AGE-RELATED DIFFERENCES IN RENAL SIDE-EFFECTS OF RADIATION AND CHEMOTHERAPY IN THE RAT

LEEFTIJDS-AFHANKELIJKE VERSCHILLEN IN DE RENALE BIJWERKINGEN VAN BESTRALING EN CHEMOTHERAPIE BIJ DE RAT

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
AAN DE ERASMUS UNIVERSITEIT ROTTERDAM
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF. DR. A.H.G. RINNOOY KAN
EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN.
DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP
DONDERDAG 30 JUNI 1988 OM 13.30 UUR

DOOR

HERMINA THEODORA MARIA JONGEJAN

GEBOREN TE ZWANENBURG

1988

Offsetdrukkerij Kanters B.V.,
Alblasserdam

PROMOTIECOMMISSIE

PROMOTOR: Prof. Dr. J.C. Molenaar

CO-PROMOTOR: Dr. A.P. Provoost

OVERIGE LEDEN: Prof. Dr. D.W. van Bekkum

Prof. Dr. M.A.D.H. Schalekamp

Prof. Dr. H.M. Pinedo

Cover design: R. Mettrop.

The financial support of Bristol-Meyers B.V. was greatly appreciated.

The animal experiments presented in this thesis have been performed at the Laboratory for Surgery. The study was supported by a grant from the Dutch Cancer Foundation (Koningin Wilhelmina Fonds), IKR 06.

Aan myn ouders.

CONTENTS

Am	Amikacin
BSA	body surface area
BW	body weight
cDDP	cis-diammine-dichloroplatinum, cis-platin
Cp	cis-diammine-dichloroplatinum, cis-platin
ED50	dose, at which 50% of the animals show the desired effect
ERPF	effective renal plasma flow
GFR	glomerular filtration rate
Gy	Gray, unit of radiation (1 Gy = 100 rad)
Hb	Hemoglobin
iv/i.v.	intranenous
PRC	plasma renin concentration
Pt	platinum
SBP	systolic blood pressure
s.c.	subcutaneously
SD	standard deviation
SEM	standard error of the mean
Uosmol	Osmolality of the urine
Vd	Volume of distribution

CHAPTER 1

INTRODUCTION

PREFACE

The improved life-expectancy of cancer patient has brought to light late sequelae of oncology therapy. This is especially true for pediatric patients. Renal damage is one of the adverse side-effects of anti-tumor therapy that may occur. Studies concerning damaging effects of radiotherapy or chemotherapy on the kidney have generally been performed in adults. There is scant experimental or clinical information on renal function after anti-tumor therapy in the young. Rapid growth occurring in the developing kidney and age-dependent differences in pharmacokinetics may influence the extent of renal damage due to oncology therapy and may induce divergent effects in young or adult individuals.

This thesis deals with experimental results from studies in rats. A comparison was made between the effects of irradiation, chemotherapy, antibiotics, or a combination of these treatment modalities on the kidneys of either young and adult rats.

INTRODUCTION

1.1 COMPARISON OF HUMAN AND RAT KIDNEYS

As this thesis is based on studies of renal function damage occurring in young and adult rats, the experimental results may not hold true for the human situation. To gain more insight in the differences and similarities between human and rat kidneys, a short review will be given of renal development, structure and function.

1.1.1 RENAL DEVELOPMENT

The rat is born after 3 weeks of gestation and renal differentiation continues postnatally until the age of approximately 3 weeks. In contrast, in humans nephrogenesis is normally completed at the gestational age of 36 weeks (Kleinman, 1982), amounting to approximately 90% of normal duration of gestation. Thus, the state of nephrogenesis in newborn babies and weanling rats is comparable. When renal differentiation is completed, all nephrons have been formed and renal growth results from cell proliferation and cell growth, with an unchanged nephron number (Larsson, 1975; Solomon, 1977).

In the rat, total kidney weight increases from about 0.6 g in the weanling rat to about 2.5 g in the adult rat (weight gain of 30%) (Provoost et al., 1983). Human kidney weight increases from 13-14 g at birth to 150 g at maturity (weight gain of 100%)(Rubin et al., 1949). The kidney weight (g) per 100 g body weight in rats decreases from 1.15 at weanling age to 0.8-0.6 g/100 g BW in adult rats (Provoost et al., 1983). In humans, renal weight (g/100 g BW) decreases from 0.77 at birth to 0.47 in adults (Chantler, 1979). During renal growth, cell proliferation is most pronounced

in the renal cortex. When cell proliferation is stimulated by renal injury, such as nephrectomy, cell proliferation increases fivefold in the renal cortex of young as well as adult rats, whereas in the renal medulla cell proliferation increases eightfold in 3 weeks' old rats and twelvefold in adult rats. This increase in proliferation may be considered as an indicator for a good regenerative capacity of both young and adult rat kidneys (Reiter et al., 1964). Cell proliferation mainly occurs during the first 48 hours after nephrectomy. After that time hypertrophy occurs. This also holds true for the human situation as indicated by the increase in kidney function and size after unilateral nephrectomy and the potential to recover from acute renal failure or acute tubular necrosis.

The number of glomeruli differ between rats and humans. One rat kidney contains about 30-34,000 glomeruli (Vimtrup, 1928; Azar et al., 1979; De Keijzer et al., 1984; Provoost et al., 1984; Provoost and Van Aken, 1985). In the adult rat, this is about 30,000 glomeruli per gram kidney. The human kidney contains about 800,000-1,000,000 glomeruli. In adults this is about 6,000-7,000 glomeruli per gram kidney (Vimtrup, 1928). Thus except for the size difference and papillary structure of the human and the rat kidney, the state of nephrogenesis, anatomy, renal function and growth rate are comparable in weanling rats and newborn babies.

1.1.2 RENAL FUNCTION

1.1.2.1 GLOMERULAR FUNCTION

In rats, the glomerular filtration rate (GFR) and effective renal plasma flow (ERPF), both expressed in ml/min, increase with age. In adult rats the GFR is about 5 times higher than in weanling rats (2 vs 0.4

ml/min). Up till the age of 6-7 weeks, the rise in GFR surpasses the increase in BW. Subsequently, there is a gradual decline in GFR relative to BW. Although body growth continues in the adult rat, the GFR (ml/min) does not increase any further after BW has reached about 300 g (Provoost et al., 1983).

In man, the GFR gradually increases from 2.5 ml/min at birth to 120-130 ml/min in adults (Chantler, 1979). Related to BW, the GFR in man increases from 0.71 ml/min per kg at birth to a maximum of about 3.1 ml/min per kg at the age of 2-4 years. From then on it declines gradually to about 1.8-2.0 ml/min per kg in adults. The changes in renal plasma flow during development are, in general, similar to the changes in the GFR.

Serum urea and creatinine concentrations are easy to measure, but unreliable as sensitive renal function parameters. Serum creatinine concentration depends on body mass, metabolic state, type and amount of food consumed and the time of measurement. The serum urea concentration highly depends on protein intake. The determination of the GFR by creatinine clearance is questionable. Difficulties in obtaining accurately timed urine collections, especially in small children, contributes to the unreliability of creatinine clearance as a renal function parameter in children (Womer et al., 1985). Both GFR and ERPF, as measured with radio-isotopes, are sensitive parameters to trace renal damage induced by either irradiation (Moss et al., 1979) or drugs (Stark and Howell, 1978). Isotope techniques are more sensitive in detecting changes in renal function than serum creatinine concentration and creatinine clearance techniques (Hall et al., 1986).

1.1.2.2 TUBULAR FUNCTION

Tubular secretion and reabsorption of solutes is little lower in young rats than in adult rats (Rubin et al., 1949). For drugs and most other solutes, the major site of transport is the proximal tubulus. It has been suggested that these transport mechanisms are less efficient in the young kidney, causing differences in renal drug accumulation and excretion in young and adult individuals (Aladjem et al., 1984).

In contrast to the multipapillary human kidney, the rat kidney has only one elongated papilla. The concentrating potency of the kidney is positively correlated to the width of the renal medulla. Due to the length of the rat renal papilla, the maximal urine osmolality amounts to over 3,000 mOsm/kg H2O, compared to about 1,500 mOsm/kg H2O in humans (Stephenson, 1983; Rahill, 1975). As urine osmolality of the healthy rat is quite constant due to stable drinking habits of these animals, urine osmolality may be used as a reliable parameter for the detection of tubular damage in the rat. As fluid intake in humans tends to be subject to more variation, this parameter is less useful in humans. Maximal urine osmolality in neonates is less than in adults, which is predominantly due to a lower urea excretion rate by the neonate (Rahill et al., 1975).

1.1.3 RENIN PRODUCTION BY THE KIDNEY

Renin is one of the hormones produced by the juxta-glomerular apparatus of the kidney. Renin converts angiotensinogen to angiotensin I, which is rapidly converted to angiotensin II by converting enzyme. Apart from being a potent vasoconstrictor, angiotensin II and also stimulates aldosterone release by the adrenals. The renin-angiotensin-aldosteron system plays an

important role in the determination of vascular tone, blood pressure and maintenance of sodium homeostasis. Plasma renin concentration (PRC) depends on many factors such as age (Fiselier et al., 1983), sodium intake (Miksche et al., 1970), and kidney perfusion (Solomon et al., 1976). In humans, renin is mainly present in plasma in an inactive form, known as prorenin (Derkx, 1987). This prorenin can be activated at low pH, low temperatures and by proteolytic enzymes. In rats such an inactive form could not be detected at our laboratory (De Keijzer et al., 1982). However, the presence of inactive renin in rats has been reported by others (Mizuno et al., 1986).

1.2 RENAL SIDE EFFECTS OF ANTI-TUMOR THERAPY

In the last decades, therapeutic modalities in oncology have developed rapidly and they keep changing. The three main stays of oncology therapy are chemotherapy, radiotherapy and surgery. Detailed information on these specific therapeutic modalities can be found in various reviews (Daly and De Cosse, 1985; Olive and Peeters, 1981; Plaschkes, 1981; Richter et al., 1985; Sandland and Barre, 1981; Wieman and Calabresi, 1985). Treatment schedules were developed empirically and intensified to achieve better local and systemic tumor control. The therapeutic ratio, defined as the ratio between therapeutic effects and side effects, is used as an indicator of treatment success. Therapeutic gain is achieved by improving therapeutic results, while side effects are kept constant or reduced. Although side effects of anti-cancer therapy are manifold, only a brief review on the renal side-effects is given here. For more detailed information, the reader is referred to extensive reviews on renal damage due to radiation (Greenberger et al., 1982; Moss et al., 1979) or chemotherapy (Garnick et al., 1983).

1.2.1 RADIATION NEPHROTOXICITY

Kidney irradiation may occur during radiotherapy for adenocarcinoma of the kidney, Wilm`s tumor, Hodgkin`s disease with abdominal involvement, and para-aortic lymphnode metastases of gonadal tumors. New radiation techniques, well-defined treatment fields and fractionation of the dose, enabled lowering the injury of healthy tissue. Extensive experimental work has recently been performed in adult animals, investigating healthy tissue reactions to irradiation (Dewit, 1986; Robbins et al., 1985; Stewart et al., 1984; Van Der Kogel, 1979; Van Rongen et al, 1987).

Immediately after irradiation subclinical cell damage occurs, which is partially repaired. The parenchymal cells are damaged and in the microvasculature there is cell swelling and vacuolation of endothelial cells and to a lesser extent tunica media cells. Vascular permeability increases. Nephron atrophy reduces total kidney mass. The kidney surface becomes irregular; the capsule thickens. The medium arteries show prominent albeit variable sclerosis. Afferent arterioli are hyalinized and there is progressive occlusion of the glomerular capillary loops. Mesangial cells proliferate and the glomeruli are eventually hyalinized. There is tubular atrophy and interstitial fibrosis (Moss et al., 1979). The number of nephrons that continue to function determine the final outcome of the radionephropathy (White, 1976).

Functionally, renal radiation damage is reflected in a decline in GFR, renal blood flow (Robbins et al., 1985; Stewart et al., 1987; Chauser et al., 1976), urinary concentrating ability (Buerkert et al., 1976; Coburn et al., 1966) and progressive proteinuria (Moss et al., 1979). Both the severity of functional renal damage and the duration before its manifestation have been shown to be dose-dependent (Robbins and Hopewell,

1987; Stewart et al., 1987).

