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BRIEF COMMUNICATION

Decreased DNA Repair Capacity in Familial, But Not in Sporadic Alzheimer's Disease

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BOERRIGTER, M. E. T. I., C. M. VAN DUIJN, E. MULLAART, P. EIKELENBOOM, C. M. A. VAN DER TOGT, D. L. KNOOK, A. HOFMAN AND J. VIJG. *Decreased DNA repair capacity in familial, but not in sporadic Alzheimer's disease.* NEUROBIOL AGING **12**(4) 367–370, 1991.—Using the alkaline filter elution technique we determined the induction and disappearance of DNA single-strand breaks (SSB) in freshly isolated peripheral blood lymphocytes (PBL) from 43 Alzheimer's disease (AD) patients and 48 normal, healthy age- and sex-matched control subjects following in vitro exposure to *N*-ethyl-*N*-nitrosourea (ENU). The mean percentage SSB disappearance in PBL from control subjects at 1 h after ENU treatment was 41.4 ± 2.9%; this was not significantly different from that found in samples from AD patients which had no (n = 16) or one (n = 12) first-degree relative with dementia (42.5 ± 8.2% and 43.0 ± 4.4%, respectively; $p > 0.75$). However, in PBL of 15 AD patients with at least two first-degree relatives with dementia the mean percentage SSB disappearance was 23.6 ± 5.8%, which was significantly lower than that found in controls ($p < 0.01$) or in the other AD patients ($p < 0.02$).

Alzheimer DNA repair Ethylnitrosourea Lymphocytes Alkaline elution

ALZHEIMER'S disease (AD) is a common neurodegenerative disorder characterized by the premature death of neurons (9). It has been found that AD patient-derived fibroblast and lymphoblastoid cell lines have an increased sensitivity to the cell killing effects of alkylating agents and X-rays, as compared to cell lines from normal age-matched controls (17, 18, 20). This phenomenon has been attributed to some defect in the capacity to remove brain-specific DNA damages (17–19). Thus in vitro expression of such a defect, i.e., cell death, in cells other than brain cells will become apparent after exposure to certain DNA-damaging agents, but in vivo the defect will be prominent only in the neuronal cells of AD patients (17). Indeed, direct comparison of DNA-damage levels in neuronal tissue of AD patients and controls indicated an at least two-fold higher level of DNA breaks in cortex of AD patients as compared to controls (15).

In keeping with the observations mentioned above, decreased levels of DNA repair in fibroblast and lymphoid cell lines of AD patients after treatment with methyl methanesulfonate or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine have been found (3, 8, 13, 19), although some of these results have been challenged (10,11). Thus far, DNA repair determinations have not been made on freshly isolated cells from AD patients. Such direct-testing for general DNA repair defects would circumvent artefacts that are associated with the use of cell lines and may offer new diagnostic criteria.

In this study a highly sensitive alkaline filter elution assay was used to determine the induction and disappearance of SSB in peripheral blood lymphocytes (PBL) of AD patients and normal age-matched controls exposed to *N*-ethyl-*N*-nitrosourea (ENU). We found that in PBL from sporadic AD patients with no or only one first-degree relative with dementia (n = 16 and n = 12, respectively) and in PBL of 48 age-matched controls the mean percentage SSB disappearance was about 40%, whereas in PBL from 15 AD patients with at least 2 first-degree relatives the mean percentage SSB disappearance of about 23% was significantly lower as compared to the former two groups.

