Li *et al* found a lower risk of Alzheimer disease with statin use for persons with at least one APOE-ε4 allele.[9] Further investigations will be needed to provide conclusive results regarding a possible effect modification by APOE genotype.

In conclusion, use of statins is associated with a decreased risk of Alzheimer disease independent of their relative lipophilicity.

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Chapter 2.2

Antihypertensive drugs and risk of Dementia

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ABSTRACT

Background: The evidence from prospective observational research for a protective effect of antihypertensive drug use on the risk of dementia is far from uniform. Duration of follow-up was limited and relied mainly on baseline drug exposure data without information on duration of use. We investigated the association between the duration of antihypertensive use and risk of dementia.

Methods: We followed 6,249 participants (mean 68.4 years, 60% women) of a prospective, population-based cohort from baseline (1990-1993) until 2005 for incident dementia. Continuous data on filled prescriptions came from pharmacy records. Total cumulative duration of antihypertensive use was expressed in years. We subtracted a latent 4-year period before the date of dementia diagnosis in the quantification of exposure duration to avoid potential bias in antihypertensive prescription due to prodromal changes in blood pressure or cognition. With Cox regression models, we calculated crude and adjusted hazard ratios (HRs) of all dementia and AD with antihypertensive use versus never use.

Results: Compared to never-use, antihypertensive use was associated with a reduced risk of all dementia (adjusted HR per year of use 0.95; 95% confidence interval (CI); 0.91-0.99). We observed an 8% (95%CI -15% to -1%) risk reduction per year of use for persons ≤75 years, whereas for persons >75 years this was 4% (95% CI -11% to 4%). Equivalent estimates were observed for AD. No apparent differences were observed among different types of antihypertensives.

Conclusions: Antihypertensive drug use was associated with 8% risk reduction of dementia per year of use for persons ≤75 years.

INTRODUCTION

Hypertension is a risk factor for cerebrovascular disease and consequently for vascular dementia. The association between hypertension and Alzheimer disease (AD) is less clear.[1] Follow-up studies generally showed that high blood pressure in midlife is associated with an increased risk of AD.[2] In contrast, high blood pressure later in life appears to lower the risk of AD.[3] It was also suggested that in the preclinical stage of AD blood pressure declines, possibly due to imminent disease.[4]

Evidence for a protective effect of antihypertensives on the risk of dementia has been far from uniform (Table 1 and 2). With the exception of two studies,[5,6] a major shortcoming of previous observational studies was the availability of merely baseline data on antihypertensive treatment. This considerably increases the chance of exposure misclassification during follow-up and, moreover, prohibits investigation of treatment duration. The intricate relation between blood pressure, age and dementia risk can also be responsible for the variance in the observed relationships. Furthermore, prodromal changes in blood pressure and cognitive decline could lead to changes in antihypertensive prescription or drug intake, both leading to bias.[4,7] Hence, the years immediately preceding clinical onset of dementia may not provide the relevant risk period to investigate the effect of antihypertensives on dementia risk.

We investigated the association between duration of antihypertensive drug use and the risk of all dementia and AD in a large prospective population-based cohort study, taking into account a latent period in the quantification of treatment duration.[8]

Table 1. Prospective observational studies on the association between antihypertensive drug use and risk of dementia

Ref.	Description	Design	Age (mean)	N	Follow-up	Outcome	Exposure assessment	Covariates	Main results
36, 37	Kungsholmen Project; 1st and 2nd phase	Cohort	≥ 75 yrs (85.3 yrs)	1301	3.0 years	Dementia (n=199)	Baseline interview.	Age, sex, MMSE, education, SBP or DBP, heart disease, stroke.	1st phase: RR all dementia with any antihypertensive use RR 0.7 (95% CI, 0.6-1.0) vs. no use 2nd phase: RR dementia 0.8 (95% CI, 0.6-1.0) and AD 0.7 (95% CI, 0.5-0.9) vs no use
38	EPESE	Cohort	≥65 yrs (~72 yrs)	634	4 years	AD (NINCDS-ADRDA) (n=90)	Baseline interview.	Age, sex, education.	4-year risk of AD vs no use with: any antihypertensive use OR 0.66 (95%CI 0.68-2.61); thiazides OR 1.33 (95%CI 0.68-2.61); K^+ sparing diuretics OR 0.63 (95%CI 0.72-0.96); loop diuretics OR 1.06 (95%CI 0.37-3.06); β-blockers OR 0.91(95%CI 0.26-3.17).
39	Rotterdam Study.	Cohort	≥55 yrs (68.1 yrs)	6416	2.2 years	Dementia (DSMIII-R, n=118) AD(NINCDS-ADRDA, n=82)	Baseline interview.	Age, sex, BMI, stroke, smoking, DM, MMSE, BP, living situation, peripheral arterial disease.	Risk of dementia vs no use with: any antihypertensive use RR 0.67 (95% CI 0.45-1.00); CCB RR 0.70 (95% CI 0.32-1.52); diuretics RR 0.83 (95% CI 0.33-1.30). Risk of AD vs no use with any antihypertensive use RR 0.77 (95% CI 0.49-1.24).
40	CSHA	Case-control	≥ 65 yrs (72 yrs)	4088	5 years	AD (DSMIV, n=194)	Baseline survey.	Age, sex, education	Risk of AD with any antihypertensive use vs no use OR 0.91 (95% CI 0.64 –1.30).
18	Kungsholmen Project	Cohort	≥ 75 yrs	966	5.7 years	AD (DSMIII-R, n=204)	Baseline interview.	Age, sex, MMSE, VD, education	Risk of AD for any antihypertensive drug use vs no use RR 0.6 (95% CI 0.5-0.9).
5	Baltimore Longitudinal Study of Aging	Cohort	≥ 60 yrs (67.1 yrs)	1092	11 years	AD (NINCDS-ADRDA, n=115)	Interview every 2 years.	Age, sex, SBP, DBP, education, smoking, heart problems.	Risk of AD vs no use of CCB's for: CCB RR 0.63 (95% CI 0.31-1.28); DHP-CCB RR 0.30 (95% CI 0.07-1.25); NonDHP-CCB RR 0.82 (95% CI 0.37–1.83).

Continued

Ref.	Description	Design	Age (mean)	N	Follow-up	Outcome	Exposure assessment	Covariates	Main results
32	Cache County Study	Cohort	≥ 65 yrs (~74.1 yrs)	3227	3.2 years	Dementia (DSMIII-R, n=185) AD (NINDS-AIRE, n=104)	Baseline interview or, medical records if institutio-nalized.	Age, sex, stroke, education, APOE-4, DM, MI, BP, hyper-cholesterolemia.	Risk of AD with; antihypertensives use vs no use HR 0.64 (95%CI 0.41-0.98), diuretics vs no diuretics HR 0.57; 95CI% 0.33-0.94), K^+ sparing diuretics vs no K^+ sparing diuretics HR 0.26; (95%CI 0.08-0.64), ACE-i vs no ACE-i HR 1.17 (95%CI 0.65-1.96), CCB vs no CCB HR 0.91 (95%CI 0.50-1.55), β-blockers vs no β-blockers HR 0.67 (95%CI 0.24-1.13)
6	Honolulu Asia Aging Study on Japanese American men	Cohort	≥72 yrs (~76 yrs)	1294	5.0 years	Dementia (n=108), AD (n=65)	Interview at each follow-up visit. Data on duration of use at last visit	Age, education; BMI, smoking, CHD, stroke, ankle-brachial index, APOE-4.	Risk of dementia: HR 0.94, 95%CI, 0.89 to 0.99) and risk of AD HR 0.96, 95%CI, 0.93 to 0.99), per year of antihypertensive use.

Abbreviations: AD - Alzheimer disease, VD - Vascular dementia, RR - relative risk, HR - hazard ratio, OR - odds ratio, CI - confidence interval, BP - blood pressure, SBP - systolic BP, DBP - diastolic BP, DM - diabetes mellitus, CCB - calcium channel blocker, (non-)DHP - (non-)dihydropyridine, ACE-i - Angiotensin-Converting Enzyme, K^+ - potassium, APOE-4 - Apolipoprotein E4 allele, BMI - body mass index, MI - myocardial infarction, CHD - coronary heart disease, VD - vascular disease

Table 2. Clinical trials of antihypertensive treatment for the prevention of dementia

Ref.	Description	Design	N	Inclusion criteria	Mean follow-up	Endpoint	Intervention and comparator	Main results
25	Systolic Hypertension in the Elderly Program (SHEP)	Double blind, placebo controlled.	4,736	>=60 years SBP 160-219 mmHg DBP <90 mmHg. Free of dementia.	5 years	Dementia (short-CARE)	Intervention (n=2,365): step 1; diuretic (chlorthalidone), step 2; β-blocker (atenolol) or reserpine. Comparator: placebo (n=2,371).	The incidence of dementia after 5 years was 1.9% in the placebo group and 1.6% in the actively treated.
26, 27	Systolic Hypertension in Europe phase 1 (Syst-Eur 1) and phase 2 (Syst-Eur 2)	Double blind, placebo controlled. (Syst-Eur 1) plus extended open label follow-up (Syst-Eur 2).	2,418 (Syt-Eur 1) 2,902 (Syst-Eur 2)	≥60 years SBP 160-219 mmHg DBP <90 mmHg Free of dementia.	2.0 years (Syst-Eur 1) and 3.9 years (Syst-Eur 2)	Dementia (DSMIII-R criteria)	Syst-Eur 1 (RCT), intervention (n=1,238): step 1: CCB (nitrendipine), step 2: ACE-i (enalapril) or diuretic (hydrochloro-thiazide) or both. Comparator: placebo (n=1,180). Syst-EUR 2 (open label): active treatment group in Syst-Eur 1 continued treatment (n=1,485). Placebo group from Syst-Eur 1 (n=1,417) treated with: step 1, CCB (nitrendipine), step 2: ACE-i (enalapril) or diuretic (hydrochlor-thiazide) or both.	Syst-Eur 1: intention to treat: active treatment reduced dementia incidence by 50% (7.7 to 3.8 cases/1000py; 21 vs 11 cases, p=0.05), compared to placebo. Per-protocol analysis active treatment decreased the rate from 6.6 to 2.7 cases/1000 patient-yrs (p=0.03). Syst-EUR 2: Compared to treated controls Syst-EUR 1, the previous actively treated group in Syst-Eur 1 had a reduced risk of dementia (55%, from 7.4 to 3.3 cases/1000 py (43 vs 21 cases, *P<0*.001)

Continued

Ref.	Description	Design	N	Inclusion criteria	Mean follow-up	Endpoint	Intervention and comparator	Main results
28	Perindopril Protection Against Recurrent Stroke Study (PROGRESS)	Double blind, placebo controlled RCT.	6,105	Normotensive and hypertensive persons Stroke or TIA in previous 5 years of enrolment. Free of dementia.	3.9 years	Dementia (DSM-IV criteria)	Intervention: flexible regimen of ACE-i (perindopril) with diuretic (indapamide) (n=3,051) Comparator: placebo, usual treatment other than the study drug was allowed (n=3,054).	Incidence of dementia for active treatment 6.3% (n=193) and 7.1% (n=217) in the placebo group (RR reduction, 12% [95% CI, −8% to 28%]; *P*=0.2). Intention to treat analyses.
29	Study on Cognition and Prognosis in the Elderly (SCOPE)	Double blind, placebo controlled RCT.	4,964	Age 70–89 years, SBP 160-179 mmHg DBP 90-99 mmHg, untreated or thiazide treated. MMSE ≥ 24.	3.7 years	Dementia (modified ICD-10 research criteria)	Intervention (n=2,477): AT2 antagonist (candesartan) Comparator (n=2,460): placebo, with open-label antihypertensive therapy if needed. (84%).	Incidence of dementia for: AT2 antagonist group: 6.8 events /1000 patient yrs. Control group: 6.3/ 1000 patient-yrs. (p>0.20). Intention to treat analyses.
30	Hypertension in the Very Elderly Trial cognitive function assessment (HYVET)	Double blind, placebo controlled RCT.	4,761	Age ≥ 80 years. SBO 160–200 mm Hg; DBP <110 mm Hg. Free of dementia.	2.2 years	Dementia (DSM-IV, CT scan, MHIS	Intervention: thazide diuretic (slow release indapamide), with the option of ACE-I (perindopril), (n=1,687). Comparator: placebo (n=1,649)	Rates incident dementias: 33 per 1000py in treatment group and 8 per 1000 py in placebo group. (HR 0.86, 95% CI 0.67–1.09). Intention to treat.

Abbreviations: RCT - randomized controlled clinical trial, AD - Alzheimer disease, VD - Vascular dementia, RR - relative risk, HR - hazard ratio, OR - odds ratio, CI - confidence interval, BP - blood pressure, SBP - systolic BP, DBP - diastolic BP, CCB - calcium channel blocker, (non-)DHP - (non-) dihydropyridine, ACE-i - Angiotensin-Converting Enzyme, K^+ - potassium, APOE-4 - Apolipoprotein -ε4 allele, BMI - body mass index, AT2 - angiotensin II receptor, TIA - Transient Ischemic Attack, py – persom years, MHIS - modified Hachinski ischaemic score

METHODS

Study population

The Rotterdam Study is a prospective, population-based cohort study of age related disorders.[9] The medical ethics committee at Erasmus MC, Rotterdam, the Netherlands, approved the study. Between 1990 and 1993 all persons aged ≥55 years living in Ommoord, a district of Rotterdam, were invited to participate. Of 10,275 eligible persons, 7,983 (78%) signed informed consent. Of these, 7,528 (94%) were screened for dementia and 7,046 were free of dementia at baseline.[10] Follow-up examinations, including screening and clinical workup for dementia, were conducted in 1993-1994, 1997-1999 and 2000-2004. In addition, the cohort was continuously monitored for major disease outcomes and death through linkage with records of general practitioners, the Regional Institute for Outpatient Mental Health Care and bimonthly updates from the municipality records. This resulted in a virtually complete follow-up for dementia until January 1st, 2005.

Nearly all persons (99.7%) were registered at one or more of seven automated pharmacies serving the Ommoord area. Records of all filled prescriptions were available from January 1st, 1991. To ensure at least 6 months medication history, we excluded persons for whom follow-up ended before July 1st, 1991.

Assessment of drug exposure

Complete data on filled prescriptions was available on a day-to-day basis from the pharmacy prescription database in automated form. This included the product name; international non-proprietary name; Anatomical Therapeutic Chemical (ATC) code; total number of delivered units (e.g. tablets/capsules); prescribed daily number of units; date of delivery and drug dosage. The duration of a prescription is calculated as the number of delivered units divided by the prescribed daily number of units.

In addition to overall antihypertensive use, we distinguished between the most commonly used types of antihypertensives in the Netherlands, as classified by ATC-code. These included β-blocking agents, thiazides and high ceiling diuretics, calcium-channel blockers, angiotensin-converting-enzym-(ACE)-inhibitors, angiotensin-2-(AT2)-antagonists and other antihypertensives (centrally acting sympathicolytics, peripheral acting sympathicolytics and agents acting on arteriolar smooth muscle).