We compared functional renal damage in young and adult rats after a single radiation dose to both kidneys. The results of these experiments will be presented in CHAPTERS 2 and 3.

The course of renal function deterioration may be complicated by hypertension. Hypertension occurring after renal irradiation is a serious condition indicating severe vascular damage. Although recovery of the kidney and disappearance of hypertension has been reported, this does not commonly occur (Luxton, 1962). If it does, hypertension may excacerbate renal injury by damaging renal vessels.

The course of systolic blood pressure (SBP) and PRC in young and adult rats is described in CHAPTER 3. The role of sodium and fluid retention in the development and maintenance of an elevated SBP after irradiation was studied using a sodium restricted diet. The activity of the renin-angiotensin system was studied by repeated PRC measurements.

1.2.2 NEPHROTOXICITY OF CHEMOTHERAPY

Chemotherapy may cause nephrotoxicity. The drugs that have reportedly caused renal damage are cDDP, some of the nitrosureas (Streptozotocin and methyl-CCNU), Methrotrexate, and occasionally, Mitomycin C, Mithramycin and 5-Azacytidine (Garnick et al., 1983). The nephrotoxicity of cDDP seems to be the most pronounced and most dose-limiting clinically. Although the use of hydration programs and diuretics have lessened the incidence of renal impairment, renal toxicity of cDDP remains a serious clinical problem.

In pediatric oncology, cDDP is used for the treatment of neuroblastomas, malignant germ cell tumors, brain tumors, retinoblastomas, sarcomas, malignant liver tumors and nasopharyngeal carcinomas (Olive et al., 1985;

Voute et al., 1981).

The availability of data concerning renal damage caused by cDDP in adult rats, coupled with the fact that this damage was clearly shown to be dose-dependent, rendered this drug eminently suitable for a comparitive investigation of funtional renal toxicity patterns in young and adult rats.

1.2.2.1 cDDP NEPHROTOXICITY

In 1965, Rosenberg et al. discovered the anti-tumor activity of cDDP, which was previously known as Peyrone`s chloride. Since the discovery of its anti-tumor properties, cDDP has succesfully been applied for ovarian and testicular cancer, head and neck cancer, neuroblastomas, sarcomas and malignant germ cell tumor (Prestayko et al., 1979; Womer et al., 1985).

The nephrotoxicity of cDDP is its major and dose-limiting side effect. Four to five days after a single dose of cDDP, dose-dependent renal damage reaches its maximum. Acute tubular necrosis is a prominent feature of cDDP nephrotoxicity in rats (Dobyan et al., 1980; Goldstein and Mayor, 1983). In humans, likewise focal tubular necrosis was found in the distal tubules and collecting tubules (Gonzales-Vitale et al., 1977). In the acute phase of cDDP nephrotoxicity the proximal tubular cells swell, loose their brush-border and become necrotic. Both glomerular perfusion and filtration decline. Serum urea and creatinine concentration rises. Proteinuria and enzymurea occurs. Electrolyte disturbances may also occur (Goldstein and Mayor, 1983).

In the chronic stage, tubules become cystic dilated with hyperplasia and flattening of tubular epithelium, atrophy of cortical tubules, interstitial fibrosis and thickening of tubular basement membranes (Choie et al., 1980; Dobyan et al., 1981). These morphological changes are

accompanied by a renal function decline. The creatinine clearance is reduced (Jones et al., 1985). There is a fall in GFR (Chopra et al., 1982; Womer et al., 1985) and ERPF (Chopra et al., 1982; Meyer, 1982)

Renal injury patterns for children and adults are quite similar (Vietti et al., 1979) but it seems that children tolerate higher doses (mg/kg BW) of c-DDP before toxicity occurs than adults (Kamalakar et al., 1977). The scarcity of information on drug-induced damage to developing kidneys, stimulated the present investigation of the nephrotoxicity of cDDP comparing young and adult rats. The results of these experiments will be presented in CHAPTER 4.

1.2.3 IRRADIATION COMBINED WITH cDDP

In modern oncology, radiotherapy rarely amounts to the sole treatment of a tumor. Recent advances in radiotherapy involve combinations with new drugs and changes in fractionation patterns of the administered radiation dose. Chemotherapy and radiotherapy are combined to improve the therapeutic effectiveness, but in practice this often leads to enhanced side effects. Theoretically, the improved therapeutic ratio can be explained by spatial cooperation, enhancement of tumor response without much enhancement of normal tissue injury or by diminution of normal tissue injury without diminution of tumor response. The therapeutic effects of the combined treatment of cDDP and irradiation exceeds expectations based on the anti-tumor effect of either treatment modality when given on its own, as shown by in vivo (Kyriasis et al., 1983) and in vitro studies (Begg et al., 1986). Pilot studies have reported acceptable toxicity of combined irradiation and cDDP administration (Pinedo et al., 1983; Reimer et al., 1981; Shipley et al., 1984). Ongoing phase III trials will provide further

information (EORTC Studies 08844, 22843. RTOG study 85-02).

As both irradiation and cDDP adminisration may cause DNA damage, combined renal irradiation and cDDP administration caries the risk of potentiation of serious renal side-effects, particularly in the developing kidney. CHAPTER 5 presents the comparitive effects on renal function of a single radiation dose to both kidneys followed by a single cDDP injection in young and adult rats.

1.3 USE OF AMINOGLYCOSIDES AFTER CHEMOTHERAPY

Clinically, the leucopenia induced by chemotherapeutic drugs such as cDDP, may cause opportunistic infections. The causative microorganisms of these infections may require the use of aminoglycoside antibiotics. Consequently, the use of aminoglycosides in patients whose kidneys have been damaged by chemotherapy, can not always be avoided (Haas et al., 1983). Aminoglycosides are nephrotoxic and cause renal damage, which develops in a characteristic pattern.

During an aminoglycoside course renal changes develop gradually, reaching a maximum after approximately 10 days of aminoglycoside administration. At that stage, the proximal tubular epithelium shows vacuolation and sometimes necrosis and desquamation, predominantly in the outer cortex. With low doses of aminoglycosides cell regeneration occurs (Tulkens, 1986). Regeneration may also take place during continuous drug administration, resulting in, at least partial normalization of renal function (Gilbert et al., 1979). A low grade tubular dysfunction may persist (Elliott et al., 1982).

1.3.1 cDDP AND AMIGLYCOSIDES

Aminoglycoside toxicity mechanisms in the kidney, which were mostly investigated using gentamicin, are very likely to be the same for all aminoglycosides (Whelton and Neu, 1982; De Broe et al., 1986). Amikacin is one of the newer aminoglycosides, clinically especially useful for the treatment of gentamicin resistant pathogens (Siegenthaler et al., 1986). At therapeutically active dose levels amikacin seems to be less nephrotoxic than gentamicin (Siegenthaler et al., 1986; Provoost et al., 1985; Rajchgot et al., 1984; Tulkens, 1986). Its antibiotic spectrum and relatively low nephrotoxicity have both contributed to the wide clinical application of Amikacin.

A few experimental studies on the nephrotoxic effects of a combination of cDDP and aminoglycosides have been reported. Two studies reported on severe renal damage after the combined administration of cDDP with an aminoglycoside (trobramycin) to rats (Kawamura et al., 1981; Bregman and Williams, 1986). Other nephrotoxins, however, such as mercuric chloride (Luft et al., 1977) or potassium-dichromate (Elliott et al., 1982) were reported to alleviate aminoglycoside-induced renal damage.

We investigated renal function after a single dose of cDDP and a course of Amikacin, lasting 14 days, in young and adult rats. The results of this investigation are presented in CHAPTER 6.

1.4 SPECIFIC AIMS OF THE STUDY

In summary, the specific aims of the present investigations are:

1. The description of acute and chronic changes in renal function and blood-pressure after several single radiation doses to both kidneys comparing young and adult rats (CHAPTER 2).

2. The evaluation of the systolic blood pressure after a single radiation dose to both kidneys in young and adult rats (CHAPTER 2 and 3).

3. The investigation of pathogenetic factors involved in the hypertension occurring after bilateral kidney irradiation in the rat (CHAPTER 3).

4. The comparison of functional renal damage after several doses of cDDP in young and adult rats (CHAPTER 4).

5. The comparison of renal function damage in case of cDDP administration immediately after bilateral kidney irradiation in young and adult rats (CHAPTER 5).

6. The comparison of possible potentiation of renal function damage, in case of amikacin administration immediately following a single cDDP dose (CHAPTER 6).

1.5 REFERENCES

Aladjem M, Aladjem Y, Koren G, Boichis H: Maturation of renal tubular transport of gentamicin.
Dev Pharmacol Ther 7:82-86, 1984

Avioli LV, Lazor MZ, Cotlove E, Brace KC, Andrews JR: Early effects of radiation on renal function in man.
Am J Med 34:329-337, 1963

Begg AC, van der Kolk RJ, Dewit L, Bartelink H: Radiosensitization by Cisplatin of RIF1 tumour cells in vitro.
Int J Radiat Biol 50:871-884, 1986

Bregman CL, Williams PD: Comparative nephrotoxicity of carboplatin and cisplatin in combination with tobramycin.
Cancer Chemother Pharmacol 18:117-123, 1986

Buerkert J, Doyle J, Ewald W, Effects of local irradiation on the excretion of sodium and water by the canine kidney.
Rad Res 66:346-362, 1976

Chantler C: The kidney.
In: S Godfrey, JD Baum (eds.), Clinical Pediatric Physiology. Alden Press, Oxford, 1979, pp. 356-398

Chauser BM, Hudson FR, Law MP: Renal function in the rat following irradiation.
Rad Res 67:86-97, 1976

Choie DD, Delcampo AA, Guarino AM, Subcellular localization of cis-dichlorodiammineplatinum (II) in rat kidney and liver.
Toxicol Appl Pharm 55:245-252, 1980

Chopra S, Kaufman JS, Jones TW, Hong WK, Gehr MK, Hamburger RJ, Flamenbaum W, Trump BF: Cis-diamminedichlorplatinum-induced acute renal failure in the rat.
Kidney Int 21:54-64, 1982

Coburn JW, Rubini ME, Kleeman CR: Renal concentrating defect in canine radiation nephritis.
J Lab Clin Med 67:209-223, 1966

Daly JM, De Cosse JJ: Principles of surgical oncology.
In: P Calabresi, PS Schein, SA Rosenberg (eds.), Medical Oncology. Basic principles and clinical management of cancer. Macmillan Publ. Comp., New York, 1985, pp 261-279

De Broe ME, Giuliano RA, Verpooten GA: Choice of drug and dosage regimen. Two important risk factors for aminoglycoside nephrotoxicity.
Am J Med 80(suppl 6B):115-118, 1986

De Keijzer MH, Provoost AP, Derks FHM: Absence of activation in vitro of renin in rat plasma.