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METHOD

Selection of Patients and Control Subjects

Patients and age-matched controls were participants in an ongoing epidemiological study of risk factors of clinically diagnosed AD at the Department of Epidemiology and Biostatistics, Erasmus University Medical School (6). All patients with AD were selected according to the criteria established by the NINCDS-ADRDA (14). Systemic disorders or brain diseases other than AD, such as multi-infarct dementia, and dementia secondary to alcoholism, depression, metabolic disorders, epilepsy, Parkinson's disease and other conditions were excluded using a clinical history, neurological examination and neuropsychological and laboratory tests (7). All patients fulfilled the following criteria: 1) slow progressive decline of intellectual function; 2) a score on the Clinical Dementia Rating scale of more than 0.5 (7); 3) a score on the Short Portable Mental Status Questionnaire (SPMSQ) of less than 20 (16); 4) a score of 7 or less on the Hachinski-scale (5); 5) no evidence for abnormalities on CT-scan other than cerebral atrophy; and 6) no evidence for focal dysfunction in the EEG. Full pedigree information was obtained by a structured interview of the next of kin of the patient or control (6). Patients and controls were classified according to the number of affected relatives. Patients were considered familial if there were 2 or more individuals known in their family with clinically diagnosed AD. Within the family of those patients, the disease was apparently inherited as an autosomal dominant disorder. Using a breakpoint of 58 years in order to distinguish between the early and late onset type of familial AD (4), all familial AD patients in the present study are classified as belonging to late-onset families. AD patients and control subjects were similar with respect to age range (51–81 and 50–79 years, respectively).

Lymphocyte Isolation and Treatment

Informed consent was obtained from the responsible family members of the subjects with AD and/or directly from the control subjects. Lymphocytes were isolated from 10 ml of coded blood samples, using Ficoll-Paque (Pharmacia) gradients (2), and washed twice in RPMI 1640 medium (Flow Laboratories) plus 2% fetal calf serum (FCS). All steps were performed at 4°C. Viability of recovered PBL was always higher than 95% as measured by trypan blue dye exclusion. ENU (Sigma) was dissolved in dimethylsulfoxide (DMSO) immediately before use. A constant number of cells (3×10^6/ml) was used for each treatment in order to exclude variations in the amount of damage initially induced. Lymphocyte suspensions were exposed to 0.5 mM ENU for 20 min in RPMI 1640 medium plus 20 mM Hepes, 2 mM glutamine and 5% FCS at 37°C. The DMSO concentration during ENU exposure was never higher than 1%. Control cells were treated with RPMI containing the same DMSO concentration. At the end of the exposure period, cells were centrifuged and resuspended in RPMI 1640 supplemented with 10% FCS and glutamine and incubated for repair at 37°C. For treatment with 4 Gy of ^{60}Co-γ-rays, PBL were suspended in RPMI 1640 medium plus 20 mM Hepes, 2 mM glutamine and 5% FCS and irradiated on ice in a Gamma-cell 100 (Atomic Energy of Canada Ltd.) at a dose rate of 6 Gy/min. After irradiation the cells were centrifuged and resuspended in RPMI 1640, 10% FCS, 2 mM glutamine for repair incubation.

Alkaline Filter Elution Assay

The technique of alkaline filter elution (12), modified for analyzing nonradioactively labelled cells (21), was used to measure ENU-induced DNA lesions, detected as single-strand breaks (SSB). In brief, cells were collected, centrifuged and resuspended in ice-cold PBS (8.1 mM Na_2HPO_4, 15 mM KH_2PO_4, 0.14 M NaCl and 2.6 mM KCl) at a final concentration of 1.5×10^6 cells/ml; 0.8×10^6 cells were applied per filter. Loading and lysing of the cells, as well as the elution of the DNA, were performed under subdued lighting in order to minimize artificial induction of SSB. Elution was carried out at a flow rate of 0.03 ml/min. Six fractions were collected at 2.5 h intervals. After the addition of Hoechst 33258, DNA in each fraction was quantitated spectrofluorometrically, as described (15). All determinations and subsequent calculations of the percentage SSB disappearance were performed in a blinded manner. The elution results were plotted as the log percent of DNA remaining on the filter as a function of elution time. Linear regression between the data points obtained at t = 2½ and t = 12½ h of elution time was used to determine the slope of the elution curves. Mean slopes of elution curves were used to calculate the percentage SSB disappearance. In all experiments, mean slopes were based on at least triplicate determinations and standardized with reference to mean slopes of untreated control cells assayed in the same experiment. Variation between the triplicate determinations of a single sample was typically less than 2%.

RESULTS

Table 1 shows the general characteristics of both the AD and control group. There were no significant differences in the mean age between AD patients and control subjects. Table 1 shows that 12 out of 43 AD patients (28%) had one first-degree relative with dementia, as compared to 13 out of 48 controls (27%). Fifteen of the 43 AD patients (35%) and none of the controls had at least 2 first-degree relatives with dementia. These 15 familial AD patients were not related in first, second or third degree.