Diagnosis of Alzheimer disease

The diagnosis of dementia was made following a three-step protocol. Screening was done with the Mini Mental State Examination (MMSE) and Geriatric Mental State schedule (GMS) organic level for all persons.[11,12] Screen-positives (MMSE score <26 or GMS organic level>0) underwent the Cambridge examination for mental disorders of the elderly.[13] Persons who were suspected of having dementia underwent more extensive neuropsychological testing. When available, imaging data were used. In addition, the total cohort was continuously monitored for incident dementia through computerized linkage between the study database and digitalized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. The diagnosis of dementia was made in accordance with internationally accepted criteria for dementia (DSM-III-R), AD (NINCDS-ADRDA), and vascular dementia (NINDS-AIREN) by a panel of a neurologist, neurophysiologist and research physician, blinded to drug exposure of the study population.[14-16]

Other covariates

Covariates included age, sex, education level, systolic and diastolic blood pressure, current smoking, total serum cholesterol, body mass index, diabetes mellitus and cardiovascular and cerebrovascular disease. Education was assessed at the baseline interview and dichotomized into low education (primary education only or low vocational training) and high education (intermediate-level vocational training, secondary education or university). Smoking status was also self-reported and categorized as ever or never. Total serum cholesterol was measured in non-fasting blood drawn at baseline. Sitting blood pressure was measured on the right upper arm using a random-zero sphygmomanometer. The average of two measurements at one occasion was used. Diabetes mellitus was defined as a non-fasting or 2-hour post load glucose level of ≥11.1mmol/l or antidiabetic medication use at baseline. Cardiovascular disease included myocardial infarction, coronary artery bypass graft, percutaneous transluminal coronary angioplasty and atrial fibrillation. Cerebrovascular disease included transient ischemic attack and stroke. Both prevalent and incident cardiovascular and cerebrovascular events were taken into account. APOE genotyping was performed on coded DNA samples, and participants were classified by presence of an APOE-ε4 allele.

Statistical analyses

We calculated the hazard ratio (HR and 95% confidence intervals (CI)) of the risk of all dementia and AD, associated with antihypertensive use using a Cox-proportional-

hazards model with antihypertensive use as time-dependent covariate. Calendar-time was used as the time-axis in the model to account for changes in prescription guidelines and availability of antihypertensive drugs over time. For all subjects, we calculated the duration of follow-up between start of study and diagnosis of dementia, death or end of the study period, whichever came first. Because lowering of blood pressure and changes in cognition occur during the latent phase of disease, physicians might change antihypertensive treatment in the prodromal period of dementia. Calculating the cumulative exposure until the date of diagnosis would not take into account such disease-related changes in prescription and this might bias our risk estimates. To avoid this type of bias, we subtracted a potential latent period from the date of diagnosis, for quantification of exposure duration.[8] Based on the current knowledge regarding the course of blood pressure[1] and cognition in the latent phase of dementia[17] we considered a 4-year prodromal phase for our main analysis. Consequently, at each date of diagnosis minus 4 years we determined cumulative duration of drug until that date for both the person who developed dementia as well as for all persons in the remainder of the cohort. Total cumulative duration of antihypertensive use was expressed in years and as categorical variable based on tertiles of total use at end of follow-up being: no use, <1.6 year use, from 1.6 to 5.3 years use and >5.3 years use. Never use of antihypertensive drugs was the reference in all analyses. In a sensitivity analysis we investigated whether associations differed if we subtracted 2 years from the date of diagnosis or if we used the original date of diagnosis.

We anticipated that the association might be modified by age, since the association between blood pressure and dementia appears to be different at older age. Therefore, we re-performed the analyses for persons ≤75 years and >75 years of age. Likewise, we investigated whether the associations were different for carriers and non-carriers of an APOE-ε4 allele, by adding an interaction term to a model, and by stratified analyses.[18] Using the same exposure definitions, we also investigated the association between separate types of antihypertensive drugs and risk of dementia. A cohort member could contribute person-time to more than one class of antihypertensive drug if a person had used more than one antihypertensive drug during follow-up.

All analyses were adjusted for age, sex, systolic and diastolic blood pressure (Model I). To adjust for potential confounders we additionally included smoking, total serum cholesterol, education, body mass index, diabetes mellitus and cardiovascular and cerebrovascular disease (Model II). Missing values for continuous variables were

imputed with linear regression analyses using sex, age and dementia outcome as determinants. For categorical variables we used a missing indicator for missing values. Analyses were performed with SPSS 16.0 and SAS 9.1 software.

RESULTS

In total 6,249 persons were included in the analysis. In Table 3 baseline characteristics of the study population are given. Persons were followed up to 13.3 years (average 8.0 years), with a total of 49,829 person years of follow up. During follow-up 527 persons developed dementia, of whom 432 were diagnosed with AD, 50 with vascular dementia and 45 with other types of dementia. The total number of filled antihypertensive prescriptions during follow-up was 54,584. Of the 527 persons who developed dementia 264 persons had used antihypertensive drugs during follow-up, whereas 263 persons never used antihypertensive drugs.

Table 3. Baseline characteristics of the study population

Characteristic	(n=6,249)
Age (± SD) in years	68.2 (± 8.3)
Sex (% women)	3,749 (60%)
Smoking, ever (%)	3,985 (63.7%)
Diabetes mellitus (%)	574 (9.2%)
Systolic blood pressure (mmHg)	139.0 (± 21.6)
Cardiovascular disease (%)*	945 (15.2%)
Cerebrovascular disease (%) †	303 (4.8%)
Total cholesterol (mmol/L)	6.7 (± 1.2)
Body-mass index, mean ± SD (kg/m^2)	26.4 (± 3.6)
Primary education, low vocational training or less (%)	2,275 (36.4%)

* Myocardial infarction, coronary artery bypass graft, percutaneous transluminal coronary angioplasty, atrial fibrillation and heart failure
† Stroke or transient ischemic attack

Compared to never-use, antihypertensive use was associated with a reduced risk of all dementia. HRs for AD were nearly equivalent (Table 4). There was an evident duration-response relationship as the risk of all dementia decreased with longer duration of cumulative use, resulting in a 5% risk reduction per year of use (Table

4). Correspondingly, the strongest risk reduction was observed with long-term use of antihypertensives (>5.3 years), whereas short-term use of antihypertensives (<1.6 years) was not associated with a reduced risk of dementia or AD.

Table 4. Hazard ratios of all dementia and Alzheimer disease with use of antihypertensives

	All dementia (n=527)			Alzheimer Disease (n=432)		
	Cases	HR (95%CI)		Cases	HR (95%CI)	
		Model I†	Model II‡		Model I†	Model II‡
Never use	263	1.00 (ref.)		214	1.00 (ref.)	
Antihypertensive drug use:						
<1.6 years	126	0.94 (0.75-1.17)	0.90 (0.72-1.13)	102	0.92 (0.73-1.18)	0.91 (0.71-1.17)
From 1.6 to 5.3 years	98	0.77 (0.60-0.99)	0.72 (0.56-0.93)	83	0.75 (0.57-0.98)	0.73 (0.55-0.96)
>5.3 years	40	0.71 (0.49-1.03)	0.68 (0.47-0.99)	33	0.70 (0.47-1.05)	0.69 (0.46-1.05)
Per year treatment	264	0.95 (0.91-1.00)*	0.95 (0.90-0.99)	218	0.95 (0.90-0.99)	0.94 (0.90-0.99)

† Model I: Age, sex, systolic and diastolic blood pressure adjusted
‡ Model II: as Model I additionally adjusted education, smoking, total serum cholesterol, body mass index, diabetes mellitus and cardiovascular and cerebrovascular disease.
* Upper limit of the confidence <1.00, p-value <0.05

The association between antihypertensive use and dementia was modified by age ($p_{interaction}$=0.003). For persons ≤75 years, antihypertensive drugs reduced the risk of dementia with 8% per year of use (Table 5). For persons >75 years, we observed a risk reduction of 4% per year of use.

Although the protective effect of antihypertensives on dementia risk seemed stronger for carriers of an APOE-ε4 allele, numbers across strata were low and the interaction between APOE-ε4 and antihypertensive drug use was not significant ($p_{interaction}$=0.9) (data not shown).

No apparent differences among the various types of antihypertensives were observed. Adjusted HRs of all dementia per year of use were: 0.97 (95% CI;0.90-1.05) for thiazide diuretics; 0.89 (95% CI;0.78-1.01) for high ceiling diuretics; 0.93 (95% CI;0.87-1.00) for β-blockers; 1.00 (95% CI;0.91-1.09) for calcium channel antagonists; 1.07 (95%

CI;0.99-1.09) for ACE-inhibitors, 0.85 (95% CI;0.44-1.66) for AT2-antagonists and 1.04 (95% CI;0.88-1.24) for other antihypertensives. Similar estimates were found for AD risk.

Table 5. Hazard ratios (HR) of all dementia and Alzheimer disease with use of antihypertensives across strata of age

	All dementia			Alzheimer Disease		
	Cases	HR (95%CI)		Cases	HR (95%CI)	
		Model I†	Model II‡		Model I†	Model II‡
	≤ 75 years of age					
Never use	139	1.00 (ref.)		110	1.00 (ref.)	
Antihypertensive use:						
<1.6 years	60	1.08 (0.80-1.47)	1.03 (0.75-1.41)	48	1.11 (0.78-1.57)	1.11 (0.78-1.56)
1.6 to 5.3 years	42	0.72 (0.50-1.03)	0.68 (0.47-0.99)	32	0.66 (0.44-1.00)	0.67 (0.55-1.02)
> 5.3 years	19	0.59 (0.36-0.99)	0.56 (0.33-0.95)	14	0.56 (0.31-1.01)	0.57 (0.41-1.04)
Per year treatment	121	0.93 (0.87-0.99)	0.92 (0.86-0.98)	94	0.91 (0.85-0.98)	0.92 (0.85-0.99)
	> 75 years of age					
Never use	124	1.00 (ref.)		104	1.00 (ref.)	
Antihypertensive use:						
<1.6 years	66	0.81 (0.60-1.10)	0.76 (0.56-1.04)	54	0.79 (0.56-1.10)	0.75 (0.53-1.06)
1.6 to 5.3 years	56	0.75 (0.53-1.05)	0.68 (0.48-0.96)	51	0.76 (0.53-1.09)	0.70 (0.48-1.02)
> 5.3 years	21	0.89 (0.52-1.52)	0.83 (0.48-1.43)	19	0.90 (0.51-1.59)	0.85 (0.48-1.51)
Per year treatment	143	0.98 (0.91-1.04)	0.97 (0.90-1.04)	124	0.97 (0.90-1.04)	0.96 (0.89-1.04)

† Model I: Age, sex, systolic and diastolic blood pressure adjusted
‡ Model II: as Model I additionally adjusted education, smoking, total serum cholesterol, body mass index, diabetes mellitus and cardiovascular and cerebrovascular disease.

In the sensitivity analyses, estimates for risk of dementia gradually attenuated if shorter prodromal periods were considered. We observed a 4% risk reduction (0% to 7%, p=0.03) if 2 years were subtracted from the date of diagnosis to 3% (0% to 6%, p=0.03) per year of antihypertensive use if the original date of diagnosis was used.

DISCUSSION

In the general population, we found that the antihypertensive use was associated with decreased risk of all dementia and AD with 8% per year of use for persons ≤75 years of age. No apparent differences were observed among the various types of antihypertensive drugs.

Strengths of our study design included its prospective design, large number of participants, a general population-based setting and the long follow-up period of over 8 years average. Moreover, we used pharmacy records for the assessment of antihypertensive drug use. This greatly reduces the chance of exposure misclassification as opposed to baseline exposure data or periodic re-assessment of drug use and allows for an accurate estimation of exposure duration. In an earlier study, we demonstrated that there was a high concordance between pharmacy filling data of cardiovascular drugs, and actual use according to a patient interview.[19] Moreover, we were able to subtract 4-years from the date of clinical diagnosis of dementia to avoid potentially biased risk estimates as a result of changes in antihypertensive prescription due to blood pressure changes or cognitive decline in the prodromal phase of dementia. Nevertheless, some issues warrant consideration. First, the 4-year period is an average estimation of the prodromal period based on the available data from literature. There is, however, limited longitudinal data on the course of blood pressure prior to clinical dementia. Data from the Kungsholmen project shows that blood pressure markedly decreases over a 3-year period preceding diagnosis of dementia.[20] The Göteborg longitudinal study revealed that a decline in systolic blood pressure between age 70 and 75 was the only predictor of dementia with onset at age 75 to 79.[21] Likewise, estimations of the intervals between cognitive decline and dementia vary from 1.5 years to up to 10 years, but generally suggest a more rapid decline in the last 2 to 3 years prior to diagnosis.[22-24] In a sensitivity analysis, we also investigated the association of antihypertensive use and dementia subtracting only two years from the date of diagnosis for quantification of exposure duration, and also the date of diagnosis itself.

Though protective effects of antihypertensive use on dementia risk were still observed, the attenuation of the protective effect in these analyses suggests that, at least in part, the protective effect of antihypertensive may be obscured in the prodromal phase of dementia. Second, we have to consider potential bias due to confounding-by-indication, since the indication for antihypertensive use, i.e. hypertension, is associated with the outcome of interest, i.e. dementia.[7] However, given the complex association between hypertension and dementia, it is difficult to predict the direction of this effect. If hypertension increases the risk of dementia[2], than our estimates would underestimate the true protective effect. Instead, if hypertension would protect against dementia in persons above ~75 years of age[3], then confounding-by-indication could lead to a spurious protective effect on dementia for antihypertensives. However, this is refuted by our finding of no benefit of antihypertensive use on dementia risk for persons >75 years. Finally, our analyses for the separate types of antihypertensive drugs must be interpreted in light of low numbers within the different antihypertensives categories. Since some antihypertensive drugs are preferred when certain comorbidities apply, the differences observed among the various antihypertensives, though small, may be explained by differences in underlying co-morbidity rather than by a true difference in physiological effect. Unfortunately, no data is available on the indication of drug use. Low numbers also prohibited further investigation across strata of age in the analyses of the separate antihypertensives.

Numerous studies have investigated the association between antihypertensive use and the risk of dementia (Table 1 and 2). Thus far, findings have been inconsistent. Considerable methodological exist amongst studies. Clinical trials mainly included patients >60 years with, in 4 out of 5 trials, a systolic blood pressure of ≥160mmHg.[25-30] Some trials had significant number of patients who were lost to follow-up[31] or allowed for usual antihypertensive treatment other than the study drug in the placebo group,[28,31] which further complicates the interpretation of their findings. The null-findings in 4 out 5 trials have also been attributed to the limited duration of follow-up. [25,28-30] Nonetheless, in the Syst-EUR trial antihypertensive treatment reduced the risk of dementia by no less than 50% after a mere 2 years of follow-up[26] and again in an open-label extension of the same trial after 3.9 years of follow-up.[27] However, as with most of the studies previously performed, the Syst-Eur trial suffered from a small number of dementia endpoints. Besides the limited duration of follow-up, most observational studies relied on baseline data on antihypertensive treatment, which can lead to considerably misclassification of drug exposure during follow-up (Table 1). Furthermore, only 2 out of

8 of the prospective observational studies investigated whether associations depended on duration of antihypertensive use.[6,32] In the Cache County Study, the protective effect of antihypertensives was observed regardless of duration of use as assessed at baseline.[32] In a cohort of Japanese men the risk of dementia was reduced by 6% with each additional year of antihypertensive treatment, based on self-reported duration of drug use at the final examination.[6] Considerable differences in both setting and design hamper a direct comparison of these findings to our study. Nevertheless, both studies suggest that the protective effect depends on the duration of antihypertensive use. Our observation of a stronger protective effect for persons ≤75 year of age corresponds with the view that an increased blood pressure earlier in life increases the risk of dementia, whereas high blood pressure in older persons may not necessarily put persons at higher risk for dementia.[1]

The pathological processes through which hypertension is thought to influence dementia pathology are many. High blood pressure can result in severe atherosclerosis, leading to cerebral hypoperfusion.[33] Changes in cerebral white matter can also occur as a result of sustained high blood pressure.[34] Other than the blood pressure lowering effect of antihypertensives, it has been suggested that certain antihypertensives exert a more direct effect on dementia pathology. For example, the intracellular build-up of calcium in neurons can be neurotoxic and thus calcium channel blockers might result in neuroprotection.[35] However, our findings do not support an advantage of one anti-hypertensive over another. Other studies have also investigated the different types of antihypertensive in relation to dementia risk, but findings have been largely inconsistent. The types of antihypertensive for which reductions were shown included ACE-inhibitors, angiotensin-II-blockers, potassium-sparing diuretics, and dihydropyridine calcium channel blockers. The fact that, across both clinical and observational studies, various types of antihypertensive drugs have been identified as a having a particular beneficial effect on dementia suggests that this is the play of chance rather than that actual differences exist. Hence, we consider it more likely that effect of antihypertensive drugs on blood pressure in itself underlies the protective effect of these drugs. For future research, a more mechanistic approach, such as the use of imaging markers of cerebral pathology, would be desirable to understand the biological basis of the association between antihypertensives and dementia.