Clin Sci 62:435-437, 1982

De Keijzer MH, Provoost AP, Wolff ED, Kort WJ, Weijma IM, Van Aken M, Molenaar JC: The effect of a reduced sodium intake on post-renal transplantation hypertension in rats.
Clin Sci 66:269-276, 1984

Derkx FHM: Human prorenin.
Thesis, Erasmus University Rotterdam, 1987, pp. 1-243

Dewit L: Tolerance of the small and large intestine to irradiation and cis-diamminedichloroplatinum (II).
Thesis, University of Amsterdam, 1986

Dobyan DC, Levi J, Jacobs C, Kosek J, Weiner MW: Mechanisms of Cisplatinum nephrotoxicity. II morphologic observations.
J Pharm Exp Ther 213: 551-556, 1980

Dobyan DC, Hill D, Lewis T, Bulger RE: Cyst formation in the rat kidney induced by Cis-platinum administration.
Lab Invest 45:260-268, 1981

Elliott WC, Houghton DC, Gilbert DN, Baines-Hunter J, Bennett WM: Gentamicin nephrotoxicity: degree and permanence of acquired insensitivity.
Lab Clin Med 100:501-512, 1982

Fiselier TJW, Lijnen P, Monnens L, van Munster P, Jansen M, Peer P: Levels of renin, angiotensin I and II, angiotensin-converting enzyme and aldosterone in infancy and childhood.
Eur J Pediatr 141:3-7, 1983

Garnick MB, Mayer RJ, Abelson HT: Acute renal failure associated with cancer treatment.
In: BM Brenner and JM Lazarus (eds.), Acute Renal Failure. W.B.Saunders Company, Philadelphia, 1983, pp. 527-554

Gilbert DN, Houghton DC, Bennett WM, Plamp CE, Reger K, Porter GA: Reversibility of gentamicin nephrotoxicity in rats: recovery during continuous drug administration.
Proc Soc Exp Biol Med 160:99-103, 1979

Goldstein RS, Mayor GH: The nephrotoxicity of Cisplatin.
Life Sci 32:685-690, 1983

Gonzales-Vitale JC, Hayes DM, Cvitkovic E, Sternberg SS: The renal pathology in clinical trials of Cis-platinum(II)diamminedichloride.
Cancer 39:1362-1371, 1977

Greenberger JS, Weichselbaum RR, Cassady JR: Radiation nephropathy.
In: RE Rieselbach, MB Garnick (eds.), Cancer and the Kidney, Lea Febiger, Philadelphia, 1982, 814-823

Haas A, Anderson L, Lad T: The influence of aminoglycosides on the nephrotoxicity of cis-Diamminedichloropatinum in cancer patients.
J Infect Dis 147:363, 1983

Hall KS, Fossa SD, Aas M: High-dose Cis-platinum combination chemotherapy in advanced non seminematous malignant germ cell tumours with emphasis on nephrotoxicity.
Cancer Chemother Pharmacol 18:4-11, 1986

Jones TW, Chopra S, Kaufman JS, Flamenbaum W, Trump BF: Cis-diammine-dichloroplatinum (II)-induced acute renal failure in the rat.
Lab Invest 52:363-374, 1985

Kamalakar P, Freeman AI, Higby DJ, Wallace HJ, Sinks LF: Clinical response and toxicity with Cis-dichlorodiammine platinum (II) in children.
Cancer Treat Rep 61:835-839, 1977

Kawamura J, Soedo A, Yoshida O: Nephrotoxicity of cis-Diamminedichloro-platinum (II) (Cisplatinum) and the additive effect of antibiotics: morphological and functional observations in rats.
Toxicol Appl Pharmacol 58:475-482, 1981

Kleinman LI: Developmental renal physiology.
The Physiologist 25:104-110, 1982

Kyriazis AP, Yagoda A, Kererakes JG, Kyriazis AA, Whitmore WF: Experimental studies on the radiation-modifying effect of Cis-diamminedichloroplatinum II (CDDP) in human bladder transitional cell carcinomas grown in nude mice.
Cancer 52:452-457, 1983

Luft FC, Nahm Yum M, Kleit SA: The effect of concomitant mercuric chloride and gentamicin on kidney function and structure in the rat.
J Lab Clin Med 89:622-631, 1977

Luxton RW, Kunkler PB: Radiation nephritis. Acta Radiol 169-174, 1964.

Meyer S: Cis-platinum and the kidney. Renal function during treatment with cis-diamminedichloroplatinum.
Thesis, 1982, Groningen, The Netherlands.

Miksche LW, Miksche U, Gross F: Effect of sodium restriction on renal hypertension and on renin activity in the rat.
Circ Res 27:973-984, 1970

Mizuno K, Watari H, Tani M, Fukuchi S: Active and inactive renin-like enzymes in the arterial wall of the spontaneously hypertensive rat.
Clin Exp Theor and Pract A7:1707-1717, 1986

Moss WT, Brand WN, Battifora H: Radiation oncology-rationale technique and results. 5th ed., CV Mosby Company, St. Louis, 1979. The Kidney, Chapter 13, pp. 366-385

Olive D, Peeters M: Chemotherapy.
In: PA Voute, A Barrett, HJG Bloom, J Lemerle, MK Neidhardt (eds.), Cancer in children. Clinical management. Springer Verlag, Berlin, 1986, pp. 21-35

Olive D, Benz-Lemoine E, Berg P: Pediatric tumors.
In: HM Pinedo, BA Chabner (eds.), Cancer chemotherapy/7. The EORTC cancer chemotherapy annual. Elsevier, Amsterdam, 1985

Pinedo HM, Karim ABMF, van Vliet WH, Snow GB, Vermorken JB: Daily Cis-dichlorodiammineplatinum (II) as a radio-enhancer: a preliminary toxicity report.
J Cancer Res Clin Oncol 105:79-82, 1983

Plaschkes J: Surgical oncology in children.
In: PA Voute, A Barrett, HJG Bloom, J Lemerle, MK Neidhardt (eds.), Cancer in children. Clinical management. Springer Verlag, Berlin, 1986, pp. 46-53

Prestayko AW, D'Aoust JC, Issell BF, Crooke ST: Cisplatin (cis-diamminedichloroplatinum II).
Cancer Treat Rev 6:17-39, 1979

Provoost AP, Adejuyigbe O, Wolff ED: Nephrotoxicity of aminoglycosides in young and adult rats.
Ped Res 19:1191-1196, 1985

Provoost AP, De Keijzer MH, Kort WJ, Van Aken M, Weijma IM, Wolff ED, Molenaar JC: The influence of the recipient upon renal function after isogeneic kidney transplantation in the rat.
Transplantation 37:55-62, 1984

Provoost AP, De Keijzer MH, Wolff ED, Molenaar JC: Development of renal function in the rat.
Renal Physiol 6:1-9, 1983

Provoost AP, Van Aken M: Renal adaptation to additional nephrons: A functional study in the three-kidney rat.
Renal Physiol 8:129-135, 1985

Rahill WJ, Renal physiology-clinical variations.
In: MI Rubin, TM Barratt (eds.), Pediatric nephrology, Chapter 2, The Williams & Wilkins Company, Baltimore, 1975, pp 10-40.

Rajchgot P, Prober CG, Soldin S, Perlman M, Good F, Harding E, Klein J. Macleod S: Aminoglycoside related nephrotoxicity in the premature newborn.
Clin Pharm Ther 35:394-401, 1984

Reimer RR, Gahbauer R, Bukowski RM, Hewlett JS, Groppe CW, Weick JK, Antunez AR: Simultaneous treatment with Cis-platin and radiation therapy for advanced solid tumors: a pilot study.
Cancer Treat Rep 65:219-222, 1981.

Reiter RJ, McCreight CE, Sulkin NM: Age differences in cellular proliferation in rat kidneys.
J Geront 19:485-489, 1964

Richter MP, Share FS, Goodman R: Principles of radiation therapy.
In: P Calabresi, PS Schein, SA Rosenberg (eds.), Medical Oncology. Basic principles and clinical management of cancer. Macmillan Publ. Comp., New York, 1985, pp 280-291

Robbins MEC, Hopewell JW: Radiation-related renal damage.
In: PH Bach, EA Lock (eds.), Nephrotoxicity in the experimental and clinical situation (Part 2), M Nijhoff, Dordrecht, 1987, pp. 817-846

Robbins MEC, Hopewell JW, Gunn Y: Effects of single doses of X-rays on renal function in unilaterally irradiated pigs.
Radiother Oncol 4:143-151, 1985

Rosenberg B, Van Camp I, Krigas T: Inhibition of cell division in Escherichia Coli by electrolysis products from a platinum electrode.
Nature 205:698-699, 1965

Rubin MI, Bruck E, Rapoport M, Snively M, McKay H, Baumler A: Maturation of renal function: childhood clearance studies.
J Clin Invest 28:1144-1162, 1949

RTOG study 85-02, EORTC studies 08844,22843

Sandland R, Barrett A: Radiation therapy.
In: PA Voute, A Barrett, HJG Bloom, J Lemerle, MK Neidhardt (eds.), Cancer in children. Clinical management. Springer Verlag, Berlin, 1986, pp. 36-45

Shipley WU, Coombs LJ, Einstein AB, Soloway MS, Wajsman Z, Prout GR and National Bladder Cancer Collaborative Group A: Cisplatin and full dose irradiation for patients with invasive bladder carcinoma: a preliminary report of tolerance and local response.
J Urol 132:899-903, 1984

Siegenthaler WE, Bonetti A, Luthy R: Aminoglycoside antibiotics in infectious diseases.
Am J Med 80:2-14, 1986

Solomon S: Developmental changes in nephron number, proximal tubular length and superficial nephron glomerular filtration rate of rats.
J Physiol 272:573-589, 1977

Solomon S, Iaina A, Eliahou H: Possible determinants of plasma renin activity in infant rats.
Proc Soc Exp Biol Med 153:309-319, 1976

Stark JJ, Howell SB: Nephrotoxicity of Cis-platinum(II)dichlorodiammine.
Clin Pharmacol Ther 23:461-466, 1978

Stephenson JL: Intoduction to Symposium: Renal concentrating mechanism.
Fed Proc 42:2379-2385, 1983

Stewart FA, Oussoren Y, Luts A, Begg AC, Dewit L, Lebesque J, Bartelink H. Repair of sublethal radiation injury after multiple small doses in mouse kidney: An estimate of flexure dose.
Int J Rad Oncol Biol Phys 13:765-772, 1987

Stewart FA, Soranson, JA, Alpen EL, Williams MV, Denekamp J: Radiation-induced renal damage: the effects of hyperfractionation.
Rad Res 98:407-420, 1984

Tulkens PM: Experimental studies on nephrotoxicity of aminoglycosides at low doses.
Am J Med 80:105-114, 1986

Van der Kogel AJ: Late effets of radiation on the spinal cord. Dose-effect relationship and pathogenesis.
Thesis, University of Amsterdam, 1979

Van Rongen E, Tan CHT, Durham SK: Late functional, biochemical and histological changes in the rat lung after fractionated irradiation to the whole thorax.
Radiother Oncol 10:231-246, 1987

Vietti TJ, Nitschke R, Starling KA, van Eys J: Evaluation of cis-Dichloro-diammineplatinum (II) in children with advanced malignant diseases: Southwest Oncology Group Studies.
Cancer Treat Rep 63:1611-1614, 1979

Vimtrup BJ: On the number, shape, structure and surface area of the glomeruli in the kidneys of man and mammals.
J Anat 41:123-151, 1928

Voute PA, Barrett A, Bloom HJG, Lemerle J, Neidhardt MK (Eds.): Cancer in children. Clinical management. Springer Verlag, Berlin, 1981

Ward JM, Fauvie KA: The nephrotoxic effects of Cis-diamminedichloroplatinum (II) (NSC-119875) in male F344 rats.
Toxicol Appl Pharm 38:535-547, 1976

Whelton A, Neu HC (Eds.): The aminoglycosides. Microbiology, Clinical use, and toxicology. Marcel Dekker Inc., New York, 1982

White DC: The histopathological basis for functional decrements in late radiation injury in diverse organs.
Cancer 37:1126-1143, 1976

Wieman MC, Calabresi P: Pharmacology of antineoplastic agents.
In: P Calabresi, PS Schein, SA Rosenberg (eds.), Medical Oncology. Basic principles and clinical management of cancer. Macmillan Publ. Comp., New York, 1985, pp 292-362

Womer RB, Pritchard J, Barratt TM: Renal toxicity of Cisplatin in children.
J Ped 106:659-663, 1985

CHAPTER 2

RADIATION NEPHROPATHY IN YOUNG AND ADULT RATS

H.T.M. Jongejan, M.D. (1), A.J. van der Kogel, Ph.D. (2), A.P. Provoost, Ph.D. (1), J.C. Molenaar, Ph.D. (1).

1. Department of Pediatric Surgery, Erasmus University Medical School, Rotterdam, The Netherlands.
2. Los Alamos National Laboratory, Life Sciences Division, Los Alamos, N.M.875545, USA.