In a previous study on DNA repair in PBL from normal human individuals we used the alkylating agent ENU at a dose of 0.5 mM, which induces approximately 4000 SSB per cell and has no significant effect on cell survival (1). In that previous study we demonstrated that PBL from about 10% of normal young subjects suffer from a low capacity to remove ENU-induced SSB. The low level of repair appeared to correlate with a low level of survival of these cells at ENU doses of 1, 2 and 5 mM (1). Figure 1 shows representative elution curves for PBL from a normal control subject and for PBL from a sporadic and a familial AD patient, directly after exposure to 0.5 mM ENU and after a 1 h repair incubation. The initial amount of SSB in PBL from these three selected subjects is not different. However, following a 1 h repair incubation the amount of SSB was significantly decreased in PBL from the control subject and the sporadic AD patient, whereas PBL from the selected familial AD patient appeared incapable of removing the ENU-induced SSB (Fig. 1, panels A–C). For PBL from these same individuals γ-ray-induced SSB were removed efficiently; repair was virtually complete within 1 h of repair incubation (Fig. 1, panels D–F).

For 48 normal controls the percentage SSB disappearance was 41.4% ± 2.9% (mean ± SEM) which was not significantly different from the 36.1 ± 4.0% found in 43 AD patients ($p > 0.75$; Table 1). AD patients which had no or only one first-degree relative with dementia (sporadic AD) had a mean percentage of SSB disappearance of 42.5% ± 8.2% and 43.0% ± 4.4%, respectively, which was not significantly different from that found in the control subjects (Table 1). However, AD patients with at least two first-degree relatives with dementia (considered as familial AD), had a significantly lower percentage SSB dis-

TABLE 1

ENU-INDUCED SSB DISAPPEARANCE IN PBL FROM AD PATIENTS AND MATCHED CONTROL SUBJECTS

Subjects	Number of First-Degree Relatives With Dementia	Number	Age (years)*	Female (%)	SSB Induction† $t=0$	SSB Induction† $t=1$	ENU Repair (%)‡
Controls	0	35	68.2 ± 1.15	72	0.181 ± 0.011	0.096 ± 0.007	40.8 ± 3.4
	1	13	70.0 ± 1.55	70	0.173 ± 0.013	0.100 ± 0.012	44.3 ± 5.5
		48	68.9 ± 0.93	71	0.179 ± 0.009	0.097 ± 0.006	41.4 ± 2.9
AD patients	0	16	67.1 ± 1.47^{ns}	88	0.164 ± 0.011^{ns}	0.084 ± 0.009^{ns}	42.5 ± 8.2^{ns}
	1	12	69.3 ± 0.82^{ns}	83	0.180 ± 0.011^{ns}	0.101 ± 0.009^{ns}	43.0 ± 4.4^{ns}
	2	15	68.8 ± 1.74^{ns}	75	0.181 ± 0.017^{ns}	0.131 ± 0.011§	23.6 ± 5.8¶
		43	68.3 ± 0.84^{ns}	81	0.176 ± 0.008^{ns}	0.105 ± 0.006^{ns}	36.1 ± 4.0^{ns}

*Mean ± SEM. †Number of SSB represented by the slope of the elution curve (mean ± SEM) at the indicated times (in h) after the 20 min ENU treatment. ‡Percentages are the mean (±SEM) of the repair values as calculated for each individual subject. ^{ns}Not significantly different from the 48 control subjects. §Significantly different from the 48 control subjects ($p<0.01$, two-sided t-test) ¶Significantly different from the 48 control subjects ($p<0.01$) and the AD patients with 0 or 1 first-degree relative with dementia ($p<0.02$).

appearance (23.6% ± 5.8%) than controls ($p<0.01$) or AD patients with no or only one first-degree relative with dementia ($p<0.02$; Table 1). In PBL from six familial AD patients that were checked at 2.5 h posttreatment the percentage ENU-induced SSB disappearance was 34.9% ± 6.7% indicating that the rate rather than the total amount of repair was lower in PBL from familial AD patients (results not shown). A similar decreased rate of SSB disappearance in PBL from some normal young subjects exposed in vitro to ENU was reported in our previous study (1).