Given the established benefit of antihypertensives on prevention of cardiovascular disease, we believe that current evidence constitutes a limited basis for advocating a

universal antihypertensive treatment policy for the sole purpose of dementia prevention. Further insight on the association between blood pressure and dementia and the effect of antihypertensives on this association could nonetheless provide us with important information on pathological pathways leading to dementia.

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Chapter 2.3

Antihypertensive drugs and progression of White Matter Lesions

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ABSTRACT

Background: Cerebral white matter lesions (WML) are frequently observed on MR-images of elderly persons and are associated with an increased risk of stroke and dementia. Hypertension is a risk factor for presence and progression of WML.
Methods: We investigated the association between antihypertensive use and WML-progression in non-demented participants from a prospective population-based cohort study. In 1995-1996, 563 participants (60-90 years; mean 72.1 years, 50% female) underwent cerebral MRI. After on average 3.3 years, 320 persons underwent a follow-up MRI. Two independent raters assessed WML-progression in a blinded fashion by side-by-side scan comparison using a validated visual rating scale. Detailed information on drug use, from 1991 to baseline MRI, came from pharmacy records. We used age and sex adjusted analysis of covariance to compare baseline characteristics between persons with and without follow-up MRI. With logistic regression analysis, we calculated odd ratios (OR) and 95% confidence interval (CI) of WML-progression, adjusted for age, sex, blood pressure (BP) and other potential confounders. Analyses were re-performed across age strata (60-70, 70-80, and 80-90 years) and for persons with and without controlled BP (systolic BP>160mmHg or diastolic BP>95mmHg).
Results: Compared to non-users, users of antihypertensive had a lower risk of WML-progression (OR 0.53; 95%CI 0.31-0.91). ORs were similar for periventricular and subcortical WML-progression. The association was modified by age and a statistically significant risk reduction was only observed in the youngest age stratum from 60 to 70 years. Antihypertensive users with a controlled BP had a lower risk of WML-progression (OR 0.44; 95%CI 0.24-0.82), than persons with hypertension despite antihypertensive treatment (OR 0.81; 95%CI 0.39-1.69).
Conclusion: The use of antihypertensives was associated with a reduced risk of WML progression. The protective effect was limited to persons <70 years of age and to those with controlled BP.

INTRODUCTION

Cerebral white matter lesions (WML) are frequently observed on magnetic resonance imaging (MRI) scans in elderly people.[1-3] These lesions are associated with an increased risk of stroke, cognitive decline, and dementia.[4-6] WML appear to progress gradually over time.[7-11] Besides age, several vascular risk factors for WML have been identified, of which blood pressure is most recognized.[12] Elevated blood pressure has consistently been associated with the presence and severity of WML in both cross-sectional[1,3,13] and longitudinal studies.[14-18] The use of blood pressure lowering agents may modify this association as persons with uncontrolled hypertension were found to have a higher prevalence of WML than persons without hypertension or with controlled hypertension.[1,15,16] Increased blood pressure has also been identified as a risk factor for WML-progression.[2,7,17,19-21] Hence, it has been postulated that blood pressure lowering agents could protect against the progression of WML. This hypothesis has been examined in few studies, with conflicting results.[8,17,20,22] Most of the previous studies were performed in the context of clinical trials or in healthy volunteers. We therefore investigated the association between the use of antihypertensives and the risk of WML-progression in a population-based prospective cohort of elderly.

METHODS

Study population

This study is based on the Rotterdam Scan Study, a large population-based cohort study in the Netherlands that aims to study the etiology and natural history of age-related brain changes in the elderly.[23] The study was approved by the medical ethics committee of the Erasmus Medical Center, Rotterdam. In 1995-1996, we randomly invited participants, aged 60 to 90 years, stratified by sex and 5-year age strata from two on-going population-based studies to participate in the Rotterdam Scan Study.[24] Participants, who were demented, were excluded based on a stepwise approach as used in the Rotterdam Study.[25] After exclusion of individuals who were demented or had MRI contraindications, 563 participants from the Rotterdam Study gave their written informed consent to participate in the study, which included undergoing an MRI scan. In total, 320 persons underwent the follow-up MRI measurement in 1999. Other than refusal, non-participation at the follow-up MRI was mainly due to death (n=46), institutionalization (n=14) and contraindication for MRI (n=13, e.g. pacemaker).

Drug exposure

Complete information on all filled prescriptions for all persons was obtained in automated format from the pharmacies from January 1st, 1991 onwards. This included the product name; international non-proprietary name; Anatomical Therapeutic Chemical (ATC) code; total number of delivered units (e.g. tablets/capsules); prescribed daily number of units; date of delivery and drug dosage. The duration of a prescription is calculated as the total number of delivered units divided by the prescribed daily number of units.

We studied exposure to antihypertensive drug as a group and, in addition, we distinguished between the various types of antihypertensives as classified by ATC-code. These included beta-blocking agents, diuretics, agents acting on the renin-angiotensin-aldosterone-system (RAAS) (angiotensin-converting-enzym-inhibitors and angiotensin receptor-antagonists), calcium-channel blockers and other antihypertensives (centrally acting sympathicolytics, peripherally acting sympathicolytics and agents acting on arteriolar smooth muscle).

White matter lesion progression

In 1995-1996, we made axial T1-, T2-, and proton density (PD)-weighted cerebral MR scans on a 1.5-Tesla scanner (MR VISION, Siemens).[26] In 1999-2000, participants underwent a follow-up MRI on the MR VISION scanner using the same sequences. For measuring change of WML severity over time, we used a specifically developed and validated WML change scale.[27,28] Two raters independently assessed the progression of WML severity on digital T2- and PD-weighted images by direct scan comparison. Raters were blinded to scans being baseline or follow-up and to all clinical information. To systematically evaluate the difference in WML severity, the raters independently scored differences in the three periventricular regions of both hemispheres (periventricular score range -6 to +6) and in the subcortical white matter of the four lobes of both hemispheres (subcortical score range: -8 to +8). Hyperintensities on PD- and T2-weighted images around an incident infarct were not considered as progression of WML. The rating showed good interobserver (intraclass correlation coefficient 0.72–0.73) and intraobserver agreement (intraclass correlation coefficient 0.70–0.93). If raters disagreed on one point or less on the scale, the mean of the ratings was used; if more, a consensus meeting was held. Adjudication by consensus meeting was required in 9% of the periventricular and 11% of the subcortical WML ratings. WML-progression

was defined as an increase of one point or more in any region, and separately for the periventricular and subcortical region.[27]

Other measures

Information on current health status was collected by interview and physical examination by trained examiners.[5] Blood pressure was measured twice in sitting position on the right arm with a random-zero sphygmomanometer with the appropriate adult cuff size after 5 minutes rest. The average of the two values measured at one occasion was used. Hypertension was defined as a systolic blood pressure (SBP) above 160 mmHg or a diastolic blood pressure (DBP) above 95 mmHg according to the Dutch national guidelines at time of recruitment.[29] Non-fasting serum total cholesterol was determined with an enzymatic procedure. Smoking habits were classified as never, former or current cigarette smoking. We considered diabetes mellitus to be present if the random glucose level was ≥11.1 mmol/l or if a person was taking oral anti-diabetics or insulin. Using automated MRI-analysis we measured volumes of WML at baseline MRI. Preprocessing steps and the classification algorithm have been described.[13]

Statistical analysis

Use of antihypertensives at baseline was categorized as ‘any’ or ‘no’ use, depending on whether a person had used antihypertensive drugs between January 1st, 1991 and date of baseline MRI (1995-1996), or not. We acknowledged that, even though antihypertensive treatment is generally used chronically, persons could have stopped therapy, for example as a result of adverse events, non-compliance or a change in life-style which rendered antihypertensive treatment no longer indicated. Therefore, we additionally defined ‘current’ use as at least one prescription of antihypertensive drugs was (re)filled ≤4 months prior to baseline MRI. No-use of any antihypertensive drug was the reference category for all analyses. A person could contribute to more than one exposure category of antihypertensive use if that person had used different types of antihypertensives prior to baseline.

We used age and sex adjusted analysis of covariance to determine whether base-line characteristics, including antihypertensive use, differed between persons with and without a follow-up MRI assessment. Logistic regression models were used to study the association between antihypertensive drug use and any progression of WML (total) and separately for periventricular and subcortical WML-progression. All analyses were

adjusted for age, sex, systolic and diastolic blood pressure (Model I). In a second model we additionally adjusted for the following cardiovascular risk factors; diabetes mellitus, total serum cholesterol, body mass index, and smoking (Model II). Subsequently, in Model III, we additionally adjusted for baseline WML volume.

We performed several subanalyses. First, we considered that the association might be biased by selective attrition, since hypertension, the underlying indication of antihypertensive treatment, is related to mortality. This could be of particular concern in elderly participants. We addressed this issue by performing the analysis in three equal age strata (60-70 years, 70-80 years and 80-90 years).[16] Second, in persons treated with antihypertensives adequate blood pressure control is not always achieved. Previously, it was shown that the severity of WML is higher in poorly controlled hypertensive patients than in successfully treated patients.[1,16] Hence, we categorized persons according to antihypertensive use and blood pressure status into 3 categories; (1) no use of antihypertensive drugs (reference), (2) antihypertensive use and controlled blood pressure (defined as <160mmHg SBP and <95mmHg DBP according to Dutch national guidelines at time of recruitment), (3) antihypertensive use and uncontrolled blood pressure (defined as ≥160mmHg SBP or ≥95mmHg DBP). Finally, we studied whether any observed effects depended on the duration of drug use by dichotomizing total duration of antihypertensive use around the mean duration of use. Analyses were done using SPSS 11.0.1, IL, USA for Windows.

RESULTS

Baseline characteristics of participants with and without a follow-up MRI assessment are presented in Table 1 and 2. Persons without a follow-up MRI were on average older at baseline, more frequently diabetic, less frequently smokers and had a higher baseline WML load than those who had a follow-up MRI assessment. However, after adjusting for age and sex, there were no statistically significant differences between participants with or without MRI at follow-up for these baseline characteristics. Notably, there was no difference in the WML-volume for persons with and without a follow-up MRI (Table 1). The mean follow-up period between the first and follow-up MRI was on average 3.3 years (SD ± 0.3 years).

Table 1. Baseline characteristics of the study population, n=563

Characteristic	Participants with a 2nd MRI at follow-up (%)	Participants without a 2nd MRI at follow-up (%)	p-value*
Number	320	243	
Age (years, ± SD)	72.1 (7.8)	75.5 (7.7)	<0.00
Sex (% female)	160 (50.0)	121 (49.8)	0.90
Systolic blood pressure (mm Hg, ± SD)	145.6 (20.4)	146.7 (20.5)	0.55
Diastolic blood pressure (mm Hg, ± SD)	76.5 (11.4)	76.6 (11.5)	0.62
Body Mass Index (kg/m^2, ± SD)	26.2 (3.7)	26.5 (3.4)	0.26
Serum total cholesterol (mmol/l, ± SD)	5.9 (1.0)	5.8 (1.1)	0.80
Smoking (% current smokers)	62 (19.9)	36 (14.9)	0.43
Diabetes mellitus (%)	13 (4.1)	17 (7.0)	0.31
White matter lesion volume (%-ICV, ± SD)	1.2 (1.4)	1.5 (1.6)	0.92

Abbreviations: MRI - magnetic resonance imaging, SD - standard deviation, ICV - intra cranial volume
* p-value for sex, age adjusted differences, if applicable

Table 2 shows the baseline characteristics of antihypertensive use. Antihypertensive use at baseline was comparable between persons with and without a follow-up MRI in the age of 60 to 70, but proportionally greater in persons above 70 without a follow-up MRI and those with controlled blood pressure.

Table 2. Antihypertensive drug use at baseline for persons with and without a follow-up MRI

Antihypertensive drug use	Participants with a follow-up MRI at follow-up (%)	Participants without a follow-up MRI at follow-up (%)	p-value*
Any use of antihypertensive drugs	134 (41.9)	141 (58.0)	0.01
60 to 70 years	53 (38.1)	28 (43.1)	0.50
70 to 80 years	43 (38.7)	51 (54.8)	0.02
80 to 90 years	38 (54.3)	62 (73.9)	0.02
With controlled BP§	87 (31.9)	96 (48.5)	0.03
Without controlled BP	45 (14.4)	46 (18.5)	0.10
Beta blocking agents	81 (25.3)	75 (30.9)	0.34
Diuretics	58 (18.1)	88 (36.2)	<0.00
Agents acting on RAAS	45 (14.1)	55 (22.6)	0.05
Calcium channel blockers	36 (11.2)	59 (24.3)	<0.00
Other antihypertensives	4 (1.3)	8 (3.3)	0.15

Abbreviations: MRI - magnetic resonance imaging, RAAS - renin-angiotensin-aldosterone system, SBP - systolic blood pressure, DBP - diastolic blood pressure

* p-value for sex, age adjusted differences

§ Systolic blood pressure ≥160mmHg or diastolic blood pressure ≥95 mmHg, according to Dutch national guidelines at time of recruitment

A total of 132 persons of the 320 persons who underwent follow-up MRI, showed progression of WML compared to baseline MRI. Of these, 51 persons had used antihypertensives between 1991 and baseline MRI (1995-1996).

In Table 3, the results are shown for any antihypertensive use and the risk of WML-progression. Compared to non-use, the use of antihypertensive drugs at baseline was associated with a reduced risk of WML-progression at follow-up (Table 3).

Table 3. Odd ratios (OR) of total, periventricular and subcortical white matter lesion (WML) progression with the use of antihypertensives

	Progression of WML								
	Total			Subcortical			Periventricular		
Antihypertensive drug use	**N***	**OR (95% CI) Model I†**	**OR (95% CI) Model II‡**	**N***	**OR (95% CI) Model I**	**OR (95% CI) Model II**	**N***	**OR (95% CI) Model I**	**OR (95% CI) Model II**
Never use	186 (81)	1.00 (ref.)	1.00 (ref.)	129 (57)	1.00 (ref.)	1.00 (Ref.)	122 (64)	1.00 (ref.)	1.00 (ref.)
Any antihypertensive use	134 (51)	0.55 (0.33-0.93)	0.53 (0.31-0.91)	134 (41)	0.63 (0.38-1.06)	0.66 (0.39-1.12)	134 (41)	0.64 (0.36-1.13)	0.65 (0.36-1.16)
60 to 70 years	53 (8)	0.28 (0.11-0.76)	0.27 (0.10-0.74)	53 (7)	0.27 (0.10-0.78)	0.26 (0.09-0.78)	53 (4)	0.33 (0.09-1.17)	0.30 (0.08-1.15)
70 to 80 years	43 (16)	0.57 (0.24-1.33)	0.64 (0.26-1.60)	43 (13)	0.67 (0.29-1.59)	0.71 (0.28-1.80)	43 (11)	0.52 (0.20-1.33)	0.63 (0.23-1.70)
80 to 90 years	38 (27)	0.98 (0.32-2.98)	1.25 (0.33-4.71)	38 (21)	1.17 (0.41-3.34)	2.22 (0.62-7.95)	38 (28)	1.36 (0.47-3.91)	2.34 (0.59-9.24)
With controlled BP§	87 (28)	0.46 (0.26-0.84)	0.44 (0.24-0.82)	87 (21)	0.50 (0.27-0.91)	0.52 (0.28-0.98)	87 (22)	0.55 (0.29-1.05)	0.53 (0.27-1.04)
Without controlled BP	46 (22)	0.85 (0.42-1.72)	0.81 (0.39-1.69)	46 (19)	1.05 (0.53-2.11)	1.08 (0.52-2.23)	46 (18)	1.02 (0.48-2.14)	1.08 (0.50-2.33)

Abbreviations: OR - odds ratio, CI - confidence intervals SBP - systolic blood pressure, DBP - diastolic blood pressure, BP - blood pressure

* Total number of persons in an exposure category. The number of persons with WML-progression in the exposure category is shown between brackets.