(This chapter has been published in: Int J Radiat Oncol Biol Phys 13: 225-232, 1987)

Abstract

The effects of bilateral kidney irradiation were compared in young and adult rats. During a 1 year period after a single dose of 0, 7.5, 10, 12.5, or 15 Gy on both kidneys, renal function (glomerular filtration rate and effective renal plasma flow), urine composition, and systolic blood pressure were measured periodically. The first changes after irradiation were observed in the glomerular filtration rate and urine osmolality. One month after 10, 12.5, and 15 Gy, glomerular filtration rate (GFR) and urine osmolality had declined below control values in the young rats. After this initial decline, renal function increased at control rate or even more during the third and fourth month after irradiation but decreased progressively thereafter. In the adult rats, GFR and urine osmolality started to decrease 3 months after 10, 12.5, and 15 Gy. A rise in systolic blood pressure and proteinuria started 2-3 months after 12.5 and 15 Gy in both age groups. Early changes in the glomerular filtration rate with a drop in urine osmolality in young rats, occurring during a period of rapid renal development indicated an irradiation-induced inhibition of glomerular and tubular development. Although renal function deteriorated at a later time in adult rats, dose-response relationships obtained in young and adult rats did not show significant differences.

Key words: Kidney-irradiation, Renal function, Blood pressure, Young and adult rat.

Introduction

The high radiosensitivity of the kidney has long been recognized (13) and has been the subject of many clinical and experimental studies. Radiation nephropathy manifests itself months to years after irradiation. Clinically, radiation nephropathy is characterized by proteinuria, oliguria, azotemia, hypertension and anemia. The urine shows albumin and cellular and hyaline casts (12, 21). Differences in latency time and severity of the symptoms have led clinicians to distinguish diverse clinical pictures (3, 12, 13). However, there seem to be no fundamental differences between the types of radiation nephropathy. Animal studies have shown that radiation nephropathy is more severe and occurs earlier with increasing dosage (23). Consequently the different types of radiation nephropathy might well be caused by different radiation doses to the kidney. Improved life expectancy of irradiated cancer patients has stressed the importance of this typically late occurring radiation nephropathy. This is especially true for young individuals.

As the radiosensitivity of a tissue or organ is usually highest during periods of proliferative activity, the growing kidney of a young individual seems to be at increased risk. However, the doses recommended for several organs, including the kidney, are in the same range for children and adults (16). As the information about the effects of radiation on developing kidneys is very limited, we compared the effects of single dose bilateral kidney irradiation in young and adult rats. During 1 year follow-up the glomerular filtration rate (GFR), effective renal plasma flow (ERPF), urine composition, systolic blood pressure (SBP), and hemoglobin (Hb) concentration were measured at regular time intervals in the same rats.

Methods and Materials

Male rats of an inbred Wistar strain (WAG/Rij) were used for this study. Young rats were just weaned, 3-4 weeks old with a body weight (BW) of 45-75 g, and adult rats were at least 12 weeks old with a BW of 180-260 g. Food and water were available ad libitum.

For the irradiation, an X ray generator (Philips-Muller, Philips, Eindhoven, the Netherlands) was used at 300 kV and 10 mA with a 1 mm Cu filter. The dose rate was 3 Gy/min. The focus-skin distance was 182 mm. The radiation dose was calculated from the midplane of the kidney. While the rat was under ethrane anesthesia, the kidney was palpated and localized on the edge of a circular (Ø= 3cm) radiation field. Both kidneys were irradiated sequentially, while the rat was in a supine position. The gut was kept outside the radiation field. Control animals were treated likewise, but not irradiated.

Renal function was determined by a radio-isotope clearance technique that permitted repeated measurements in the same animal (15). After anesthetizing the rats with pentobarbital (6 mg/100g), they were injected i.v. (vena sublingualis in young rats, dorsal vein of the penis in the adults) with 125-I-hippuran and 51-Cr-EDTA (Amersham International, Amersham, England). After 1 hour a blood sample was taken from the orbita. The GFR and the ERPF were calculated from the activity of respectively 51-Cr-EDTA and 125-I-hippuran left in the plasma. The formula used for these calculations was:

$$C = Vd/t.\ln P0/Pt$$

where C is the clearance of 51-Cr-EDTA or 125-I-IOH, Vd is the distribution

volume of each substance (ml/min), Pt is the amount of radioactvity (cpm/ml) in the plasma sample taken at t=60 min and P0=I/Vd in which I is the amount of injected radioactivity (cpm). The relationship between Vd and BW for rats of this strain was determined in a previous study (15):

for Cr-51-EDTA:

$$Vd=0.264BW-1.92x10^{-4}BW^2+1.03$$

for I-125-IOH:

$$Vd=0.439BW-6.17x10^{-4}BW^2-1.19$$

To eliminate differences in BW occurring after irradiation and to facilitate the comparison between young and adult rats, GFR was normalized for BW. In the following text GFR is expressed in ml/min/100g unless otherwise specified.

During the renal function assay, blood was collected for hemoglobin (Hb) determination. Hb was measured as a cyanomethemoglobin-complex (TOA microcellcounter, Kyoto, Japan).

To collect 24-hour-urine the rats were placed in metabolic cages (Techniplast metabolic cages, Buguggiate (VA), Italy). After an acclimatization period of 2 days, 24 hour-urine was collected during 3 days, and the urine measurements over these 3 days were averaged. Apart from urine volume, urine osmolality and the concentrations of creatinine, urea, sodium, potassium, and protein were determined. Urine osmolality was determined by freezing point depression (Vogel Micro Osmometer, Roebling, Giessen, FDR), the protein concentration was measured spectrophotometrically (Bio Rad Chemical Division, Richmond, CA). Concentrations of creatinine, urea, sodium, and potassium were determined using standard assay techniques.

The SBP was measured plethysmographically in the unanesthetized rat, using the tail cuff method (Narco Bio Systems, Houston, Texas) (6). Rats were prewarmed at an environmental temperature of 32°C during half an hour. They were placed in restraining cages during the blood pressure measurements. To familiarize to this procedure the rats were trained during 2-3 weeks. After raising the tail cuff pressure above the systolic blood pressure the pulsations disappeared. Reappearance of arterial pulsations in the tail were monitored plethysmographically and recorded. The cuff pressure at which the first arterial pulse emerged was taken as the SBP. To obtain a reliable blood pressure value from each rat, the mean of 3 consecutive readings, taken 3-4 times in one week, was calculated.

Experimental protocol

At the start of the experiment each dose group contained 10 young and 10 adult rats. All rats were subjected to measurements of renal function and SBP. Urine collection was performed in subgroups of 7 out of 10 rats of each dose group.

Renal function was measured 0.5, 1, 2, 3, 4, 5, 6, 8, and 12 months after irradiation. Simultaneously, 24-hour-urine specimens were collected, starting 1 month after irradiation. The SBP measurements were started after a training period, 8 weeks after irradiation, and were continued weekly for a period of half a year and monthly thereafter.

Statistics

Within each age group, dose groups were compared by one way analysis of variance. When the F-value indicated a significant difference ($p<0.05$) the Newman-Keuls test was applied to reveal which groups differed. Correlation between two parameters at one time point was calculated by linear regression analysis. Dose-effect curves were constructed by Probit-analysis.

Results

Renal function measurements

Young rats

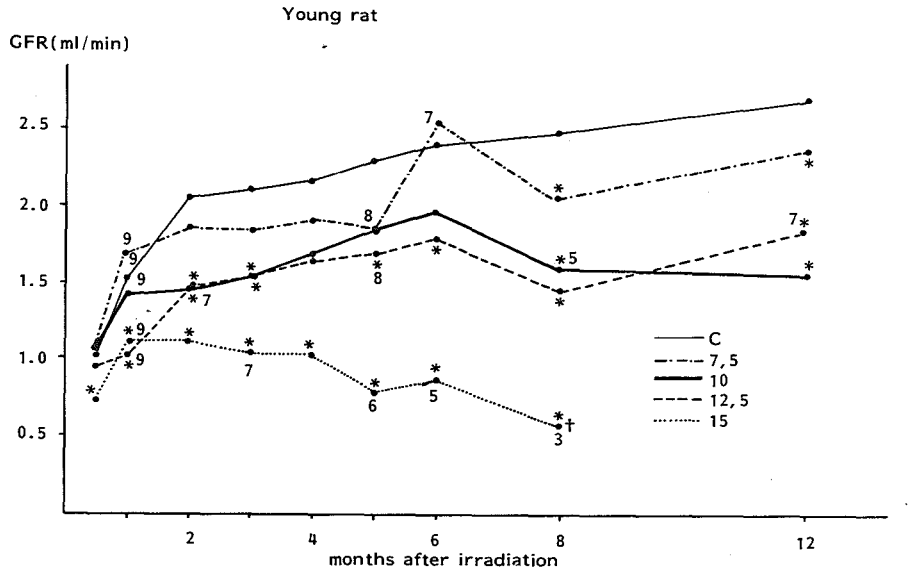


Fig 1
GFR (ml/min) during 1 year after bilateral kidney irradiation in the young rat. * Significantly different from control value, $p<0.05$. At the start of the experiment each group contained 10 rats. A change in the number of surviving rats is indicated by the figures along the lines.

In young rats the GFR (ml/min) normally increases rapidly until the age of 12 weeks, whereupon there is a slow further increase (15). This rapid renal function development is shown in the sham irradiated rats during the first 2 months after irradiation (Fig. 1). As early as 1 month after irradiation the GFR (ml/min) was significantly below control values, after doses of 10 Gy and higher. In the 15 Gy group GFR (ml/min) even declined

from 1 month after irradiation. All these rats had died by 8 months after irradiation. After the second month, renal function in the 10 and 12.5 Gy groups increased at the same rate or even faster than control values. However, 6 months after irradiation the GFR in these young rats showed a further decrease. A dose of 7.5 Gy had no effect on renal function (GFR in ml/min/100g) until at least 1 year after irradiation, when the rats were killed. After normalization for BW, the GFR in controls increased slightly during the first 2 months after irradiation, but declined slowly thereafter. After 10 Gy or more, the GFR was significantly below control values starting 1 month after irradiation (Fig. 2a).

Adult rats

In adult rats the GFR did not change significantly until 3 months after irradiation. After 3 months there is a gradual, slowly progressive decline in GFR in the 12.5 and 15 Gy group. In the 10 Gy group this decline started half a year after irradiation. As in young rats, the 7.5 Gy dose did not affect the GFR within 1 year after irradiation (Fig. 2b).

In both young and adult rats, changes in ERPF paralelled changes in GFR, but were less marked.

Thus, after irradiation, a decline of renal function started earlier in young rats compared to adult rats. However, in both age groups the decline was progressive, and after 5 months had reached a severe, but not fatal, level of renal function in young and adult rats. At this time point, dose-effect curves were constructed. For each dose group, the percentage of rats with a GFR of 75% or less of the control value, was calculated. The dose-effect curves thus constructed 5 months after iradiation were not significantly different for young and adult rats. Probit analysis of these curves yielded an ED50 (dose which causes the defined effect in 50% of the rats) of 11.4 Gy in young rats and of 12.2 Gy in adult rats, which was not

significantly different.

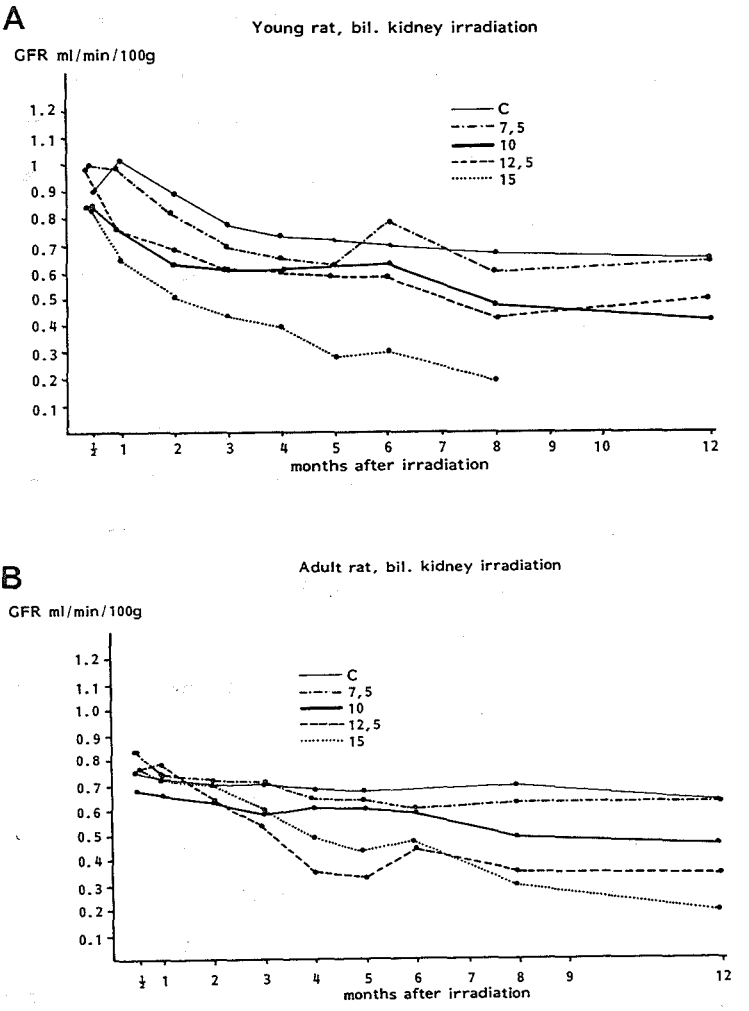


Fig 2
GFR (ml/min/100g) during 1 year after bilateral irradiation in young rats (a) and in adult rats (b). * Significantly different from control value, $p<0.05$. At the start of the experiment each group contained 10 rats. A change in the number of surviving rats is indicated by the figures along the lines.