There was no statistically significant correlation between age and the percentage SSB disappearance at 1 h after exposure to ENU for either the AD groups, the control groups, or all groups combined. The amount of SSB disappearance was not associated with the age of onset, the degree or the duration of the disease. Also, no differences were observed between males and females of either group (results not shown).

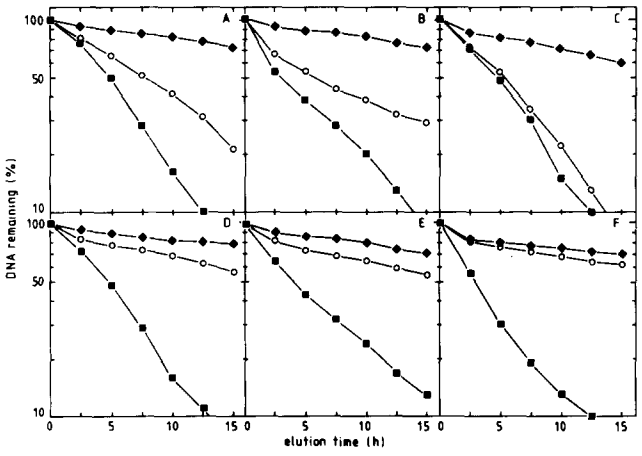

FIG. 1. Representative elution curves of PBL from a normal control subject (A and D), a sporadic AD patient (B and E) and a familial AD patient (C and F) exposed in vitro to 0.5 mM ENU for 20 min at 37°C (A, B and C) or irradiated with 4 Gy of γ-rays at 4°C (D, E and F). Untreated control cells (◆); treated PBL, no repair incubation (■); treated cells, 1 h repair incubation (○).

DISCUSSION

A deficiency in the ability of cells to repair alkylating agent-induced SSB could reflect a hypersensitivity to one or more specific DNA lesions induced by those agents. The initial observations by Robbins et al. (17,18) and Scudiero et al. (20) of a significantly lower survival of fibroblast and lymphoid cell lines from AD patients appeared to be confirmed by recent reports of DNA repair deficiencies in AD cell lines treated with methyl methanesulfonate (MMS) or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) (3, 8, 13, 19). However, the limited number of patient-derived cell lines used in those studies (n = 4–7), the application of long-term cell culture instead of using freshly isolated cells and some conflicting evidence reported later (10,11) made it necessary to confirm and extend these early observations.

Our present set of data on large groups of AD patients and matched control subjects definitely confirm and considerably extend earlier findings of a DNA repair defect in familiar AD (8,13). These earlier determinations have not been made on freshly isolated cells from AD patients, but on a small number of cell lines derived from AD patients. The use of freshly isolated cells in the present study refutes the possibility that observed differences among individuals within a group or between different groups are an artefact of culture. However, it should be realized that in our study no histopathological examination has as yet confirmed the clinical diagnosis of AD. This could partly explain the large individual variation observed in the groups.

In a previous study, we have used the excision repair inhibitor 1-β-arabino-furanosylcytosine to provide evidence that the most likely type of defect responsible for the low ENU-induced SSB repair observed in some individuals is a lesion-specific step in excision repair, e.g., glycosylation (1). In this regard it should be noted that different alkylating agents induce a different spectrum of DNA lesions, the removal of which may require different repair pathways and possibly different glycosylases. Therefore, the previously reported DNA repair defect in cells from sporadic AD patients, detected after treatment with MMS (19), is not necessarily in conflict with our present results but could be the consequence of the different alkylating agents used.

Whether defective DNA repair is related to or closely linked

to the etiology and/or pathophysiology of familial AD is not clear at this time. A diminished DNA repair in familial but not in sporadic AD could either be a consequence of a different pathogenesis of familial AD or could represent a genetic defect only present in familial AD. Medical treatment was similar for familial and sporadic AD patients. Therefore, the decrease in DNA repair cannot be ascribed to differences in medication. Although the repair of ENU-induced DNA lesions in sporadic AD patients is quantitatively similar to that observed in normal control subjects, this does not necessarily exclude differences in other pathways, or qualitative differences in, for example, the fidelity of DNA repair processes. Both a decreased rate of repair and/or a lower fidelity of DNA repair in nondividing cells, such as neurons, could have adverse effects on the expression of genes important for neuronal function and survival.

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