† Model I: adjusted for sex, age, systolic and diastolic blood pressure.

‡ Model II: as model I and additionally adjusted diabetes, total serum cholesterol, body mass index, and smoking.

§ Systolic blood pressure ≥160mmHg or diastolic blood pressure ≥95 mmHg, according to Dutch national guidelines at time of recruitment.

The odds ratios were equivalent for periventricular and subcortical WML-progression and effect sizes were comparable to the overall estimate. The association was modified by age and the statistically significant risk reduction was only observed in the youngest age stratum from 60 to 70 years, while in the higher age categories the risk was non-significantly reduced, or not present at all. Users of antihypertensives with a controlled blood pressure had a reduced risk of WML-progression, whereas persons who had hypertension despite antihypertensive treatment did not (Table 3). Additional adjustment for baseline WML did not alter the odds ratios (data not shown). Similar odds ratios were observed when restricting the exposure definition to current users who had filled at least one prescription ≤4 months prior to baseline MRI (OR 0.59 95% CI (0.33-1.04). This was applicable for 42 of the 51 exposed cases at baseline. Here, ORs for the different age strata were 0.27 95% CI (0.09-0.83) for 60 to 70 years, 0.91; 95% CI (0.34-2.43) for 70 to 80 years and 1.19; 95% CI (0.27-5.26) for 80 to 90 years.

Between January 1st 1991 and date of first MRI the mean duration of antihypertensive use was 3.2 years (±1.8 years). Compared to no use, we observed that persons with less than 3.2 years of use prior to baseline had a lower risk of WML-progression (OR 0.33; 95%CI 0.16-0.68) than persons who had used antihypertensives for more than 3.2 years (OR 0.78; 95%CI 0.41-1.47). The limited number of exposed cases, however, prohibited further stratification across age or achieved blood pressure control for the separate duration categories.

There were only slight differences among the various types of antihypertensives, although the risk reduction was only statistically significant for beta-blockers and RAAS inhibitors (Table 4).

Table 4. Odd ratios (OR) of white matter lesion (WML) progression with any use of the individual types of antihypertensives

	Progression of WML		
Antihypertensives drug use	**N***	**OR (95% CI), Model I†**	**OR (95% CI), Model II‡**
Never use	186 (81)	1.00 (ref.)	1.00 (ref.)
Any use of: §			
β- blocking agents	81 (27)	0.51 (0.28-0.93)	0.52 (0.28-0.96)
Diuretics	58 (28)	0.56 (0.27-1.17)	0.63 (0.31-1.28)
Agents acting on RAAS	45 (15)	0.38 (0.17-0.82)	0.37 (0.17-0.81)
Calcium channel blockers	36 (11)	0.45 (0.19-1.04)	0.43 (0.18-1.03)

RAAS - renin-angiotensin-aldosterone system
* Total number of persons with follow-up MRI-measurement. The number of persons with WML-progression in the exposure category is shown between brackets.
† Model I: adjusted for sex, age, systolic and diastolic blood pressure
‡ Model II: as Model I and additionally adjusted diabetes, total serum cholesterol, body mass index, and smoking
§ There were no persons with WML-progression in the exposure category of other antihypertensives.

DISCUSSION

In the general elderly population, we found a protective effect of antihypertensive use on the progression of WML. The protective effect was limited to persons younger than 70 years of age and to those with controlled blood pressure.

Strengths of our study include the population-based setting, and the large sample size of elderly persons aged 60 years and older. A particular asset of our study is that we had complete pharmacy records of all filled prescriptions, providing us with very specific and detailed information on drug use. In an earlier study, we demonstrated that there was a high concordance between pharmacy filling data of cardiovascular drugs, and actual use according to a patient's interview.[30] In view of the observational nature of our study, we should consider the possibility of selection bias and confounding. First, progression of WML could only be assessed in persons with a follow-up MRI. For the youngest age stratum of 60 to 70 years of follow-up we showed that antihypertensive use was unrelated to non-participation at follow-up and, hence, this could not have confounded the observed risk reduction for WML-progression. For the age groups

above 70 years of age, users of antihypertensive were less likely to have a follow-up MRI. Individuals in these age groups who did have a follow-up MRI may thus pertain to a 'healthier' selection of this study population. We can not exclude that selective participation biased our risk estimates in these higher age groups, although such a bias would tend to lead to a decreased risk. Second, we have to consider potential bias due to confounding-by-indication, since the indication for drug use, i.e. hypertension, is associated with the outcome of interest, i.e. progression of WML. The fact that we did not find an increased but decreased risk of progression indicates the absence of confounding by indication. Would the risk estimates still be confounded by the indication of hypertension, it would mean that the true protective effect is even higher than we measured. Third, WML-progression was rated visually, which gives less precise estimates of total lesion progression than a volumetric measurement but these were unavailable in our study.[31] Independent validation showed, however, that our visual rating scale had good correlation with volumetric measurements.[27,28] Finally, the indication of drug use is unknown in our study. Certain types of antihypertensives are preferred when certain comorbidities apply, such as beta-blockers in the case of angina pectoris. Hence, from our study we cannot conclude whether the differences observed among the various antihypertensives, though small, may be explained by differences in underlying co-morbidity or by a true difference in physiological effect. Our observations, particular for the individual antihypertensive drugs, should be interpreted in context of limited case numbers.

Our finding of a protective effect of antihypertensive use on WML-progression is compatible with three earlier studies that reported a lower risk of more severe WML among users of antihypertensive drugs, particularly also among persons with controlled blood pressure.[1,15,16] Only few studies have prospectively investigated the effect of antihypertensive treatment on the progression of WML. Differences in study populations, imaging techniques, lesion ratings and assessment of drug exposure limits the comparison of previous studies to our results. Sachdev *et al.* investigated the course of WML-progression over 3 years in 51 healthy volunteers and found no association between the use of antihypertensives and change in WML volume.[8] However, only 17 users of antihypertensives were available in the analysis and no distinction was made between persons with controlled and uncontrolled blood pressure. Within the cohort of the Cardiovascular Health Study investigators found that the use of diuretics was associated with a worsening of WML grade.[17] Two studies on antihypertensive use and WML-progression were performed in the context of two clinical trials; the Study on

COgnition and Prognosis in the Elderly (SCOPE)[20] and the Perindopil Protection Against Recurrent Stroke Study (PROGRESS).[22] Both studies found a reduced risk of WML-progression with the use of either an angiotensin receptor blocker[20] or an ACE-inhibitor with or without a diuretic compared to placebo.[22] However, in the treatment and control groups the study protocol of both studies allowed for usual antihypertensive treatment, not containing the study drug. In both studies lower blood pressure levels were attained in the treatment groups for which the risk of WML-progression was reduced. In our study, we did not observe a difference in the effect of antihypertensive use on periventricular and subcortical WML-progression. This observation is in line with the results from a previous report from our cohort that showed that increased blood pressure was associated with both periventricular and subcortical WML.[16] In all of the previous investigations on the association between antihypertensives and WML-progression, none distinguished between periventricular and subcortical WML-progression.

Contrary to what would be expected, persons with shorter duration of antihypertensives use had a lower risk of WML-progression than persons with longer duration of antihypertensive use. We speculated that persons who have required antihypertensive treatment for longer periods of time are also long-standing hypertensive patients and adequate blood pressure control may not be achieved at all times. Hence, though antihypertensive treatment might slow down the process that leads to increasing WML volume, with time the effect of increased blood pressure surpasses the protective effect of antihypertensives. Furthermore, hypertension treatment guidelines were introduced in the early 1990s and persons who started antihypertensive therapy more recently may have had better surveillance of their hypertension from the start of therapy. Limited case numbers prohibited further stratification across other characteristics, such as age or blood pressure, to study whether these may have played a role.

Antihypertensives may offer potential therapeutic possibilities in preventing the risk of cerebrovascular events and dementia associated with WML-progression. Confirmation of our findings by other studies is needed. Furthermore, insight into the mechanism by which antihypertensive drugs prevent WML-progression will help us better understand and substantiate these observations. Future studies could also focus on whether differences among the various types of these drugs exist or who might particular benefit from antihypertensive use.

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Chapter 2.4

Antithrombotic drugs and presence of Cerebral Microbleeds

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ABSTRACT

Background: Cerebral microbleeds are hemosiderin deposits in the brain that are indicative of microangiopathy. Microbleeds in strictly lobar brain locations have been related to cerebral amyloid angiopathy, a bleeding-prone state.
Objective: To investigate the relation between antithrombotic drug use and presence of cerebral microbleeds, especially those in strictly lobar locations.
Setting: The Rotterdam Scan Study, a population-based imaging study in a general elderly community in the Netherlands.
Participants: A population-based sample of 1,062 persons from a longitudinal cohort, aged 60 years and older, free of dementia.
Design: Population-based cross-sectional analysis. We used magnetic resonance imaging (MRI) to assess presence and location of microbleeds. Complete information on outpatient use of platelet aggregation inhibitors and anticoagulant drugs prior to MRI was obtained from automated pharmacy records.
Results: Compared to non-users of antithrombotic drugs, cerebral microbleeds were more prevalent among users of platelet aggregation inhibitors (adjusted odds ratio (OR) 1.71; 95% confidence interval (CI) 1.21-2.41). We did not find a significant association for anticoagulant drugs and microbleed presence (OR 1.49; 95%CI 0.82-2.71). Strictly lobar microbleeds were more prevalent among aspirin users (adjusted OR compared to non-users 2.70; 95%CI 1.45-5.04) than among persons using carbasalate calcium (adjusted OR 1.16; 95%CI 0.66-2.02). This difference was even more pronounced when comparing persons who had used similar dosages of both drugs.
Conclusions: In this cross-sectional study, we show that use of platelet aggregation inhibiting drugs is related to presence of cerebral microbleeds. Furthermore, our data suggest that aspirin use and carbasalate calcium use may differently relate to presence of strictly lobar microbleeds.

INTRODUCTION

Cerebral small vessel disease is common in the elderly, and well-studied markers on magnetic resonance imaging (MRI) include lacunar infarcts and white matter lesions. In the past decade, cerebral microbleeds have become acknowledged as new markers of small vessel disease in the brain. These microbleeds, consisting of hemosiderin deposits in macrophages,[1] can be visualized on T2*-weighted gradient-recalled echo (GRE) MRI as small areas of hypointensity. Generally, microbleeds are thought to occur on the basis of either cerebral amyloid angiopathy[2] or arteriolosclerotic microangiopathy.[3] There is accumulating evidence that microbleed location in the brain is reflective of their underlying etiology. Microbleeds in deep or infratentorial locations are thought to be suggestive of hypertensive or arteriolosclerotic microangiopathy, whilst those occurring in strictly lobar brain sites are indicative of cerebral amyloid angiopathy.[4] Cerebral amyloid angiopathy is characterized by accumulations of amyloid in the vessel wall, causing degeneration of smooth muscle cells resulting in vessels that are more susceptible to ruptures and haemorrhages.[5] This suggests that especially strictly lobar microbleeds may be indicative of the presence of bleeding-prone brain vessels.[2] In cerebral amyloid angiopathy, the use of platelet aggregation inhibitors and anticoagulants has been found to be related to increased occurrence of symptomatic hemorrhage.[6-8] In parallel, the development of asymptomatic lobar microbleeds in these persons may perhaps also be accelerated by use of these antithrombotic drugs.

We hypothesized that microbleeds, especially those in strictly lobar locations, occur more often in persons using antithrombotic drugs. We therefore studied the association between the use of platelet aggregation inhibitors or anticoagulant drugs, with presence of microbleeds in different brain locations in a large elderly population.

METHODS

Study population

The study population was derived as described previously.[4] In short, we randomly selected 1,073 members of the first cohort expansion of the Rotterdam Study and in addition invited all Rotterdam Study participants who underwent brain imaging in the context of a previous round of the Rotterdam Scan Study (n=302).[9] Of these, a total of 1,229 persons were eligible, of whom 1,114 participated and gave written informed

consent (response 91%). A total of 1,098 complete MRI examinations were performed between August 2005 and August 2006, of which 36 scans had to be excluded because of artifacts,[4] leaving a total of 1,062 scans to be analyzed.

Brain MRI and rating of cerebral microbleeds

We performed a multi-sequence MRI protocol on a 1.5-Tesla MRI scanner (General Electric Healthcare, Milwaukee, USA) as described previously,[4] including a T1-weighted, proton density weighted and fluid-attenuated inversion recovery (FLAIR) sequence. For microbleed detection, we used a high-resolution 3-dimensional T2*-weighted gradient recalled echo (3D T2* GRE) sequence,[10] optimized to increase the conspicuity of cerebral microbleeds (repetition time= 45 ms, echo time= 31, matrix size= 320*224, flip angle= 130, field-of-view= 25 x 17 cm2, parallel imaging acceleration factor= 2, 96 slices encoded with a slice thickness of 1.6 mm zero padded to 192 slices of 0.8 mm, acquisition time 5 minutes 55 seconds).

All 3D T2* GRE scans were reviewed as described before[4] by one of two trained raters (MWV, MAI), who recorded the presence, number and location of cerebral microbleeds. Microbleeds were defined as focal areas of very low signal intensity on the 3D T2* GRE scan,[11-13] and they were categorized into one of three locations: lobar (cortical grey and lobar white matter), deep (deep grey matter: basal ganglia and thalamus, and white matter of internal/external capsule and corpus callosum), and infratentorial (brainstem and cerebellum).[4,11-13] All potential cerebral microbleeds were reviewed with an experienced neuroradiologist (AvdL). Intra-observer and inter-observer reliabilities for microbleed detection were very good.[4]

Antithrombotic drug use

Participants were registered at one or more of seven community pharmacies serving the study area. Of these pharmacies, complete records of all outpatient filled prescriptions in automated format were available as of January 1st, 1991. This included the product name; international non-proprietary name; Anatomical Therapeutic Chemical (ATC) code; total number of delivered units (e.g. tablets/capsules); prescribed daily number of units; date of delivery and drug dosage.

We determined the use (categorized into yes/no) of antithrombotic drugs from January 1st 1991 onwards. We classified antithrombotic drugs based on the ATC system according to pharmacological subgroup into platelet aggregation inhibitors and

anticoagulant drugs. Anticoagulant drugs were furthermore classified according to chemical subgroup (vitamin K antagonists and heparins). Among platelet aggregation inhibitors, we distinguished between aspirin and carbasalate calcium preparations. Carbasalate calcium has a lower risk of gastrointestinal mucosal damage and bleeding than aspirin.[14] Though this bleeding-risk difference is generally attributed to differences in local effects on gastrointestinal mucosa,[14] a recent report postulated that there may be differences in systemic effects between aspirin and carbasalate calcium.[15] We therefore hypothesized that aspirin and carbasalate calcium may differentially relate to microbleed presence. To take into account that aspirin and carbasalate calcium can also be used as pain medication, we concluded whether these drugs were used for inhibition of platelet aggregation on the basis of prescribed dose and regimen. National guidelines dictate the use of aspirin and carbasalate calcium up to 80 mg and 100 mg, respectively, for the purpose of platelet aggregation inhibition.[16] The dosage criterion was overruled only if the regimen clearly indicated the indication of use or if used as loading dose at start of therapy 3.4% of all prescriptions). Dosages of aspirin or carbasalate calcium around 30 or 38 mg, respectively, are generally specifically advised in case of previous cerebrovascular events ('neuro dosage'), whereas dosages around 80 mg to 100 mg, respectively, are considered cardiac dosage'.[16]

Confounding by indication

Associations between drug use and certain outcomes may be confounded by the indication for which the drugs are prescribed. Antithrombotic drugs are usually prescribed in persons at risk for, or with a history of, ischemic cardiovascular or cerebrovascular disease, which in turn can be related to the risk of cerebral microbleeds. We therefore assessed cardiovascular risk factors as potential confounders. Furthermore, we determined whether persons had a known history of cerebrovascular disease. Finally, we recorded the presence of infarcts and the volume of white matter lesions on MRI, as these are known markers of ischemic cerebrovascular disease, and therefore more likely to be influenced by confounding by indication.