Urine measurements

Of all urine parameters measured, the osmolality showed the first clear changes in both age groups. Figure 3a and b show the mean values of urine

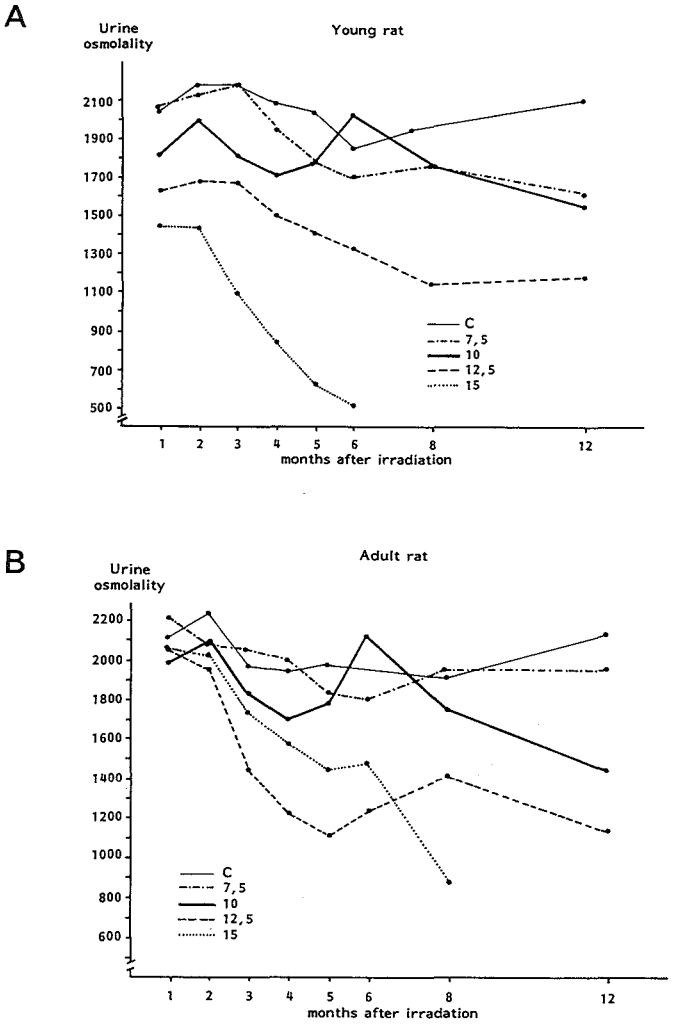


Fig 3
Urine osmolality (mOsmol/kg) during 1 year after bilateral kidney irradiation in young rats (a) and in adult rats (b). *Significantly different from control value, $p<0.05$. At the start of the experiment each group contained 7 rats. A change in the number of surviving rats is indicated by the figures along the lines.

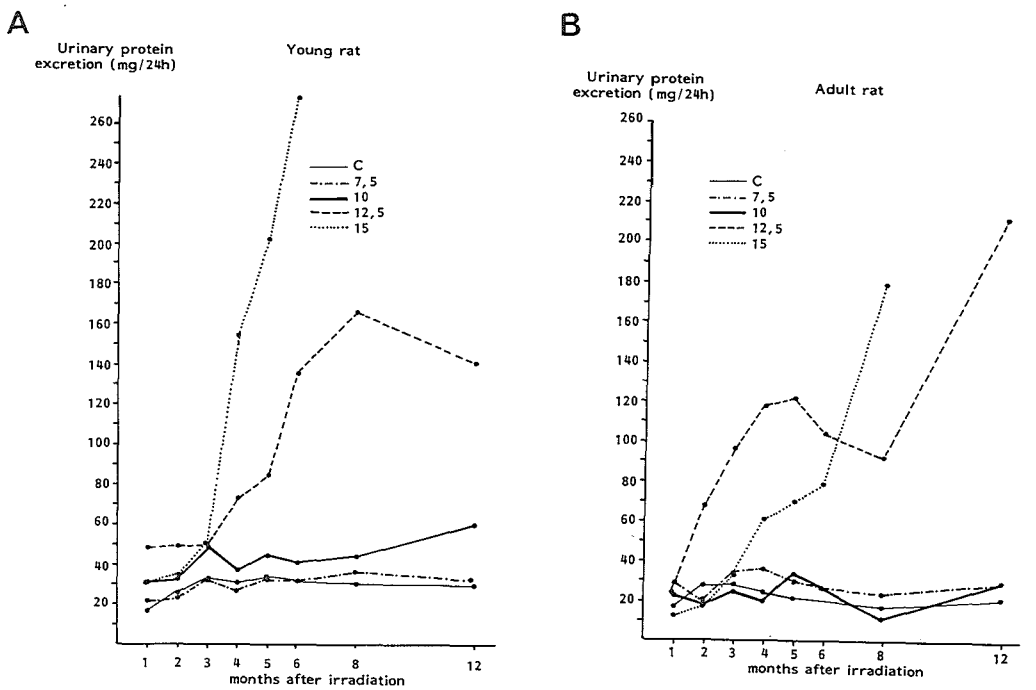


Fig 4
Urinary protein excretion (mg/24 hr/100g BW) during 1 year after bilateral kidney irradiation in young rats (a) and adult rats (b). *Significantly different from control value, $p<0.05$. At the start of the experiment each group 7 rats. A change in the number of surviving rats is indicated by the figures along the lines.

osmolality. In young rats the first urine measurements, at 1 month after irradiation, showed a significantly ($p<0.05$) lower urine osmolality in the three highest dose groups (10, 12.5 and 15 Gy) (Fig. 3a). In adult rats (Fig. 3b) a significant reduction in urine osmolality started to occur 3 months after irradiation. In both age groups the reduction in urine osmolality was progressive. There was a strong correlation between the level of GFR and urine osmolality. The correlation coefficient ranged from 0.72 to 0.93 at different time points. As the urine osmolality decreased, the

concentrations of sodium, potassium, urea, and creatinine declined simultaneously, whereas urine production and water intake increased. The osmolar clearance (= plasma volume cleared from particles per 24 hours) did not show consistent changes in either age group after irradiation. The urinary protein excretion (mg/100g/24 hr) significantly increased in the 12.5 and 15 Gy group in young and adult rats (Fig. 4a and b), 2 to 3 months after irradiation.

Systolic blood pressure measurements

The first SBP-measurements were obtained 2 months after irradiation. In the control animals, the mean value of the SBP varied from 115-135 mmHg. In both age groups the SBP rose above 150 mmHg 3 months after irradiation in the highest dose groups (Fig. 5). The latency time for the rise in SBP varied considerably between rats in each dose group. At higer doses, the SBP rose earlier and reached a higher level.

The Hb-measurements in adult and young rats showed a decline of 5-14% of control values in the two highest dose groups at 8 months after irradiation, when renal function had declined to 28-64% of control value. Early changes were not observed for this parameter.

To determine which of the parameters changed simultaneously, correlation coefficients were calculated between GFR, SBP, urine osmolality, and urinary protein excretion. As would be expected a good correlation (r=0.78) was found between GFR and urine osmolality, 1 month after irradiation in young rats. In adult rats a good correlation between these 2 parameters was reached after 3 months. Five months after irradiation, correlation coefficients between all parameters were more than .85 in both age groups, indicating consistent changes of these parameters in the individual rat after irradiation.

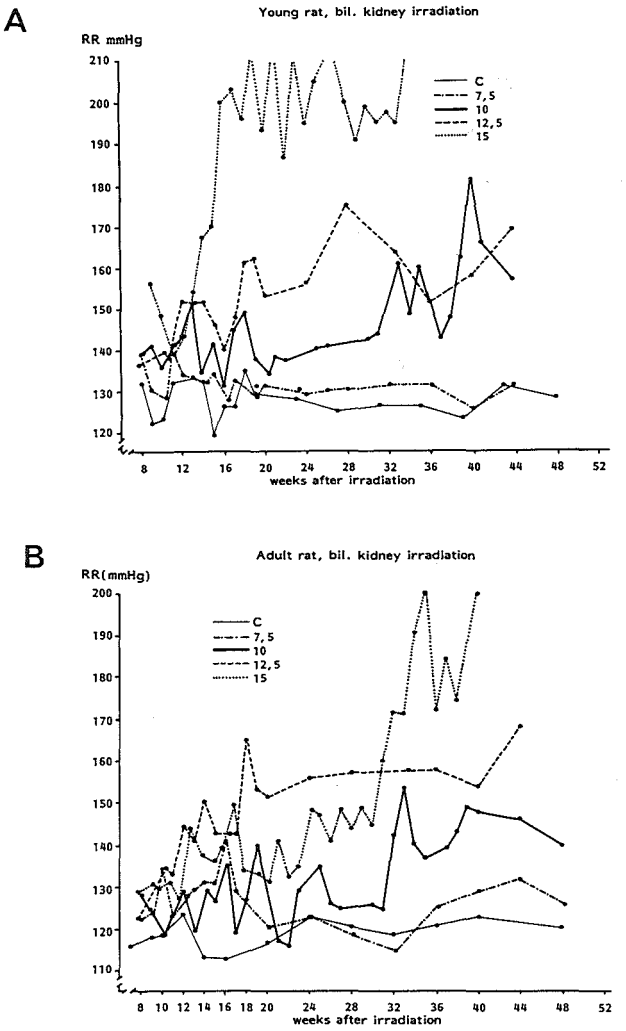


Fig 5
SBP (mmHg) during 1 year after bilateral kidney irradiation in young rats (a) and in adult rats (b). At the start of the experiment each group contained 10 rats. A change in the number of surviving rats is indicated by the figures along the lines.

Discussion

To compare the radiation nephropathy which develops after bilateral kidney irradiation in young and adult rats, renal function, urine composition, and SBP were measured during 1 year after irradiation. With the described technique of localized kidney irradiation, the small intestine and other abdominal organs were not included in the radiation field. We did not observe any gastro-intestinal problems, nor adhesion formation in our rats.

From the parameters measured, the GFR and urine osmolality showed the earliest changes in both age groups after irradiation, indicating a glomerular as well as a tubular component of damage. The urine osmolality decreased whereas the osmolality clearance remained relatively constant, indicating that the free water reabsorption was depressed. This early decline in free water reabsorption has also been reported to occur within 1 month after unilateral kidney irradiation in the dog, whereas the GFR was still normal (5, 7). These authors concluded that the tubule was the primary site of radiation damage. Our results showed a comparable and simultaneous decline in GFR and urine osmolality and hence do not permit conclusions about differences in radiation sensitivity between tubules and glomeruli. Using other parameters, radiation doses and schemes or animal models, other investigators suggested the glomeruli (11) or the microvasculature (19) as the site of initial pathologic changes. The mild changes in ERPF we observed do not indicate the presence of early gross abnormalities in renal perfusion, but tell us nothing about the microvasculature perfusion. Consequently, the pathogenesis of the radiation nephropathy remains controversial. The direct functional relationships between tubuli, glomeruli, and blood vessels will not enable conclusions on damage developing in separate compartments.

For the calculation of GFR and ERPF from radio-activity left in the plasma sample, the distribution volume (Vd) was determined using formulas derived from previous studies in healthy rats (15). Although Vd could be influenced by irradiation, differences in the actual Vd from that used in the formulas will have relatively small effect on the calculated GFR (14).

The systemic parameters, the SBP as well as Hb-concentration, only showed relatively late changes occurring well after the development of functional kidney damage. Thus, these parameters do not seem to be indicative of early changes, and appear to be secondary to other functional impairments. The SBP rose after the GFR had declined to 50-60% of control values. This occurred between the third and fourth month in the highest dose-groups in young as well as in adult rats. With increasing dosage, higher SBP values were reached at shorter time intervals after irradiation. In the literature, comparable latency times and SBP-levels at similar dose levels were found (10, 24). The relationship between hypertension, the development of renal damage, and functional or morphologic renal vascular alterations is complex. Wachholz and Casarett (19) showed that whole body irradiation with shielded kidneys caused a similar degree of hypertension as bilateral kidney irradiation with twice the dose. The vascular and parenchymal damage was more pronounced in the irradiated kidneys than in the shielded ones. These authors surmised that irradiation causes vascular and renal parenchymal damage. In the kidney, these two types of radiation injury may potentiate each other when irradiation sensitizes the vessels to hypertensive injury, as was found by Asscher et. al. (2). Hypertension occurring after predominant unilateral renal irradiation has been alleviated by nephrectomy of the diseased kidney, both clinicaly (4, 8) and experimentally (10, 24). The resemblance to the Goldblatt hypertension, induced by renal artery constriction, has focused the attention on the causative role of the

renin-angiotensin system in the development of hypertension and renal damage after irradiation. In the clinical reports mentioned above (4, 8) the renal artery of the diseased kidney did not function. The renal artery obstruction may be due to extensive vascular damage caused by the high radiation dose (Crummy:40Gy; Bloomfield:55Gy). The high renin concentrations in plasma and kidney found by Bloomfield et. al. (4) thus could be explained by the Goldblatt mechanism and are not specific for radiation nephropathy. Experimentally, Wilke et. al. (22) found no rise in plasma renin activity after low dose whole body irradiation of the newborn dog. However, these dogs only showed a transient rise in SBP. Hypergranulation of the juxta-glomerular apparatus of the irradiated kidney in hypertensive rats was only present after unilateral kidney irradiation, not after bilateral kidney irradiation (10). In this experiment the renin concentrations were not measured. Our own preliminary data after bilateral kidney irradiation do not show impressive changes in plasma renin activity, when SBP rises, nor was there an early decline in ERPF. The earliest decrement in ERPF occurred 1 and 4 months after a dose of 15 Gy in young and adult rats, respectively. Likewise, Zaruba (25) did not find any consistant changes in BP, GFR, or ERPF until 6 weeks after bilateral kidney irradiation in the dog. However, after unilateral irradiation of the rabbit kidney, microangiographic studies showed a shunting of blood from outer cortical glomeruli to juxta-medullary glomeruli, as early as 2 weeks after 10 Gy. No hypertension occurred in these rabbits (17). Consequently, despite clinical and experimental indications, it has not been proved unequivocally that the renin-angiotensin system is involved in the development of radiation induced hypertension. Perhaps the renin-angiotensin system only plays a role in hypertension occurring in serious, fully developed unilateral radionephropathy.

In contrast to our observation in the rat, early and dose dependent

changes in Hb-concentration were reported after kidney irradiation in the mouse (1). The irradiation model used by these authors included a part of the small intestine in the radiation field. Gastro-intestinal damage and resulting malabsorption might have contributed to the anemia. Hematologic characterization of this anemia should make clear whether it is comparable with the normochromic, normocytic anemia, typically occurring in renal insufficiency (12). The difference in species may also be responsible for the divergence in hematologic response to renal irradiation between mice and rats.

The changes in GFR and urine osmolality occurred earlier when the rats were irradiated at weanling age than when this was done at an adult age. Particularly during the first 2 months after irradiation of the young rats, the GFR did not increase as rapidly as in control rats. This may represent an inhibition of renal growth, coinciding with the rapid renal development in the young rat. At the time of the irradiation, the kidneys were already completely differentiated, all nephrons having been formed. The proximal tubular length, single nephron GFR (18), and total GFR (15) increase rapidly during this period. This growth render both glomeruli and tubuli sensitive to irradiation.

In earlier studies of the radiation effect on weanling kidneys no direct comparison with adult animals was made, and no functional data were obtained. Wachtel et. al. (20) found the mitotic activity of the weanling mouse kidney to be reduced dose dependently 2 days after whole body irradiation (0.5-40 Gy) and controlateral nephrectomy. Of the remaining kidney, weight and DNA content were reduced 3 weeks after 20 Gy. Doses of 5 and 10 Gy only temporarily depressed kidney weight in unilateral nephrectomized weanling rats (9). Higher doses caused a permanent renal weight depresssion, starting 3 weeks after irradiation. Our data on renal

function measurements after 10 Gy to the weanling kidney also show a slight increase 3 to 4 months after irradiation. Thus, it appears that after low doses of irradiation the weanling kidney may keep some capacity for regeneration. Interestingly, in the chronic phase there was only a slight difference between the dose-response curves for a 25% or more reduction in GFR for young and adult rats. The weanling kidney manifested damage faster than the adult kidney but eventually there was only little difference in renal damage between young and adult kidneys. The early radiation nephropathy we observed in young rats differed from the early phase of the radiation nephropathy described clinically in adults. Early clinical radiation nephropathy is characterized by malignant hypertension and a rapid decline in renal function with poor prognosis (13). In the young rats renal function was less than 50% lower than that of controls in the highest dose group. The decline in GFR was progressive in the 15 Gy group, but in the 10 and 12.5 Gy group renal function remained stable during several months, after which the GFR decreased further.

In conclusion, the GFR and urine osmolality are sensitive markers of radiation nephropathy in the rat. After bilateral kidney irradiation, an early inhibition of GFR development and urine osmolality depression occurred in the young rats but not in the adults ones. This early deterioration of renal function in young rats might be caused by an inhibition of renal growth or development. In both age groups, the decline in renal function was progressive and dose-dependent. No marked differences emerged between the radiosensitivity of young and adult rat kidneys from the dose-response curves.

References

1. Alpen, E.L., Stewart, F.A.: Radiation nephritis and anaemia: a functional assay for renal damage after irradiation. Brit. J. Radiol. 57:185-187,1984.

2. Asscher, A.W., Wilson, C., Anson, S.G.: Sensitisation of blood-vessels to hypertensive damage by X-irradiation. Lancet 1:580-583,1961.

3. Avioli L.V., Lazor, M.Z., Cotlove, E., Brace, K.C., Andrews, J.R.: Early effects of radiation on renal function in man. Am. J. Med. 34:329-337,1963.

4. Bloomfield, D.K., Schneider, D.H., Vertes, V.: Renin and angiotensin II studies in malignant hypertension after X-irradiation for seminoma. Annals Int. Med. 68:146-151, 1968.

5. Buerkert, J., Doyle, J.,Ewald, W.: Effects of local irradiation on the excretion of sodium and water by the canine kidney. Rad. Res. 66:346-362, 1976.

6. Bunag, R.D.: Validation in awake rats of a tail-cuff method for measuring systolic pressure. J. Appl. Physiol. 34:279-282, 1973.

7. Coburn, J.W., Rubini, M.E., Kleeman, C.R.: Renal concentrating defect in canine radiation nephritis. J. Lab. Clin. Med. 67:209-223, 1966.

8. Crummy, A.B., Hellman, S., Stansel, H.C., Hukill, P.B.: Renal hypertension secondary to unilateral radiation damage relieved by nephrectomy.Radiol. 84:108-111, 1965.

9. Donaldson, S.S., Moskowitz, P.S., Canty, E.L., Efron, B.: Radiation-induced inhibition of compensatory renal growth in the weanling mouse kidney. Radiol. 128:491-495,1978.

10. Fisher, E.R., Hellstrom, H.R.: Pathogenesis of hypertension and pathologic changes in experimental renal irradiation. Lab. Invest. 5:530-538, 1968.

11. Glatstein, E., Fajardo, L. F., Brown, J.M. Radiation injury in the mouse kidney-I Sequential light microscopic study. Int. J. Rad. Oncol. Biol. Phys. 2:933-943, 1977.

12. Greenberger, J.S., Weichselbaum, R.R., Cassady, J.R.: Radiation nephropathy. In: Cancer and the kidney. Editors: R. E. Rieselbach, M. B. Garnick, Philadelphia, Lea Febiger, 1982, pp.814-823.

13. Luxton, R.W., Kunkler, P.B.: Radiation Nephritis. Acta Radiol.2:169-178,1964.

14. Provoost, A.P., de Keyzer, M.H., Kort, W.J., Wolff, E.D., Molenaar, J.C.: The glomerular filtration rate of isogeneically transplanted rat kidneys. Kidney Int. 21:459-465,1982.

15. Provoost, A.P., de Keijzer, M.H., Wolff, E.D., Molenaar, J.C.: Development of renal function in the rat. Renal Physiol. 6:1-9,1983.

16. Rubin, P., Van Houtte, P., Constine, L.: Radiation sensitivity and organ tolerances in pediatric oncology: a new hypothesis. Front. Radiat. Ther. Onc. 16:62-82,1982.

17. Scanlon, G.T.: Vascular alteration in the irradiated rabbit kidney. Radiol. 94:401-406,1970.

18. Solomon, S.: Developmental changes in nephron number, proximal tubular length and superficial nephron glomerular filtration rate of rats. J. Physiol. 272:573-589, 1977.

19. Wachholz, B.W., Casarett, G.W.: Radiation hypertension and nephrosclerosis. Rad. Res. 41:39-56,1970.

20. Wachtel, L.W., Cole, L.J., Rosen, V.J.: Abscopal and direct effects of whole-body X-irradiation in weanling rats: kidney mitotic activity and DNA-content after uninephrectomy. Int. J. Rad. Biol. 10:75-82, 1966.

21. White, D.C.: The histopathological basis for functional decrements in late radiation injury in diverse organs. Cancer 37:1126-1143, 1976.

22. Wilke, W.L., Jaenke, R.S., Phemister, R.D.: Neonatal irradiation nephropathy in the growing dog II Plasma renin activity and arterial pressure following neonatal, sublethal, whole-body irradiation. Rad. Res. 78:72-81, 1979.

23. Williams, M.V., Denekamp, J.: Radiation induced renal damage in mice: influence of fraction size. Int. J. Rad. Oncol. Biol. Phys. 10:885-893, 1984.

24. Wilson, C., Ledingham, J.M., Cohen, M.: Hypertension following X-irradiation of the kidneys. Lancet 1;9-16, 1958.

25. Zaruba, K. Effect of local irradiation of the kidneys on the renal function of the dog. Rad. Res. 35:661-667, 1968.

Acknowledgements
We thank TNO (Rijswijk, the Netherlands) (head: Prof Dr D.W. van Bekkum) for the use of their X ray equipment. The technical assistance of Mrs E. Fierret was appreciated. Mrs A. Ribbink-Goslinga was of great help as a stylistic editor.

CHAPTER 3

HYPERTENSION AFTER BILATERAL KIDNEY IRRADIATION IN YOUNG AND ADULT RATS

H.T.M. Jongejan, M.D. (1), A.J. van der Kogel, Ph.D. (2), A.P. Provoost, Ph.D. (1), J.C. Molenaar, M.D., Ph.D. (1).

1. Department of Pediatric Surgery, Erasmus University Medical School, Rotterdam, The Netherlands.
2. Los Alamos National Laboratory, Life Sciences Division, Los Alamos, N.M. 87545, U.S.A.