Cardiovascular risk factors

For cardiovascular risk factors, we used information that was obtained by interview, laboratory and physical examination at the preceding regular visit of study participants to the research center.[4] We computed the Framingham Risk Score for each participant using age, sex, systolic and diastolic blood pressure, serum total cholesterol, serum HDL cholesterol, presence of diabetes and smoking status.[17]

History of cerebrovascular disease

A known history of cerebrovascular disease was assessed as follows. Upon entry in the Rotterdam Study, history of stroke is assessed.[18] Subsequently, participants are continuously monitored for incident stroke through automated linkage of the study database with files from general practitioners and hospital discharge information. All reported events are validated by an experienced neurologist.[19]

Infarcts and white matter lesions on MRI

Infarcts were rated on FLAIR, PD-weighted and T1-weighted sequences by the two raters who had scored the cerebral microbleeds, as described previously.[20] All infarcts were reviewed in a consensus meeting with an experienced neuroradiologist. For white matter lesion volume quantification, we used a validated tissue classification technique. In brief, we used the k-nearest neighbor classifier[22] to automatically segment MRI scans into grey matter, white matter, CSF and white matter lesions. White matter lesion volumes were calculated by summing all voxels of the white matter lesion-class across the whole brain, to yield volumes in milliliters.

Statistical analysis

We categorized persons based on the location of their microbleeds as described previously.[4] In short, we made a category of persons who had one or more microbleeds restricted to a lobar location (‘strictly lobar’). Persons who had at least one microbleed in a deep or infratentorial brain location were assigned to the category ‘deep or infratentorial microbleeds’.

We analyzed the relation between exclusive use of platelet aggregation inhibitors and anticoagulants prior to MRI and the presence of microbleeds using multiple logistic regressions. We redid these analyses for microbleeds in specific locations (strictly lobar and deep or infratentorial) and in addition studied all separate drug types (aspirin, carbasalate calcium, vitamin K antagonists and heparins). Non-users of antithrombotic drugs served as reference, unless specified otherwise. All analyses were adjusted for age and sex. To adjust for cardiovascular risk without overfitting the model,[23] we additionally adjusted all analyses for the Framingham risk scores. For comparison we also studied the association between antithrombotic drug use and presence of brain infarcts and volume of white matter lesions (dichotomized at the 75th percentile). We redid all analyses excluding persons with a known history of cerebrovascular disease. Furthermore, we tested whether the mean daily dose that was prescribed for aspirin and

carbasalate calcium differed. To control for differences in dosage of these drugs, and thus for possible differences in indication,[16] we performed a direct comparison between users of aspirin or carbasalate calcium who had only been prescribed so-called 'cardiac dosages' (>50 mg/day). All analyses were performed using the statistical software package SPSS, Illinois, USA, version 11.0.1 for Windows.

RESULTS

Population characteristics are shown in Table 1. Mean age of the population was 69.6 years (range 60.7-96.7 years) and 543 (51.5%) were women. There were 363 persons (34.2%) who had used any kind of antithrombotic drug in the years prior to MRI scanning. Of these, 245 persons (23.1%) had exclusively used platelet aggregation inhibitors and 61 (6.3%) had exclusively used anticoagulant drugs. Platelet aggregation inhibitors comprised aspirin (n=67) and carbasalate calcium (n=141). Anticoagulant prescriptions were mainly for vitamin K antagonists (n=51), and only few persons had used heparin (n=5). No prescriptions for other antithrombotic drugs were filled at any of the community pharmacies.

Users of antithrombotic drugs significantly more often had cerebral microbleeds than non users of antithrombotic drugs (Table 2). This was especially true for persons using platelet aggregation inhibiting drugs (age- and sex-adjusted OR for users compared to non-users 1.68; 95% confidence interval (CI) 1.20-2.35). There was no significant association between anticoagulant drug use and presence of microbleeds (Table 2). Use of antithrombotic drugs was also related to presence of brain infarcts and to high white matter lesion volume (Table 2). Additional adjustment for cardiovascular risk did not change any of the associations (Table 2). Exclusion of persons with a known history of cerebrovascular disease (n=36) attenuated the associations between antithrombotic drug use and infarcts or white matter lesions, but did not alter the relation with cerebral microbleeds (data not shown).

When analyzing antithrombotic drug use in relation to microbleed location, we found that aspirin users more often had strictly lobar microbleeds (adjusted OR 2.70; 95%CI 1.45-5.04) than non-users. This was not seen for users of carbasalate calcium (OR for presence of strictly lobar microbleeds 1.16; 95%CI 0.66-2.02); Table 3). In contrast, both aspirin and carbasalate calcium were equally strongly related to presence of deep or infratentorial microbleeds (Table 3). The pattern for infarcts and white matter lesion load was reversed: users of carbasalate calcium more often had infarcts (OR 2.64; 95%CI 1.52-4.95) or high white matter lesion load (OR 1.80; 95%CI 1.16-2.79) than non-users; but this was not true for aspirin users (OR of aspirin users compared to no-users, for infarcts 1.73; 95% CI 0.78-3.83) and for white matter lesions OR 1.01; 95%CI 0.52-1.95). Further investigation of anticoagulant drugs into exposure to vitamin K antagonists versus heparin was less informative due to low numbers of heparin

users; though use of vitamin K antagonists seemed related to deep or infratentorial microbleeds, but not to strictly lobar microbleeds (Table 3).

Table 1. Characteristics of the study population (n=1,062)

Characteristic	
Age, years	69.6 ± 7.2
Women	543 (51.1)
Cerebral microbleeds	250 (23.5)
Strictly lobar	146 (13.7)
Deep or infratentorial*	104 (9.8)
Any use of antithrombotic drugs	363 (34.2)
Exclusive use of platelet aggregation inhibitors	245 (23.1)
Aspirin	67 (6.3)
Carbasalate calcium	141 (13.3)
Exclusive use of anticoagulant drugs	61 (5.9)
Vitamin K antagonists	51 (4.9)
Heparin	5 (0.5)
Systolic blood pressure, mmHg	144.4 ± 18.7
Diastolic blood pressure, mmHg	80.2 ± 10.3
Current smoker	310 (29.7)
Past smoker	445 (42.6)
Diabetes	95 (8.9)
Total serum cholesterol, mmol/l	5.7 ± 1.0
HDL-cholesterol, mmol/l	1.4 ± 0.4
Known history of cerebrovascular disease	36 (3.4)
Infarcts on MRI	119 (11.2)
WML volume, ml†	3.4 (2.0-7.3)

Values are numbers (%) or means ± standard deviation, unless 462 specified otherwise.
* With or without microbleeds in a lobar location.
† Median (interquartile range).
Data missing for: smoking (n=17) and for use of anticoagulant drugs (n=24).

Table 2. Use of platelet inhibitors or anticoagulant drugs and presence of cerebral microbleeds, infarcts and white matter lesions

	Odds ratio (95% CI)					
	Any microbleed (n=250)		Infarct (n=119)		High WML volume† (n=66)	
Antithrombotic use*	**Model I**	**Model II**	**Model I**	**Model II**	**Model I**	**Model II**
No use (n=699)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Any use antithrombotic drugs (n=363)	1.55 (1.14-2.09)	1.56 (1.15-2.12)	2.55 (1.69-3.84)	2.68 (1.77-4.08)	1.69 (1.24-2.31)	1.70 (1.24-2.35)
Platelet aggregation inhibitors (n=245)	1.68 (1.20-2.35)	1.71 (1.21-2.41)	2.31 (1.46-3.67)	2.46 (1.54-3.93)	1.46 (1.02-2.09)	1.49 (1.03-2.15)
Anticoagulant drugs (n=61)	1.47 (0.81-2.67)	1.49 (0.82-2.71)	1.41 (0.59-3.36)	1.43 (0.60-3.42)	1.69 (0.92-3.11)	1.58 (0.85-2.93)
Both platelet aggregation inhibitors and anticoagulant drugs (n=57)	1.10 (0.59-2.08)	1.06 (0.56-2.03)	5.16 (2.72-9.81)	5.46 (2.84-10.5)	3.03 (1.66-5.53)	3.12 (1.69-5.77)

* Exclusive users of given drug categories. Persons with a history of users of more than one type of antithrombotic drug are investigated separately.

† Dichotomized at the 75th percentile.

Model I = adjusted for age & sex.

Model II = adjusted for age, sex and Framingham Risk Score.

Table 3. Use of antithrombotic drugs and presence of cerebral microbleeds according to location

	Odds ratio (95% CI)			
	Strictly lobar microbleeds (n=146)		Deep or infratentorial (n =104)	
Antithrombotic use*	**Model I**	**Model II**	**Model I**	**Model II**
No use (n=699)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Any use antihrombotic drugs (n=363)	1.43 (0.98-2.08)	1.42 (0.97-2.08)	1.72 (1.11-2.64)	1.78 (1.15-2.76)
Platelet aggregation inhibitors (n=245)	1.66 (1.10-2.50)	1.63 (1.08-2.48)	1.69 (1.03-2.75)	1.81 (1.11-2.96)
Aspirin (n=67)	2.67 (1.44-4.97)	2.70 (1.45-5.04)	2.03 (0.92-4.49)	2.10 (0.95-4.66)
Carbasalate calcium (n=141)	1.12 (0.65-1.93)	1.16 (0.66-2.02)	1.62 (0.90-2.89)	1.80 (1.00-3.23)
Anticoagulant drugs (n=61) †	1.37 (0.66-2.87)	1.39 (0.67-2.91)	1.71 (1.04-2.75)	1.75 (0.77-3.97)
Vitamin K-antagonists (n=51)	1.52 (0.68-3.42)	1.53 (0.68-3.45)	2.19 (0.95-5.03)	2.22 (0.96-5.13)
Both platelet aggregation inhibitors and anticoagulant drugs (n=57)	0.59 (0.22-1.58)	0.61 (0.23-1.62)	1.84 (0.86-3.91)	1.72 (0.78-3.76)

* Exclusive users of drug categories. Users of more than one type of antithrombotic are investigated separately.

† Only few cases (n=5) were available for exclusive use of heparin, prohibiting separate investigation of this exposure group.

Model I = adjusted for age & sex.

Model II = adjusted for age, sex and Framingham Risk Score.

Among users of platelet aggregation inhibitors, the mean prescribed daily dose of aspirin was higher than the dose of carbasalate calcium (89.0 mg versus 72.2 mg (equivalent to 57.8 mg aspirin), p< 0.001). This was mainly due to carbasalate calcium being more often prescribed in low doses ('neuro dosage')[16]: 56% of persons using carbasalate calcium had been prescribed at any time a low dose preparation (<50 mg daily dose), versus none of the persons using aspirin. Restricting our analysis to users of high dosages ('cardiac dosage')[16] of either aspirin or carbasalate calcium, we found an even more marked difference between aspirin use and carbasalate use and presence of strictly lobar microbleeds (Table 4), whilst mean prescribed daily dose did no longer differ significantly (89.0 mg for aspirin versus 102.8 mg for carbasalate calcium (equivalent to 82.2 mg aspirin)). This again indicated that aspirin users more often had strictly lobar microbleeds compared to users of carbasalate calcium. Again, this discrepancy was not present for microbleeds in deep or infratentorial locations or for infarcts and high white matter lesion load.

Table 4. Use of cardiac dosage aspirin or carbasalate calcium and cerebral microbleeds, infarcts and white matter lesions

		Odds ratio (95% CI)				
Antithrombotic use		**Any microbleed**	**Strictly lobar microbleeds**	**Deep or infratentorial microbleeds**	**Infarct**	**High WML volume[a]**
Use of carbasalate calcium in "cardiac" dose (n=61)		1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Use of aspirin in "cardiac" dose (n=66)	Model I	2.05 (0.94-4.49)	4.02 (1.34-12.04)	1.00 (0.36-2.76)	1.28 (0.43-3.83)	0.53 (0.22-1.29)
	Model II	1.88 (0.84-4.18)	3.83 (1.25-11.70)	0.95 (0.34-2.68)	1.26 (0.42-3.82)	0.59 (0.24-1.47)

Model I = adjusted for age & sex.
Model II = adjusted for age, sex and Framingham Risk Score.[17]

DISCUSSION

We found that cerebral microbleeds were more prevalent in persons who had used, or were using, platelet aggregation inhibitors. Furthermore, our data show a higher prevalence of strictly lobar microbleeds among aspirin users than among people using carbasalate calcium.

Before discussing the implications of our findings, we need to address some methodological considerations. Firstly, our study was not prospective, limiting our ability to draw conclusions regarding cause and effect. Presence of cerebral microbleeds on MRI does not provide information on when these bleeds actually occurred, as hemosiderin deposits can remain visible in the brain for an undefined period of time. Therefore, it is possible that some of the microbleeds we assessed actually occurred prior to use of antithrombotic drugs. If this occurred, this may have influenced our results. Secondly, there is the issue of confounding by indication. As cerebral microbleeds may be related to presence of cardiovascular or cerebrovascular disease in general, it may be that antithrombotic drugs are more often prescribed to persons with an increased risk to develop microbleeds, confounding our results. We have tried to minimize confounding by indication by adjusting our analyses for cardiovascular risk and furthermore by excluding persons with a known history of cerebrovascular disease from our analyses.

We found that anticoagulant drug use was not significantly related to presence of microbleeds, whilst platelet aggregation inhibitor use was. Lack of statistical significance for anticoagulants can be due to the lower number of anticoagulant users in our population. Alternatively, it could be speculated that this difference can be explained by the different pathways of action on haemostatis of anticoagulant versus antiplatelet drugs. Platelet aggregation inhibiting drugs interfere with the intravascular aggregation of platelets into haemostatic plugs at the site of fissures or ruptures in atherosclerotic plaques or vessel walls.[24] Anticoagulant drugs such as vitamin K antagonists or heparins act on inhibiting the cascade of enzymatic reactions that ultimately stabilize the platelet clot. It may be that microbleed formation is more dependent on the sealing of small vessel wall defects by platelet aggregation than it is on clot stabilization.

We furthermore found a differential relation between use of aspirin or carbasalate calcium and presence of strictly lobar microbleeds, microbleeds which are thought to be indicative of cerebral amyloid angiopathy.[2,4] This difference is not likely caused by confounding by indication as we did not observe such a difference for deep or infratentorial microbleeds or for infarcts and white matter lesions, all of which are known to be more strongly related to vascular risk than strictly lobar microbleeds.[4,25,26] The difference was even more pronounced when comparing persons who had used only the ‘cardiac dosage’ of these drugs, indicating that prescribed doses did not play a role either. Though it is possible that this is a chance finding, it may also be that this difference is caused by an underlying biological mechanism. Replication of this finding

is needed to further evaluate, though we could speculate that aspirin and carbasalate calcium may perhaps differentially affect bleeding-risk or microbleed development in cerebral amyloid angiopathy. Although aspirin and carbasalate calcium have the same active component, acetylsalicylate, (i.e. carbasalate calcium is a complex of calcium acetylsalicylate and urea), it has been described that under certain circumstances bioavailability of aspirin may differ from carbasalate calcium[27] or from other salt-forms of acetylsalicylate[28] and that both drugs may not have similar systemic effects.[15] Hypothetically, a difference in (time to) peak concentration of active acetylsalicylate, or a difference in plasma concentrations of the drug, for example, could cause aspirin to achieve a critical level of inhibition of platelet aggregation and induce leakage of blood from amyloid-laden vessels, whilst this may not occur using carbasalate calcium at similar dosages. Alternatively, it may be that the mode of administration of aspirin and carbasalate calcium, for example in effervescent tablets or enteric-coated tablets, influences the (differences in) plasma concentrations and biological effect.[29] Unfortunately, our data set lacked power to investigate whether mode of administration may have played a role in our observations. Thus, more research is needed to explore the difference in microbleed prevalence we assessed between both chemical substances. There is currently major interest in bleeding-risks of antithrombotic or thrombolytic treatment in persons who have microbleeds on MRI,[30-35] as this may affect clinical management in patients with cardiovascular or cerebrovascular disease. Our data show an association between use of platelet aggregation inhibitors and presence of cerebral microbleeds. The cross-sectional design of our analyses prohibited to investigate whether persons with cerebral microbleeds are at increased risk of symptomatic hemorrhage when using platelet aggregation inhibitors. Of note is that the beneficial effects of well-indicated antithrombotic drugs in persons at risk of myocardial infarction or ischemic cerebrovascular disease should not be disregarded, as these have shown to outweigh any risks of bleeding.[36] Nevertheless, it may be that in selected persons, e.g. those with signs of cerebral amyloid angiopathy, this risk-benefit ratio may differ for certain drugs (e.g. aspirin), thus influencing treatment decisions. The cross-sectional associations between antithrombotic drugs and microbleeds in the general population that we report on would therefore justify further longitudinal research into this relation.