(This chapter has been published in: Rad Res 111: 474-487, 1987)

Abstract

The mechanism of a rise in blood pressure after kidney irradiation is unclear but most likely of renal origin. We have investigated the role of the renin-angiotensin system and dietary salt restriction on the development of systolic hypertension after bilateral kidney irradiation in young and adult rats. Three to 12 months after a single X-ray dose of 7.5, or 12.5 Gy to both kidneys of young and adult rats, the systolic blood pressure (SBP) and plasma renin concentration (PRC) were measured regularly. A single X-ray dose of 12.5 Gy caused a moderate rise in SBP and a slight reduction in PRC in both young and adult rats. A dose of 7.5 Gy did not significantly alter the SBP or PRC during the follow-up period of one year. In a second experiment the kidneys of young rats received an X-ray dose of 20 Gy. Subsequently, rats were kept on a standard diet (110 mmol sodium/kg) or a sodium-poor diet (10 mmol sodium/kg). On both diets, SBP started to rise rapidly three months after kidney irradiation. Sodium balance studies carried out at that time revealed an increased sodium retention in the irradiated rats compared to controls on the same diet. In rats on a low sodium intake there was neither a delay nor an alleviation in the development of hypertension. Compared to controls, the PRC tended to be lower in irradiated rats up to four months after irradiation. Subsequently, malignant hypertension developed in all 20 Gy rats, resulting in pressure natriuresis, stimulating the renin-angiotensin system. Our findings indicated that hypertension after bilateral kidney irradiation was not primarily the result of an activation of the renin-angiotensin system. Although there were some indications that sodium retention played a role, dietary sodium restriction did not influence the development of hypertension.

Introduction

Hypertension as a complication of renal radiation therapy is a serious clinical problem (1, 2, 3). The latency period after which hypertension occurs and the severity of the hypertension vary with radiation dosage and the amount of renal tissue irradiated. Subsequently, renal radiation damage and hypertension may lead to a vicious circle, in which the hypertension accelerates the development of renal damage.

In previous experiments we studied the effect on renal function and systolic blood pressure (SBP) of a single X-ray dose to both kidneys in young and adult rats (4). In both age groups the SBP started to rise when renal function, as indicated by the glomerular filtration rate (GFR), was already severely impaired. The latency period after which the rise in SBP occurred was inversely related to the dose.

The pathogenesis of radiation hypertension is unclear. However, after irradiation of the kidneys it is most likely that the mechanisms underlying the rise in SBP are of renal origin. The kidney plays an important role in blood pressure regulation in at least three different ways. First, the kidney is crucial for maintaining fluid and electrolyte homeostasis (5). Second, renin, which is produced and released by the kidney, is an important component of the vasoconstricting renin-angiotensin system (6). Finally, the kidney produces substances that have a blood pressure-lowering effect (7). In the current view on the pathophysiology of hypertension, blood pressure may be elevated by volume retention and/or vasoconstriction (8, 9, 10). The relative contribution of each factor to the hypertension may vary with time and underlying pathology. Vascular lesions, resulting from hypertension, cause ischemia and may potentiate the hypertension.

We felt the need for more insight into the renal contribution to the development of hypertension after bilateral kidney irradiation. The activity of the renin-angiotensin system as well as the influence of sodium balance were investigated in two experiments. In the first experiment, the plasma renin concentration (PRC), the SBP and sodium balance were determined regularly in young and adult rats during a 1-year follow-up period post irradiation. In the second experiment the effect of a dietary sodium restriction on these parameters as well as on renal function was studied in young rats only, during half a year post irradiation.

MATERIALS AND METHODS

Animals

Male rats of an inbred Wistar strain (Wag/Rij, TNO, Rijswijk, The Netherlands) were used for this study. The young rats were just weaned and 3 weeks old (with body weight (BW) of 45-65g); the adult rats were over 12 weeks old (with BW of 180-250g).

The animals were fed with standard rat chow (AMII, Hope Farms, Linschoten, The Netherlands) containing 110 mmol sodium/kg and 190 mmol potassium/kg. In the second experiment half of the animals were fed with a sodium-poor rat chow (Hope farms, Linschoten, The Netherlands) containing 10 mmol sodium/kg and 100 mmol potassium/kg. There was also a small difference in protein content between the diets (18% in sodium restricted diet, 24% in standard diet). Tap water contained 2.2 mmol sodium/L and 0.1 mmol potassium/L.

Irradiation

For irradiation of the kidney, a Philips-Muller X-ray generator with a 1 mm Cu filter was used, at 300 kV and 10 mA (3 Gy/min). The focus-skin distance was 182 mm. The radiation dose was calculated from the midplane of

the kidney. The kidneys were irradiated sequentially. The kidney was palpated, moved laterally and fixed in a circular radiation field by placing a cylinder-like mall over the kidney. At the kidney hilus a semi-circled excision permitted the passage of vasculature and ureter. The position of the kidneys in the radiation field was checked radiographically several times. During irradiation the rat was under ethrane anesthesia. No small intestine or other organs were in the radiation field (4). Controls underwent a similar, sham procedure excluding irradiation.

Systolic blood pressure

The SBP was measured plethysmographically using the tail-cuff method in the unanesthetized rat (Electro Sphygmo Manometer PE 300, Narco Bio Systems, Houston, Texas). Rats were prewarmed at an environmental temperature of 32*C for 30 min. During the blood pressure measurement the rat was placed in a restraining cage. The tail cuff was inflated until the pressure exceeded the SBP. During gradual deflation of the cuff, the pressure at which the first arterial pulsation occurred was taken as the SBP. The rats were trained for 2-3 weeks to familiarize them with this procedure. To obtain a reliable blood pressure value from each rat, the mean was calculated of three consecutive readings, taken three to four times in one week.

Plasma renin concentration

For the PRC measurement, a venous blood sample was collected by orbital puncture in ice-cold EDTA syringes, shortly after inducing anesthesia with pentobarbital (6 mg/100g BW). Care was taken to excite the animals as little as possible. After centrifugation, plasma was stored at -20*C. To determine the PRC a radioimmunoassay modified for the use of rat plasma was applied(11). This method determines the production of angiotensin I (ng AI/ml/hr) after three hours incubation of the plasma sample at pH=6.5

(phosphate buffer) in the presence of an excess of rat angiotensinogen.

Metabolic studies

The sodium balance was determined from rats placed in metabolic cages (UNO mice cages for young rats; Techniplast rat cages for adult rats). After two days of acclimatization, daily intake of tap water and food were measured and urine was collected for two days. At the start of each collecting period, cages were cleaned with demineralized water. Urine osmolality was determined by freezing point depression (Vogel Micro Osmometer, Roebling, Giessen, FRG.). Urinary sodium and potassium concentration was measured by flame photometry (Klina flame, Beckman Instruments Inc, Fullerton, CA) using lithium as an internal standard. The protein concentration in the urine was measured spectrophotometrically (Bio-Rad Chemical Division, Richmond, CA) The daily sodium intake was calculated from the ingested amount of food and drinking water. Sodium excretion was calculated from urinary sodium. Nonurinary sodium loss, e.g. in the feces, was considered to be a constant fraction (12) and not taken into account.

Renal function

Renal function was measured using a radioisotope technique (13). The activity left in the plasma 1 hour after a single i.v. injection of Cr-51-EDTA and I-125-Iodohippurate (Amersham International, Amersham, England) was used to calculate the GFR and effective renal plasma flow (ERPF), respectively.

Experimental protocol

First experiment

For 1 year after bilateral kidney irradiation of young and adult rats with a single X-ray dose of 7.5 or 12.5 Gy, the SBP, sodium balance, and PRC were studied at regular time intervals in the same rats. The same measurements were performed in control subjects, which were not irradiated.

At the start of the experiment, each group consisted of 10 rats. Urine was collected on 2 consecutive days at 1, 2, 3, 4, 5, 6, 8 and 12 months after irradiation. Starting 3 months after irradiation, the SBP and PRC were determined at the same time points.

Second experiment

Metabolic studies were performed for 24 weeks after a 20 Gy X-ray dose to both kidneys of young rats kept on a normal and a sodium-poor diet. The SBP and PRC were determined repeatedly in the same rats. There were 10 rats in each group at the start of the experiment. Urine was collected 2, 4, 8, 12, 16 and 24 weeks after irradiation. At the same time PRC was determined. SBP measurement was initiated after 5 weeks. BW was determined regularly during the follow-up period. Renal function was measured 8 and 24 weeks after irradiation.

Statistical Analysis

In the first experiment one way analysis of variance was performed within each age group to see which groups differed. When a difference was indicated ($p<0.05$) the Newman-Keuls test was used to indicate which groups differed. In the second experiment Student's t-test was applied to compare irradiated rats and controls on the same diet. A statistically significant difference was indicated by $p<0.05$. The PRC and SBP were correlated by linear regression analysis. The statistical analyses were performed using SPSS/PC-software.

RESULTS

First experiment

Except for a transient elevation at 4 months after irradiation in adult rats, a 7.5 Gy X-ray dose to both kidneys of young or adult rats did not significantly raise SBP. In both young and adult rats the mean SBP in the

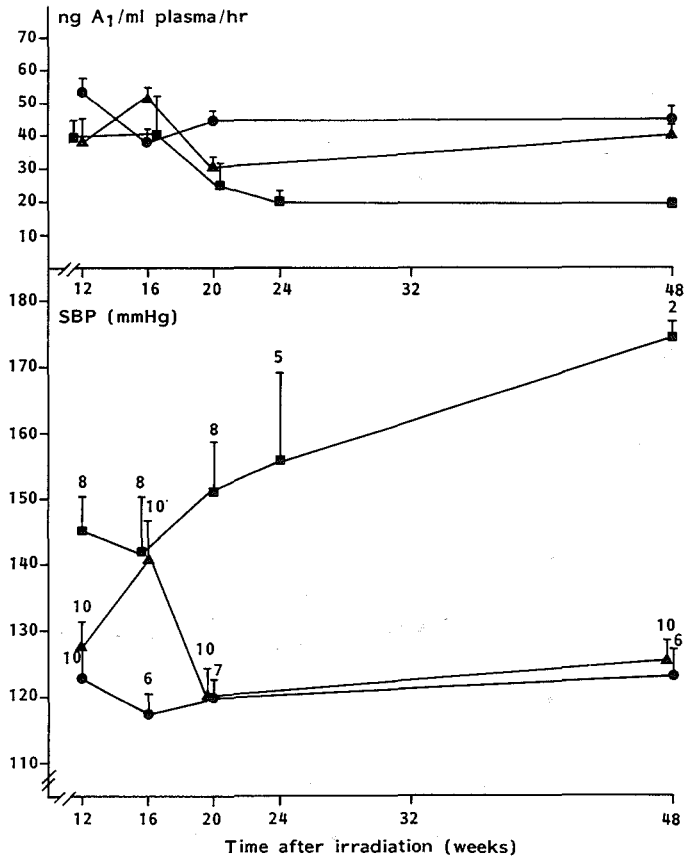


Fig 1
Longitudinal changes in PRC (ng AI/ml/hr) (upper panel) and SBP (mm Hg) (lower panel) during one year in controls (●) or after a single X-ray dose of 7.5 (▲) or 12.5 (■) Gy to both kidneys of adult rats. Data represent means and SEM. Figures indicate the number of rats in each group.
* $P<0.05$ vs controls.

12.5 Gy group was higher than control values, 3 months after irradiation and gradually increased thereafter (Fig 1 and 2). The mean PRCs in these groups were lower than control values. The mean PRCs in the 7.5 Gy groups were in the same range as control values (Figs 1 and 2). There was a slight but

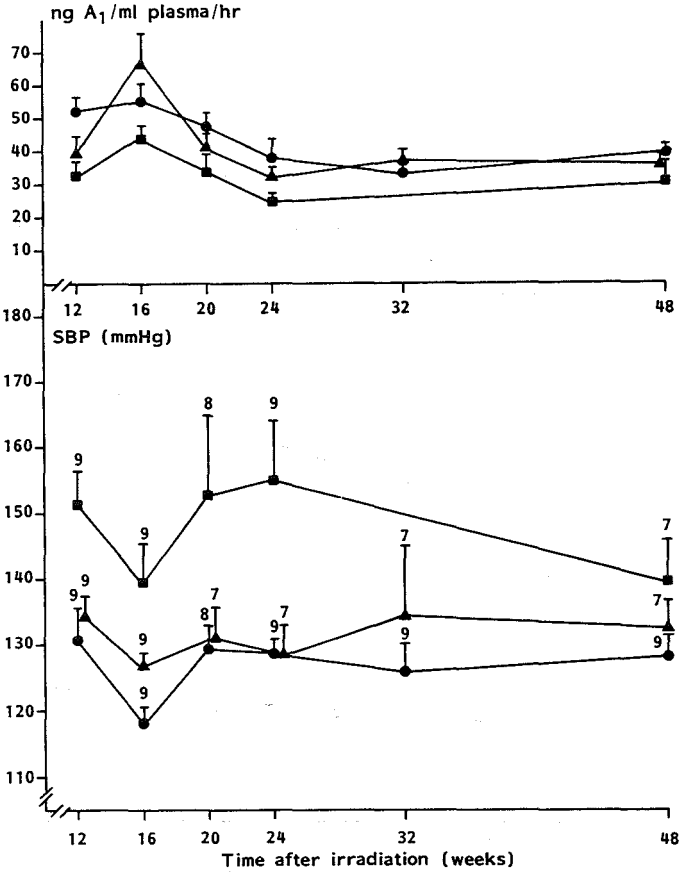


Fig 2
Longitudinal changes in PRC (ng AI/ml/hr) (upper panel) and SBP (mm Hg) (lower panel) during one year in controls (●) or after a single X-ray dose of 7.5 (▲) or 12.5 (■) Gy to both kidneys of young rats. Data represent means and SEM. Figures indicate the number of rats in each group.
*P<0.05 vs controls.

significant negative correlation between SBP and PRC (r=0.28, n=113, p<0.05 in young rats; r=0.32, n=91, p<0.05 in adult rats).