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General discussion

The general objective of this thesis was to study the effects of nonsteroidal anti-inflammatory drugs (NSAIDs) and cardiovascular drugs on neurodegenerative and cerebrovascular disease. In this discussion, the main findings are summarized in the context of recently published evidence. Next, the methodological issues of these studies will be discussed, focusing on specific aspects of pharmacoepidemiological research that may have consequences for an accurate interpretation of our findings. Finally, the potential (clinical) implications of our observations will be discussed and suggestions for future research are given.

MAIN FINDINGS AND INTERPRETATION

NSAIDs, Stroke and Transient Ischemic Attack

In the general population, we found an increased risk of ischemic stroke with current use of any NSAID. The increased risk was observed with current use of COX2-selective and non-selective NSAIDs.[1] Transient ischemic attack (TIA) is an outcome strongly related to ischemic stroke and essentially considered an ischemic stroke of 'transient' nature also caused by focal cerebral or retinal ischemia. Concordantly, we also observed an overall greater risk of TIA with the use of NSAIDs. Our observations are largely in agreement with previous findings from clinical trials and observational investigations which also showed an increased risk with use of NSAIDs of (ischemic) cerebrovascular endpoints.[2,3] The mechanism by which NSAIDs induces thrombotic events is yet unclear. It was initially thought that selective inhibition of COX2 caused a prothrombotic state due to a disturbance in the COX2 and COX1 mediated balance of prostacyclin, which inhibits platelet aggregation, and respectively thromboxane, which facilitates platelet aggregation and acts as a vasocontrictor.[4,5] But, since both COX-enzymes are involved in this vascular homeostasis it could also be that any inhibition of COX could disturb this equilibrium.[6-8] Alternatively, the stroke hazard across COX-classes might be plausibly explained by effects of NSAIDs that are not unique to selective COX2-inhibition, such as increased blood pressure.[9,10] In conclusion, we should be critical of the widespread idea that only COX2-selective NSAIDs increase the risk of cardiovascular and cerebrovascular events.

NSAIDs and Parkinson disease

In the Rotterdam Study we did not find evidence for a protective effect of NSAIDs in Parkinson disease (PD). This was contrary to the hypothesis that NSAIDs may

protect against PD based on the results from human postmortem and animal studies which suggested a role for inflammatory processes in PD pathogenesis.[11] Previous observational studies also found that NSAID use was associated with a reduced risk of subsequent PD.[12,13] Several explanations can be given for the null finding in our study. For instance, it is likely that the effect is related to the duration of exposure. Unfortunately, in our study the relatively low number of exposed incident PD cases resulted in unstable estimates in the category of long-term users of NSAIDs. For the same reason, analyses across subtypes of NSAIDs were not possible, whereas in reality differences among subtypes may exist.[14] It could also be that inflammation is not a cause but a consequence of neurodegeneration in PD. Only recently it was prospectively examined whether human plasma concentrations of inflammatory biomarkers assessed before PD diagnosis were predictive of future risk of the disease.[15] A positive association was observed for interleukin-6 (IL-6), but not for C-reactive protein, fibrinogen, or tumor necrosis factor-α receptors. The specific role of IL-6 in PD pathogenesis is as yet unclear. Furthermore, the lack of associations with other inflammatory biomarkers prevented a solid conclusion about a role of inflammation and PD. These recent findings do not contradict our observations from the Rotterdam Study.

NSAIDs and Alzheimer disease

In Chapter 1.4 we reported that long-term use of amyloid-β42 (Aβ42)-lowering NSAIDs was associated with a decreased risk of AD, whereas this was not observed for non-Aβ42-lowering NSAIDs. Amyloid-β42 protein is the major constituent of the senile plaques, a hallmark of Alzheimer pathology.[16] Our observations suggest that NSAIDs influence the risk of AD through an amyloid-processing related mechanism. These findings could explain why recent trials using non-Aβ42-lowering NSAIDs found no beneficial effect on the prevention of AD[17] and may have important consequences for the development of preventive drugs for AD. Whilst two more recent observational studies and a pooled cohort study again confirmed the protective effect of NSAIDs in AD, they did not provide support for a different effect of Aβ42-lowering and non-Aβ42-lowering NSAIDs in AD.[18-20] The classification of NSAIDs was the same across studies and identical to our classification. However, there were considerable differences in study design, particularly in terms of source of information on drug exposure and exposure definition, which could explain the discrepancies. No clinical trial has tested Aβ42-lowering NSAIDs for the prevention of AD nor has the effect of NSAIDs on Aβ42-load in the human brain been studied.

Statins and Alzheimer disease

We found that use of statins but not of other cholesterol-lowering drugs was associated with a 43% lower risk of AD compared to never use of cholesterol lowering drugs. The protective effect was observed regardless of lipophilicity of statins. The therapeutic benefit of these agents in AD prevention is the topic of much controversy in the literature. Cross-sectional observational studies have suggested an association between the use of cholesterol lowering drugs, particularly statins, and the risk of AD.[21-26] Thus far, prospective studies provided inconsistent evidence for a protective effect of statins on the risk of AD.[23,27-30] Important limitations of these studies, however, included use of interview data on drug exposure or cross-sectional exposure assessment, and short duration of follow-up. Furthermore, it has been hypothesized that only lipophilic statins, that cross the blood-brain-barrier more easily, would affect the risk of AD.[31] Our observation of a protective effect of statins regardless of lipophilicity could be explained by the fact that properties other than lipophilicity also determine their tissue distribution or that passage of the blood-brain-barrier is irrelevant to exert their effect. Randomized controlled clinical trials of cholesterol lowering agents to date have not revealed a reduction in the incidence of dementia in patients on statins.[32,33] However, these studies were not designed to identify dementia or AD. We await the results of primary prevention trials to provide conclusive results as to whether statins reduce the risk of AD.

Antihypertensives and Alzheimer disease

We found that the use of antihypertensive drugs was associated with 8% risk reduction of dementia per year of use for persons below 75 years of age. The effect of antihypertensive drug use on the risk of dementia or AD has been investigated in several prospective observational studies and clinical trials with conflicting results.[34] However, duration of follow-up in these studies was limited and observational studies relied mainly on baseline data on drug exposure without information on duration of use. By using continuous information on antihypertensive use from pharmacy records we limited the chance of potential misclassification of drug exposure. Furthermore, to avoid potential bias in antihypertensive prescription due to prodromal changes in blood pressure[35] or cognition as a result of developing disease, we subtracted a 4-year latent period before the date of dementia diagnosis in the quantification of exposure duration. Our observation of a stronger protective effect for persons below 75 year of age is in line with the view that increased blood pressure earlier in life increases the risk of dementia[36,37], whereas high blood pressure in older persons may not

necessarily put persons at higher risk for dementia.[38,39] Since there were no apparent differences among the various types of antihypertensive drugs, we consider it likely that the class effect of lowering blood pressure underlies the protective effect of these drugs. We conclude that the currently available evidence for a protective effect of antihypertensives on AD constitutes a rather limited basis for advocating a universal antihypertensive treatment policy for the sole purpose of dementia prevention.

Antihypertensives and Progression of White Matter Lesions

In Chapter 2.3 we studied the use of antihypertensives in relation to progression of cerebral white matter lesions (WML) and observed that users of antihypertensives had a lower risk of WML-progression. Cerebral WML are frequently observed on magnetic resonance imaging (MRI) scans in elderly people.[40,41] These lesions are associated with an increased risk of stroke, cognitive decline, and dementia.[42-44] Hypertension is the most recognized risk factor for the presence, severity and progression of WML.[45] We investigated the association between antihypertensive use and WML-progression in a sample of 563 non-demented participants from our cohort who underwent MRI. For 320 persons follow-up MRI measurements were available to asses WML-progression. Compared to non-users, users of antihypertensive had a 50% risk reduction of WML-progression at follow-up, particularly in the age group of 60 to 70 years. For persons older than 70 years of age our analyses were compromised by selective attrition due to which a spurious protective effect could be expected. However, we did not observe a protective effect of antihypertensive users above 75 years of age, which suggests that selective attrition may not have played a major role, if any, in this age group. Furthermore, we found that users of antihypertensives with controlled blood pressure (<160 mmHg) had a lower risk of WML-progression, than persons with elevated blood pressure despite antihypertensive treatment. Control of blood pressure with antihypertensive agents could provide a possible strategy for the prevention of WML progression.

Anticoagulants and cerebral Microbleeds

In Chapter 2.4, we hypothesized that microbleeds, especially those in strictly lobar locations, occur more often in persons using antithrombotic drugs. We studied the association between antithrombotic drug use and presence of cerebral microbleeds in 1,062 persons from the population-based Rotterdam Scan Study. We found that use of antithrombotic drugs, particularly platelet aggregation inhibitors, was related to presence of cerebral microbleeds.

Furthermore, we observed that aspirin users significantly more often had lobar microbleeds than users of carbasalate calcium, suggesting that these drugs may differently relate to presence of strictly lobar microbleeds. Cerebral microbleeds are emerging as a very important topic in neurology research as well as clinical practice; not in the least because their clinical relevance is still largely unknown, yet considered potentially high. In stroke patients, microbleed presence has been associated with an increased risk of stroke recurrence or hemorrhagic transformation of ischemic brain areas.[46] Very little is known on risk factors and prognosis of microbleeds in the general population. Recently, we reported that microbleed prevalence in the general elderly population is as high as 18% in persons aged 60-70 years, and increases up to 38% in those over the age of 80.[47] There is accumulating evidence that microbleeds in strictly lobar brain locations are indicative of cerebral amyloid angiopathy,[48] a bleeding prone state, in which antithrombotic drug use was shown to increase the risk of symptomatic brain hemorrhage.[49,50] In parallel, the development of asymptomatic lobar microbleeds in these persons may perhaps also be accelerated by use of antithrombotic drugs. The cross-sectional nature of this final study does not allow for the assessment of a temporal relationship between drug exposure and occurrence of microbleeds. Also, confounding-by-indication is likely to have played a role since persons with cardio-vascular disease are more likely to have microbleeds and, at the same time, are also more prone to receive antithrombotic treatment. Therefore, our findings should be regarded as exploratory and confirmation by prospective studies is needed.

Methodological considerations

Experimental clinical research is the corner stone for studying benefit and harm of drug therapy. However, for ethical and practical reasons, experimental clinical research cannot always be employed and observational research may provide an alternative and, in certain instances, better tool. In contrast to experimental clinical research, observational research draws inferences about the effect of a treatment in a population where the assignment of subjects into a treated versus a control group is outside the control of the investigator.[51,52] Treatment selection is done on the basis of clinical need or preference, which can result in differences in clinical outcomes solely because of differences between those who do and do not receive treatment. Major challenges in conducting observational research are therefore to draw inferences that are acceptably free from influences from these biases, but also, in first instance, to assess and define drug exposure.[53,54]

The studies described in this thesis are based on the prospective cohort of the Rotterdam Study.[55] Methodological strengths include its general population based setting, the large number of participants and long-duration of follow-up. A particular asset of the Rotterdam Study cohort in the context of the current thesis is the availability of information on drug exposure from community pharmacy records. From January 1st 1991 onwards we had detailed information on all dispensed drugs in automated format, collected irrespective and independent of the outcome under study. The source, methods of collection and completeness makes information and selection bias for these data unlikely. Also, pharmacy derived data on drug exposure considerably reduces the chance of exposure misclassification as opposed to studies in which (self-reported) baseline drug exposure data, periodical assessment of drug exposure or prescription records are used.[56] Furthermore, the filled prescription data provided us with information on drug use at each point in time during follow-up. This is important information because, unlike constantly present determinants such as gender or history of stroke, exposure to drugs changes constantly over time due to initiation, stopping and switching of medication. By using a Cox-regression model with time-dependent variables for our prospective analyses we were able to take changes in exposure status into account.[57] In addition, this model allowed us to investigate timing of drug use in relation to the occurrence of the event, i.e. the 'risk-window'. An appropriate 'risk-window' would capture the relevant period of increased or decreased risk of an outcome following an exposure.[58,59] The 'risk-window' depends on the pharmacological effect of the drug and on the induction and latency period of the disease under study. Selection of an appropriate risk-window results in exposure misclassification and thereby underestimation of the true association. In our first report we studied the effect of NSAIDs on stroke, for which inhibition of COX-enzymes appears relevant. The effect of NSAIDs on the COX-enzymes is considered reversible. With cessation of the drug, the effect on COX-subsides as the drug is cleared from the body and the physiological balance of COX-mediated processes re-establishes. Since stroke is also of acute onset, a causal relationship is therefore only plausible if the event occurs during periods of drug intake or shortly after cessation of the drug. This is in contrast to the effect of NSAIDs on amyloid-β42 processing where every period of drug use is thought to impede the accumulation of amyloid-β42-protein and adds up to a sum of period of protection, delaying the onset of AD. In this study we thus calculated cumulative use during a theoretical life-time risk-window as exposure. Yet other considerations for the exposure definition had to be born in mind when we investigated the associations between both statins and antihypertensive on the risk of AD. As with the effect of

NSAIDs on COX, the main effects of these drugs on human physiology, i.e. decreasing blood pressure and cholesterol reduction respectively, are not considered permanent. Nevertheless, if during each period of use the process to disease is halted or slowed, users of these drugs will have a lower risk of disease than non-users, despite the fact that they may not use continuously. Hence, in these studies our main analyses pertained to cumulative use. However, in subanalyses we excluded persons who did not refill their prescription in certain time-periods prior to diagnosis to take into account that the risk may return to the background incidence rate after cessation of drug use.

Despite the many advantages of pharmacy prescription records to determine drug exposure, assumptions are still required. For example, if two of more prescriptions for similar medications overlap and it is unlikely that a person would use these drugs simultaneously, one has to assume discontinuation of one of the drugs. These assumptions may not always represent the actual situation of drug use. Furthermore, pharmacy data on filled prescriptions do not provide the guarantee that a drug is actually taken. Drug compliance is a general issue in efficacy research and influenced by many factors. Compliance can differ from person to person, but also depends on the drug itself (adverse effects, ease of administration and regimen) making it difficult to predict the magnitude of non-compliance. By varying exposure definitions we attempted to determine whether and how this affected our findings. Fortunately, for chronically taken medication timely refill at the pharmacy mostly means that the patient is compliant. For incidental medication, such compliance checks can not be performed with pharmacy data. This applies to NSAIDs, which besides being chronically used, such as for arthritis, are also used incidentally. Another limitation is that pharmacy registrations do not include ‘over-the-counter’ (OTC) drugs, i.e. drugs for which prescriptions are not required. If investigating prescription drugs that are (also) available as OTC drugs, like NSAIDs and aspirin, this can lead to misclassification of exposure. Exposed persons can be incorrectly classified as unexposed, or the true cumulative duration of exposure may be underestimated. During most of our study periods, OTC available medication was reimbursed in the Netherlands if prescribed for chronic conditions and it is thus safe to assume that outside of chronic indications, OTC drugs are used incidentally. Under this assumption, lack of information on OTC drug use may have limited consequences for studies were long-term use is required for the drug to have an effect, such as in our study of NSAIDs and AD. However, this may have implications for the acute effect of NSAIDs in stroke. There we may have randomly missed exposure in some of the cases and non-cases and thereby caused an underestimation of the true risk.

In non-experimental research the allocation of treatment is by definition not random.[54] If drug prescription is rational and, for the sake of argument we ignore undertreatment, the indication of the drug will only be present in treated persons and not in non-treated persons. Unavoidably, this results in an imbalance in prognostic factors between those with and without the indication for treatment. Two forms of bias that typically originate from the indication of drug use are confounding-by-indication (or contra-indication) and protopathic bias.[60] Confounding-by-indication occurs if the indication of the drug under investigation is also an independent risk factor for the outcome. Essentially, the conceptual basis of our studies on antihypertensives and cholesterol-lowering drugs and risk of AD and WML progression lies in the association between the indication of these drugs and the outcomes, as both hypertension and cholesterol-levels are thought to be related to the outcomes under investigation. Since in these associations confounding-by-indication leads to increased risk estimates and because we found a risk reduction, we apparently underestimated the true protective effect. Contrary, in our study the effect of confounding-by-indication on anticoagulants and risk of microbleeds could lead to spuriously increased risk estimates, since users of anticoagulants are at risk for vascular disease, which in turns increases the risk of microbleeds. To minimize confounding-by-indication, we adjusted our analyses for the Framingham risk score and furthermore by excluding persons with a known history of cerebrovascular disease from our analyses. For our studies on NSAIDs and AD or PD confounding-by-indication is less likely to have played a role since the indications of pain or joint disease are generally not considered risk factors for AD or PD. However, during the prodromal phase of these diseases preferential prescribing or change in drug use behavior may have occurred. If, like in these studies, not the drug's indication acts as a confounder but the early symptoms of the outcome is the reason for prescribing the drug, this bias is referred to as 'protopathic bias'. To avoid protopathic bias in our studies on AD and PD we subtracted a four year period before the date of diagnosis from the potential period for exposure assessment to ensure that changes in drug use patterns due to preclinical signs of the disease are not erroneously associated with the disease of interest .[61] Finally, we must consider the possibility that the indication of a drug can give rise to selection bias if the use of the drug of interest differs among study participants and non-participants at baseline or follow-up. In our cross-sectional study on antithrombotics and microbleeds we anticipate that the response rate among users of antithrombotics is lower than among non-users. Again, since antithrombitic use is related to cardiovascular morbidity, which in turn is related to microbleeds, this may have biased our estimates. The magnitude of this bias is, however, difficult to predict since no imaging data are

available for these participants. The same holds for our study on antihypertensives and WML-progression, where we showed that the non-response observed in the older age groups at follow-up was related to the drug under investigation. However, this did not result in a spurious protective effect as we would have expected.

(Clinical) implications

In general, the extent to which our findings may have (clinical) implications depends in part on the direction of the observed associations, i.e. benefit or harm. In this thesis, the drugs for which we observed a potential beneficial effect on neurological disease have all been studied in relation to outcomes outside their currently registered indications and clinical use. For drugs to be imbedded in clinical practice guidelines, registration for these indications is essential. However, the registration of a drug for a particular indication is a time-consuming and intensive process and, by legislation, generally requires evidence from randomized clinical trials. Hence, the direct clinical implications of these findings will be limited, at least on the short-term. For the drugs that we found to have adverse effects on neurodegenerative and cerebrovascular disease, the immediate clinical relevance is more obvious.

We showed that the use of non-selective and COX2-selective NSAIDs increases the risk of stroke. In response to the previous reports on the cardiovascular risks of COX2-selective NSAIDs certain measures have already been put into effect by national and international authorities to prevent future events. This involved changes in drug labeling and 'dear health care professional letters', which have been less stringent for non-selective NSAIDs than for COX-selective NSAIDs.[2,62] Our data, and those of others, suggest that with respect to cardiovascular risk, similar labels are appropriate for all NSAIDs. Also, OTC availability of NSAIDs may need reconsideration.

Besides the potential harm of NSAIDs we also observed that a certain subgroup, namely Aβ42-lowering NSAIDs, seems to reduce the risk of AD. However, the knowledge regarding the thrombotic risk of NSAIDs and their risk of gastro-intestinal bleeding makes it very unlikely that they will be of clinical importance in the prevention of AD. Likewise, this also limits the feasibility of future clinical trials of NSAIDs in AD or experimental studies in humans. Questions, such as whether and how NSAIDs affect amyloid-β in humans may thus not easily be answered in experimental settings. However, our findings do provide an indication for future drug development that therapeutics targeted at Aβ42 can be worthwhile.

We found that both statins and antihypertensives may protect against AD. Currently, both drugs are extensively used in vascular risk management. Based on our findings, vascular risk management could also be viewed in the light of preserving 'neurological health'. The question, of course, remains if these drugs are also of value to AD prevention outside their current indication areas and if specific patient groups could be recognized who would benefit most. Our study on antihypertensives and WML-progression suggests that presence of WML could prove to be one of the indication areas of interest.

The final report in this thesis actually resulted, in part, from clinical questions regarding antithrombotic treatment in persons with microbleeds on MRI. Our finding of a higher prevalence of cerebral microbleeds among users of platelet aggregation inhibitors has no direct consequence for clinical practice yet. One, because of the cross-sectional nature of the study does not allow for causal inference. Two, because the beneficial effects of well-indicated antithrombotic drugs in persons at risk of myocardial infarction or ischemic cerebrovascular disease have thus far shown to outweigh any risks of bleeding.

FUTURE RESEARCH

Despite the substantial re-evaluations and continuing flow of evidence from clinical and epidemiological studies regarding the thrombotic risk of NSAIDs, various issues remain which hamper adequate treatment and regulatory decision making. Among others, this concerns the lack of exact knowledge about the risk of each individual NSAID for the various thrombotic events, particularly for cerebrovascular events. Also, we should gain better insight into dose-dependency and timing of risk and whether certain patient groups are at higher risk than others. Unraveling these details will require large population numbers not usually available in single medical databases or from prospective cohort studies. In general, the late recognition of the cardiovascular risk associated with NSAID use also raised concerns as to whether current pharmacovigilance systems, which are based on spontaneous reporting from physicians and patients, are able to detect adverse events rapidly enough. Current initiatives to combine population databases to attain a more rapid detection system of adverse drug reactions are in progress. At the same time, these extensive databases can also be deployed for more detailed investigation of the aforementioned issues on

the cardiovascular safety of NSAIDs. The feasibility of such studies will rely on the available data to control for confounding, the outcomes of interest and, foremost, timing and details on drug exposure.

Consensus is growing that Aβ-peptide has a role in AD pathogenesis. Our work on Aβ42-lowering NSAIDs and risk of AD again provides indirect support hereof. Pharmaceutical industries are already pursuing a variety of other amyloid-based therapeutics for the prevention and treatment of AD.[63,64] Only few of the newly developed compounds have reached the stage of human testing and, unfortunately, success has yet failed to materialize. One of these compounds under study for the prevention of AD progression, and of particular relevance to our study, concerns the R-enantiomer of the NSAID flurbiprofen. R-flurbiprofen is a potent reducer of amyloid-β levels but without COX-inhibiting properties.[65] Initial results based from a Phase III trial looked promising, but the investigators recently concluded that the drug had no beneficial effects on AD progression.[66] Though lack of effect on progression of AD would not exclude efficacy in the prevention of AD, the development of the drug was nevertheless halted. Another approach that had reached the clinical trail stage comprised that of passive or active immunization with amyloid antibodies.[67] Preliminary results showed that patients with a higher antibody titer had a slowing of the progression of cognitive loss. However, the program was discontinued prematurely after signs of aseptic meningoencephalitis developed in some treated patients.[68] Despite these setbacks, hopes for amyloid directed therapy in AD are high. Future research directed to unravel the AD pathology, will be necessary to clarify whether amyloid-β remains a rational target for future drug development in AD.

Though our studies showed that there may be a role for statins and antihypertensives in the prevention of AD, there is still limited evidence available from primary prevention trials. Most trials performed to date did not include a systematic clinical cognitive assessment or did not have sufficient power to detect a beneficial effect on AD. Also, in the view of health care cost-containment it may not be a very attractive outlook to expand the, already substantial, number of persons eligible for cholesterol lowering or antihypertensive treatment. This would lead to considerable increase in health care costs.[69,70] Therefore, treatment with these drugs for the sole purpose of AD prevention would only by attainable if not merely proven effective, but also cost-effective.[71] Also, if further primary prevention trials were to be conducted we should first focus on patient groups who are already eligible for treatment with these drugs or who at greater risk

of developing AD. Moreover, we could gain power and efficiency by using biologic and imaging markers that predict future risk of disease for the recruitment of participants as well as outcome parameters.[72]

We described that antihypertensive use may prevent progression of WML. WML progression represents one of the most convincing and readily measurable markers of progressing vascular brain disease. WML changes are objective and quantifiable as compared to the more subjective clinical diagnosis of subsequent disease. Furthermore, WML changes can be detected early in the disease process and thus shorten the duration of follow-up which in turn is expected to reduce selection bias. The European Task Force on Age-Related White Matter Changes[73] has suggested that trials on vascular brain disease may use lesion load progression as a secondary outcome variable to evaluate therapeutic effects.[74,75] Thus far, this endpoint has been adopted in several trials, including two trials on antihypertensives.[76,77]

These trials provide the initial insights into the potential role of these therapeutic agents in prevention progression of WML. However, because these trials allowed for usual antihypertensive treatment in both treatment and control arms, it remains unclear whether the beneficial effect of antihypertensive treatment on WML progression was due to a stronger reduction in blood pressure in the treatment arms or whether some agents may provide (more) beneficial effects above others. Placebo-controlled studies of antihypertensives on WML-progression are thus needed to provide conclusive evidence. Clinical trials may also overcome some methodological issues encountered in current observational research investigating this association, such as confounding-by-indication and selective attrition of study participants.

The cross-sectional investigation of antithrombotic drugs and microbleeds that we report on showed that, in the general population, cerebral microbleeds were more prevalent in persons who had used, or were using, antithrombotic drugs. In view of broadening indications for antithrombotic medication in prevention of ischemic disease, we feel that our results justify further longitudinal research into this association.

The research presented in this thesis illustrates that observational research on the effect of drugs on disease can also be used to provide us with clues on the pathway to disease which in turn can provide a basis for further basic scientific or clinical research.

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Summary

The aging global population will create an unsurpassed burden of the age-related neurological conditions such as Alzheimer disease (AD), Parkinson disease (PD) and stroke. Despite intensive research in the last decades no effective therapy for the prevention or treatment of these disorders is currently available. On the basis of previously proposed risk factors and current knowledge of pathogenesis, we sought to investigate two drug groups as determinants of neurodegenerative and cerebrovascular disease and of potential imaging markers of these disorders. In **Chapter 1** nonsteroidal anti-inflammatory drugs (NSAIDs) are studied in relation to the outcomes of stroke, TIA, PD and AD. NSAIDs are among the most widely used drugs by elderly persons, mainly employed for their anti-inflammatory and analgesic effects. In **Chapter 2** we attend to cardiovascular medication as determinant of AD, and two markers of vascular brain disease as seen on magnetic resonance imaging scans (MRI), namely white matter lesion (WML) progression and cerebral microbleeds. We considered three chronically used cardiovascular drugs which are all essential drugs in vascular risk management, namely antihypertensives, cholesterol-lowering drugs and anti-thrombotics. The studies presented in this thesis are based on the prospective population-based Rotterdam Study which was initiated in 1990-1993 in 7,983 persons of 55 years and over, living in a well-defined suburb of Rotterdam, the Netherlands. Our final study also included a randomly selected sample from the first expansion of the Rotterdam Study cohort of persons 60 years and older. We had detailed information on prescription drug exposure from local automated pharmacy records from 1991 and during the complete follow-up.

Following the previous reports from clinical and observational research on the increased risk of thrombotic events with cyclooxygenase (COX)-2 selective NSAIDs we first examined the relation between NSAID use and stroke in **Chapter 1.1**. NSAIDs were grouped according to their relative selectivity for the two isoforms of cyclooxygenase-enzymes as COX1-selective, non-selective and COX2-selective NSAIDs. We found an increased risk of stroke with current use non-selective and COX2-selective NSAIDs at time of the event. Using the same approach we also investigated the association between NSAID use and risk of TIA. Like for stroke, we found an increased risk of TIA with current use of NSAIDs that appeared not to be restricted to COX2-selective NSAIDs (**Chapter 1.2**). Our data contests the notion that the risk of thrombotic events, including risk of ischemic cerebrovascular events, is not restricted to the use of COX2-selective NSAIDs.

There is growing evidence for a role of inflammation in PD pathogenesis. Hence, it was hypothesized that NSAIDs may have a beneficial effect on PD risk. In **Chapter 1.3** we investigated whether NSAID use reduced the risk of PD. In this study we found no significant beneficial effect of NSAID use on the risk of PD. A drawback of this study was the limited power due to low case numbers. Consequently we were unable to investigate the effects of long-term use of these drugs or differences across subtypes of NSAIDs. These kinds of subanalyses are potentially interesting, as the protective effect may depend on the duration of use or differ across NSAIDs subtypes.

A leading hypothesis regarding AD pathogenesis postulates a central role for amyloid-β42 (Aβ42) peptide, the major constituent of senile plaques observed in the AD diseased brain. Several lines of evidence suggest that certain NSAIDs interfere with Aβ42-processing and ultimately lower Aβ42 levels. In **Chapter 1.4** we report that the reduced risk of AD associated with long-term use of NSAIDs was confined to the subgroup of NSAIDs that interfere with Aβ42-processing. Our observation corroborates *in vitro* and animal studies which suggest that this group of NSAIDs influence the risk of AD through an amyloid-processing related mechanism, rather than an inflammatory effect. Moreover, these data shed new light on the interpretation of recent trials for no beneficial effect of long-term treatment with NSAIDs on AD whilst the trial included only non-Aβ42-lowering NSAIDs. Further investigation of the role of Aβ42 in AD pathogenesis is of importance, as it could prove a target for development of preventive treatment strategies in AD.

Evidence from observational studies has suggested that cholesterol-lowering agents, including statins, may prevent against AD. High cholesterol-levels have been associated with AD risk as well. However, brain cholesterol is synthesized *in situ* and exchange over the blood-brain-barrier (BBB) is limited. Hence, it was suggested that only lipophilic statins, those that cross the BBB, would affect brain cholesterol. In **Chapter 2.1** we showed that the protective effect of statins was observed irrespective of their lipophilicity. Many other factors besides lipid solubility determine a drug's distribution in different tissues, which may explain our findings. It may also be that brain penetration is not essential for statins to exert their effect on AD pathology.

Increased blood pressure has also shown to be a risk factor for the development of AD. Therefore, we investigate in **Chapter 2.2** if the use of antihypertensives would conversely reduce the risk of AD. We found that the use of antihypertensive drugs was

associated with 8% risk reduction of dementia per year of use for persons below 75 years of age. This is in line with the view with the view that increased blood pressure earlier in life increases the risk of dementia, whereas high blood pressure in older persons may not necessarily put persons at higher risk for dementia.

Apart from the association between antihypertensives and AD, we also examined whether antihypertensive use would reduce the risk of WML-progression (**Chapter 2.3**). WML are established imaging markers of vascular brain disease, which have been related to an increased risk of stroke and dementia. Hypertension has been identified as a well known risk factor for the presence of WML and a potential target for treatment strategies. In a subset of 320 persons from the Rotterdam Study with follow-up MRI data, we found that users of antihypertensive use prior to baseline MRI had a reduced risk of WML progression at follow-up in persons younger than 70 years of age or with controlled blood pressure. Above 70 years of age, no benefit of antihypertensives was observed and our findings were subject to selective loss-to-follow-up.

For our final study on antithrombotics and presence of microbleeds, described in **Chapter 2.4,** we also included a random sample from the first extension of the original Rotterdam Study of persons aged 60 years and over. By means of a cross-sectional study design we determined whether the use of antithrombotic drugs, between start of the Rotterdam Study in 1991 and first MRI scan (2005-2006), was related to presence of cerebral microbleeds. We show that use of antithrombotic drugs is related to a higher prevalence of cerebral microbleeds, thereby confirming our a priori hypothesis. The possible implications of our findings to clinical management in patients with cardiovascular or cerebrovascular disease, justifies longitudinal research into this relation.

The research presented in this thesis illustrates that observational research on the effect of drugs on disease can be used to provide us with clues on the pathway to disease. This in turn can provide a basis for further basic scientific or clinical research and the development of new drugs.

Samenvatting

De ‘vergrijzing’ van de wereldbevolking zal een ongekende toename in ziektelast van ouderdomsgerelateerde neurologische en cerebrovasculaire aandoeningen zoals de ziekte van Alzheimer (AD), de ziekte van Parkinson (PD) en beroerte tot gevolg hebben. Ondanks intensief onderzoek in de laatste decennia is tot op heden geen preventie van deze ziekten mogelijk of effectieve behandeling voorhanden. Op basis van eerder geïdentificeerde risicofactoren en kennis van de pathogenese, hebben wij twee groepen geneesmiddelen onderzocht als mogelijke determinanten van neurodegeneratieve en cerebrovasculaire aandoeningen en daaraan verwante afwijkingen in het brein zoals gezien op magnetic resonance imaging (MRI). **Hoofdstuk 1** omvat het onderzoek naar non-steroïde anti-inflammatoire geneesmiddelen (NSAIDs) in relatie tot beroerte, transient ischemic attack (TIA), PD en AD. NSAIDs behoren tot een van de meest gebruikte geneesmiddelen onder ouderen, voornamelijk toegepast bij bewegingsapparaat gerelateerde pijn en vanwege hun anti-inflammatoire effecten. In **Hoofdstuk 2** besteden we aandacht aan cardiovasculaire medicatie in relatie tot AD en twee markers van vasculaire schade in het brein, namelijk wittestofafwijkingen (WML) en cerebrale micro-bloedingen zoals waargenomen op MRI scans. We richten ons op drie chronisch gebruikte cardiovasculaire middelen die veelal toegepast worden in vasculair risicomanagment, namelijk antihypertensiva, cholesterolverlagers en antitrombotica. De studies in dit proefschrift zijn uitgevoerd binnen het in 1990-1993 gestartte prospectieve Erasmus Rotterdam Gezondheid Onderzoek (ERGO/Rotterdam Study) onder 7,983 mannen en vrouwen uit de algemene bevolking van 55 jaar en ouder, woonachtig in de Rotterdamse wijk Ommoord en, voor onze laatste studie, een willekeurige sample van de eerste uitbreiding van dit cohort onder mensen van 60 jaar en ouder. Gedetailleerde informatie van voorgeschreven geneesmiddelen uit geautomatiseerde gegevens van de lokale openbare apotheken was vanaf 1991 en gedurende de complete looptijd beschikbaar.

In navolging van eerdere publicaties over een verhoogd risico van thrombotische accidenten met het gebruik van cyclooxygenase (COX)-2 selectieve NSAIDs, in klinisch en observationeel onderzoek, onderzochten wij in **Hoofdstuk 1.1** als eerste de relatie tussen NSAID gebruik en beroerte. NSAIDs werden aan de hand van hun relatieve selectiviteit voor beide isovormen van het COX-enzym gegroepeerd in COX1-selectieve, niet-COX-selectieve en COX2-selectieve NSAIDs. We vonden een verhoogd risico van beroerte voor personen die ten tijde van het optreden van de beroerte gebruikers waren van niet-selectieve en COX2-selectieve NSAIDs. Op dezelfde wijze onderzochten wij tevens de relatie tussen NSAIDs en het optreden van TIA in Hoofdstuk

1.2. Net als voor beroerte hadden gebruikers van NSAIDs een verhoogd risico op TIA. Dit verhoogde risico leek eveneens niet beperkt te zijn tot COX2-selectieve NSAIDs. Onze bevindingen spreken de huidige opvattingen tegen dat het verhoogde risico van trombo-embolische incidenten, inclusief ischemische cerebrovasculaire incidenten, beperkt is tot de COX2-selectieve NSAIDs.

Er zijn meer en meer aanwijzingen dat ontsteking een rol speelt in de pathogenese van de ziekte van Parkinson (PD). Derhalve bestond de hypothese dat NSAIDs mogelijk het ontstaan van PD zouden kunnen voorkomen. In **Hoofdstuk 1.3** gingen wij na of NSAID gebruik het risico van PD zou verlagen. In dit onderzoek vonden we geen beschermend effect van NSAIDs bij PD. De studie werd beperkt door het kleine aantal personen met PD. Hierdoor was nader onderzoek, naar de lange termijn effecten van NSAIDs of verschillen tussen de diverse NSAIDs, niet mogelijk. Deze analyses zijn mogelijk interessant aangezien het beschermende effect af kan hangen van duur of het type NSAID.

Een vooraanstaande hypothese met betrekking tot Alzheimer pathogenese stelt een centrale rol voor het amyloïd-β42-eiwit. Amyloid-β42 (Aβ42) is het voornaamste bestandeel van de seniele plaques kenmerkend voor de hersenen van AD patiënten. Resultaten van verschillende onderzoeksgebieden suggereren dat bepaalde NSAIDs interfereren met de amyloïd homeostase en de concentraties van dit eiwit verlagen. De resultaten van **Hoofdstuk 1.4** laten zien dat het beschermende effect van langdurig gebruik van NSAIDs in AD beperkt is tot de NSAIDs met een Aβ42 verlagend effect. Onze bevindingen sluiten aan bij eerdere aanwijzingen uit *in vitro* en diermodel studies waaruit bleek dat deze groep NSAIDs het risico van AD middels een aan amyloïd gerelateerd proces beïnvloedden, in plaats van een via een anti-inflammatoir effect. De resultaten geven bovendien een andere kijk op de resultaten van recent klinisch onderzoek waarin werd geconcludeerd dat langdurig gebruik van niet-Aβ42 verlagende NSAIDs niet zou beschermen tegen AD. Additioneel bewijs voor een rol van Aβ42 in AD is belangrijk, aangezien het een interessant aanknopingspunt kan zijn voor de ontwikkeling van preventieve behandelingen voor AD.

Uit eerder verricht observationeel onderzoek is gebleken dat cholesterolverlagers, waaronder statines, het risico op AD zouden kunnen verlagen. Tevens is er een verband aangetoond tussen concentraties van cholesterol en het risico van AD. Echter, de synthese van brein cholesterol vindt vrijwel geheel *in situ* plaats en cholesterol wordt

nauwelijks over de bloed-hersen-barriere (BHB) uitgewisseld. Daarom werd gedacht dat alleen lipofiele statins, degene die de BHB makkelijker zouden passeren, een effect zouden hebben op cholesterol in het centrale zenuwstelsel. In **Hoofdstuk 2.1** laten we zien dat statines het risico op AD verlagen, maar dat dit onafhankelijk is van de lipofiliteit van het statine. Naast dat veel andere factoren de verdeling van statines in verschillende weefsel bepaalt, kan het ook zo zijn dat passage van de BHB niet essentieel is voor statines om een effect te kunnen hebben op AD pathogenese hetgeen onze bevindingen zou kunnen verklaren.

Net als cholesterol, is een verhoogde bloeddruk ook in verband gebracht met het risico op dementie. Daarom onderzochten wij in **Hoofdstuk 2.2** of het gebruik van antihypertensiva het risico op dementie kon verlagen. Wij vonden dat het gebruik van antihypertensiva geassocieerd was met een 8% reductie van het risico op dementie per jaar gebruik bij personen van 75 jaar en jonger. Dit is in lijn met eerdere bevindingen die suggereren dat met name verhoogde bloeddruk op jongere leeftijd geassocieerd is met een verhoogd risico op dementie, terwijl op latere leeftijd het mogelijk andersom geldt.

Naast het verband tussen antihypertensiva en het risico op AD bekeken wij tevens, in **Hoofdstuk 2.3**, of het gebruik van antihypertensiva het risico op progressie van cerebrale wittestofafwijkingen kon verlagen. Wittestofafwijkingen zijn erkende markers van vasculair hersenlijden en zijn in verband gebracht met een verhoogd risico op beroerte en dementie. Hypertensie is een bekende risicofactor voor de aanwezigheid en progressie van wittestofafwijkingen en daarmee een potentieel aanknopingspunt voor therapeutische preventie. In een subset van de ERGO onderzoeksgroep met MRI-scan data vonden wij dat gebruik van antihypertensiva in de periode tussen 1991 en de eerste MRI-scan (1995-1996) minder kans hadden op progressie van wittestofafwijkingen bij personen jonger dan 70 jaar of met een gecontroleerde bloeddruk. Bij personen ouder dan 70 jaar bleken antihypertensiva de progressie van wittestofafwijkingen niet te voorkomen en werd meer non-respons geconstateerd onder gebruikers van antihypertensiva dan niet gebruikers hetgeen onze resultaten kan hebben beïnvloed.

Onze laatste studie in dit proefschrift, beschreven in **Hoofdstuk 2.4**, is gebaseerd op een willekeurig geselecteerde subpopulatie van deelnemers van het originele ERGO cohort en een uitbreiding van het cohort onder personen van 60 jaar en ouder. Middels een cross-sectionele studie hebben wij in deze populatie onderzocht of gebruik van

antitrombotica, tussen aanvang van ERGO (1991-1994) en de eerste MRI scan in (2005-2006), gerelateerd was aan de aanwezigheid van cerebrale microbloedingen. We vonden dat gebruikers van plaatjes aggregatie remmers, middelen die de bloedstolselvorming remmen, een verhoogd risico hadden op deze microbloedingen hetgeen onze a priori hypothese bevestigde. Dezelfde relatie vonden wij niet voor anticogulantia, middelen die de stabilisatie van het stolsel tegengaan. Daarnaast zou er een verschil kunnen bestaan tussen de relatie van aspirine enerzijds en carbasalaat calcium anderzijds tot strikt lobaire microbloedingen. De mogelijke implicaties van deze bevindingen voor klinische besluitvorming bij patiënten met cardiovasculair en cerebrovasculair lijden illustreert de noodzaak voor verder longitudinaal onderzoek naar deze associatie.

Het onderzoek gepresenteerd in dit proefschrift illustreert dat observationeel onderzoek naar de effecten van geneesmiddelen op een ziekte belangrijke aanwijzingen kan geven over een ziekteproces en een basis vormen voor verder basaal wetenschappelijk of klinisch onderzoek en ontwikkeling van nieuwe geneesmiddelen.

ABOUT THE AUTHOR

Mendel Haag was born on July 13th, 1976 in Amsterdam, the Netherlands. In 1994 she graduated from the Montessori Lyceum, Rotterdam. She studied Pharmacy at the University of Utrecht, were she obtained her doctoral in 2000 and her pharmacist degree in 2002. During her studies she performed a research project in clinical chemistry at the Canadian Reference Laboratory (currently CEQAL) directed by Prof. D.W. Seccombe. As part of her internships she spent 3 months at the Groote Schuur Hospital in Cape Town, South-Africa. After a short period in public pharmacy, she joined Pfizer bv as Outcomes Research Associate in 2003. In 2005 she started the work presented in this thesis with the neuroepidemiology (Prof. M.M.B. Breteler) and the pharmacoepidemiology (Prof. B.H.C. Stricker) group at the Department of Epidemiology, Erasmus MC, Rotterdam. In 2007, she obtained her Masters degree in Clinical Epidemiology at the Netherlands Institute for Health Sciences. As of November 2008 she holds a position as a post-doc researcher with the Integrated Primary Care Information at the Department of Medical Informatics, Erasmus MC, Rotterdam.
She lives together with her partner Kilian and their son, Zeger, born April 2007, in Amsterdam.

PHD PORTFOLIO

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Erasmus MC Department	Epidemiology
Research School	Netherlands Institute for Health Sciences
PhD period	2005-2008
Advisors	Prof. M.M.B. Breteler and Prof. B.H.C. Stricker

1. PHD TRAINING

Research skills

Statistics and methodology

2005-2007 Masters of Science in Clinical Epidemiology, Netherlands Institute for Health Sciences, Erasmus University, Rotterdam, the Netherlands. 70 ECTS

Presentations

Oral presentations

2007 COX-Selectivity of Nonsteroidal Antiinflammatory Drugs and the Risk of Stroke, 23rd International Conference on Pharmacoepidemiology and Therapeutic Risk in Montreal, Canada. 0.5 ECTS

2006 Amyloid-β42-Level Lowering Nonsteroidal Anti-Inflammatory Drugs and the Risk of Alzheimer's Disease, 10th International Conference on Alzheimer's Disease and Related Disorders in Madrid, Spain. 0.5 ECTS

2006 Amyloid-β42-Level Lowering Nonsteroidal Anti-Inflammatory Drugs and the Risk of Alzheimer's Disease, 22nd International Conference on Pharmacoepidemiology and Therapeutic Risk in Lisbon, Portugal. 0.5 ECTS

Poster presentations

2008 Antihypertensives and White Matter Lesion progression, a population-based cohort study. 24th International Conference on Pharmacoepidemiology and Therapeutic Risk in Copenhagen, Denmark, August. 0.5 ECTS

International conferences

2008 24th International Conference on Pharmacoepidemiology and Therapeutic Risk in Copenhagen, Denmark. 0.8 ECTS

2007 23rd International Conference on Pharmacoepidemiology and Therapeutic Risk in Montreal, Canada. 0.8 ECTS

2006 10th International Conference on Alzheimer's Disease and Related Disorders in Madrid, Spain. 0.8 ECTS

22nd International Conference on Pharmacoepidemiology and Therapeutic Risk in Lisbon, Portugal. 0.8 ECTS

Seminars and workshops

2006-2008 Research seminars, Department of Epidemiology, Erasmus MC, Rotterdam, the Netherlands. 1 ECTS

2006-2008 'Advanced topics in Pharmacoepidemiological methods', special skills workshops, International Conference on Pharmacoepidemiology and Therapeutic Risk. 0.2 ECTS

2007 Networking and Negotiating, workshops, NWO Talent days, Utrecht The Netherlands. 0.3 ECTS.

Other

2005-2007 Organisation and programme coordination of the Research seminars, Department of Epidemiology, Erasmus MC, Rotterdam, the Netherlands. 8 ECTS

2007 Organisation and programme coordination of the "Advances in Clinical Neuroepidemiology" course, Prof. M.M.B. Breteler, NIHES, Rotterdam The Netherlands. 1 ECTS

2. TEACHING ACTIVITIES

Lecturing

2007 Clinical epidemiology, 4th year medical students, Erasmus MC, Rotterdam, the Netherlands. 0.6 ECTS

Supervising practicals

2006-2008 Data-analysis in pharmacoepidemiology, 4th year medical students. Prof. B.H.C. Stricker, Erasmus MC, Rotterdam, the Netherlands. 0.5 ECTS

2007 Statistics, 4th year medical students, Department of Biostatistics, Erasmus MC, Rotterdam, the Netherlands. 0.5 ECTS.

Supervising Master's theses

2007-2008 'Prevalence of migraine', Joyce van de Ende, Department of Epidemiology, Erasmus MC, Rotterdam, the Netherlands. 8 ECTS