From the monthly metabolic studies, the sodium balance and sodium/potassium excretion ratio were calculated. These calculations

revealed neither a sodium retention nor a change in sodium/potassium excretion ratio, coinciding with changes in the SBP or PRC in irradiated rats.

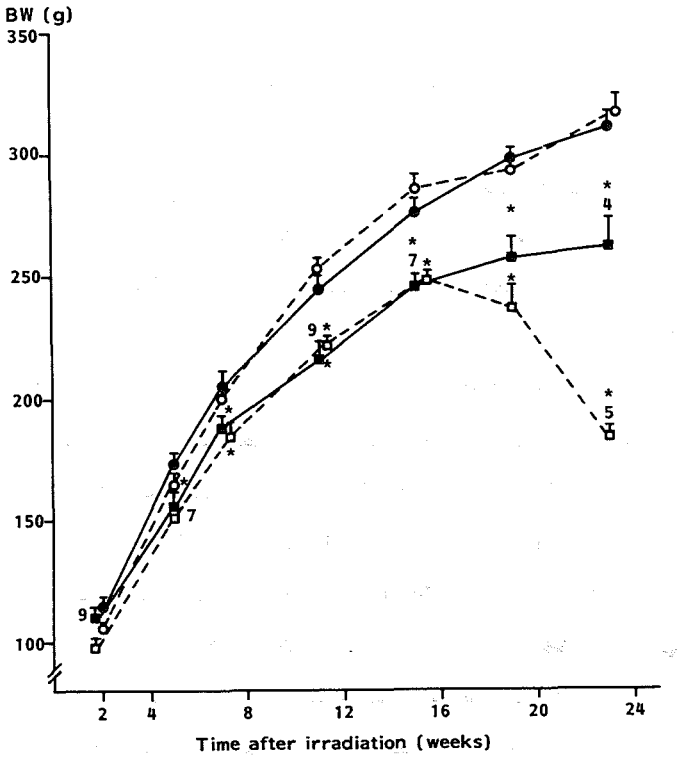


Fig 3
Longitudinal changes in BW during 24 weeks in controls (●,○) or after a single X-ray dose of 20 Gy (■,□) to both kidneys in young rats, on a standard diet (solid lines) or a sodium restricted diet (dashed lines). At the start of the experiment mean BW in the groups varied from 49 to 52g. Data represent means and SEM. Each group initially consisted of 10 rats. Figures indicate changes in group size due to death.
* $P<0.05$ vs controls.

Changes in PRC and SBP were similar in time and magnitude in young and adult rats.

Second experiment

After a single X-ray dose of 20 Gy to both kidneys of young rats, the influence of a dietary sodium restriction on SBP, PRC, sodium balance, BW and renal function were investigated. During the first 8 weeks after irradiation, the low sodium diet influenced the urine parameters and PRC in the sodium-poor diet groups. Therefore, this period will be dealt with seperately.

0-8 weeks after irradiation

Body growth was attenuated by irradiation but not influenced by the diet, indicating that the dietary sodium supply was sufficient for normal body growth (Fig 3).

Two weeks after the start of the experiment PRC was five to six times higher in controls on a sodium-poor diet than in those on a standard diet (Fig 4). At the same time, in control rats on a sodium-poor diet, water intake and urine volume per 100 g BW increased by 50-90%, compared to controls on the normal diet. Hence, urinary osmolality in the sodium-poor control group decreased to 40% of the value in the standard diet group (Fig 5). After 8 weeks, PRC in the sodium-poor control group had come down to the level of the standard diet control group, and remained at this level during the follow-up period (Fig 4). Water consumption, urine production and osmolality were also the same in both control groups by that time.

In irradiated and control rats, similar changes were induced by dietary sodium restriction in PRC and urine osmolality (Figs 4 and 5). Two weeks after renal irradiation, however, PRC was lower (fig 4) and sodium excretion was higher (Table I) in the irradiated rats compared to the controls on the sodium-poor diet. After 4 weeks PRC and sodium excretion were the same in

irradiated and control rats on a sodium-poor diet. The high PRCs 24 weeks after irradiation indicate that a high renin secreting potency remained present.

With a standard diet no differences occurred in PRC and sodium excretion between irradiated rats and controls during the first 4 weeks after irradiation. Eight weeks after irradiation, PRC was the same in the irradiated rats on either diet and lower than control values (Fig 4).

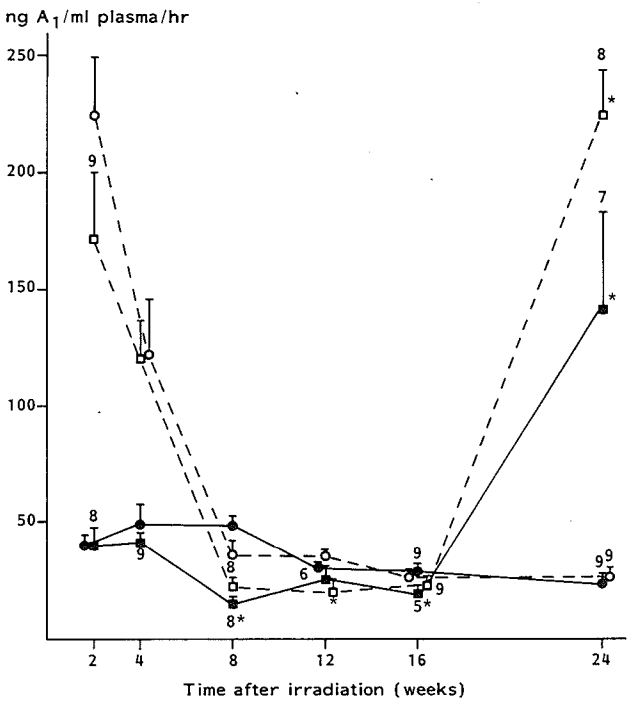


Fig 4
Longitudinal changes in PRC during 24 weeks in controls (●,○) or after a single X-ray dose of 20 GY (■,□) to both kidneys in young rats, on a standard diet (solid lines) or a sodium restricted diet (dashed lines). Data represent means and SEM. Each group initially consisted of 10 rats. Figures indicate changes in group size due to death.
* $P<0.05$ vs controls.

Urine osmolality started to decline progressively 2 weeks after irradiation in irradiated rats on a standard diet. After 8 weeks, urine osmolality was the same for irradiated rats on either diet and significantly lower than control values (Fig 5).

The mean SBP value in the irradiated rats varied from 149 to 156 mmHg in the sodium-poor diet and from 145 to 149 mmHg in the standard diet group from 5 to 11 weeks after irradiation. In controls, the SBP was lower and varied from 134 to 146 mmHg for both diets.

The GFR and ERPF in irradiated rats were significantly lower than control values on the same diet 8 weeks after irradiation. The GFR was also significantly lower in irradiated rats on a low sodium diet than in irradiated rats on a standard diet. The GFR values expressed as a percentage of the mean GFR value of control rats on the standard diet are shown in Fig. 7. On both diets the GFR was more affected than the ERPF, resulting in a fall in filtration fraction in the irradiated rats compared to control values. Urine osmolality and GFR had already declined to 40-60 % of control values 8 weeks after irradiation, when the SBP was only mildly elevated.

Eight to twenty-four weeks after irradiation

The high X-ray dose of 20 Gy to both kidneys of young rats resulted in a mild elevation of the SBP at 5 weeks after irradiation. It remained stable until 11 weeks after irradiation, increasing rapidly thereafter. This sharp rise in SBP in irradiated rats was even more pronounced in the sodium-poor diet group than in the standard diet group (Fig. 6). Coinciding with this rise in SBP, 12 weeks after irradiation, the irradiated rats on either diet excreted less sodium (expressed as a percentage of the intake) than controls, indicating sodium and water retention. However, this difference was significant only for rats on a sodium-poor diet. Also, the ratio of urinary sodium and potassium excretion was less in irradiated rats than in

controls on the same diet 8 and 12 weeks after irradiation (Table I). Again, this was significant only for irradiated rats on a sodium-poor diet.

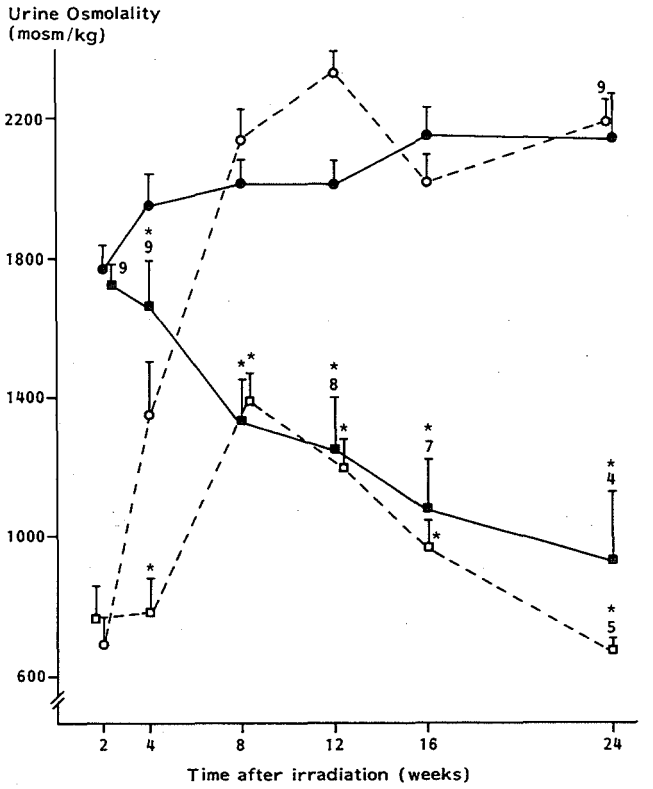


Fig 5
Longitudinal changes in Urine osmolality (Uosmol) during 24 weeks in controls (●,○) or after a single X-ray dose of 20 Gy (■,□) to both kidneys in young rats, on a standard diet (solid lines) or a sodium restricted diet (dashed lines). Data represent means and SEM. Each group initially consisted of 10 rats. Figures indicate changes in group size due to death.
* $P<0.05$ vs controls.

TABLE 1.

	Week 2		Week 4		Week 8		Week 12		Week 16		Week 24	
	C	R	C	R	C	R	C	R	C	R	C	R
						Standard diet						
No. of rats	10	9	10	9	10	9	10	9	10	7	10	7
% Na exc.	73	59	69	71	76	75	78	67	74	68	75	123*
Na/K exc.	0.60	0.64*	0.60	0.61	0.62	0.59	0.60	0.52	0.58	0.56	0.54	1.11*
						Na-poor diet						
No. of rats	10	9	10	10	10	10	10	10	10	10	10	8
% Na exc.	36	89*	27	25	46	37	53	33*	57	89	36	57*
Na/K exc.	0.10	0.22	0.05	0.05	0.07	0.04*	0.08	0.04*	0.07	0.12	0.05	0.69*

Note. The dialy urinary sodium excretion expressed as a percentage of sodium intake (% Na exc.) and the urinary sodium/potassium excretion ratio (Na/K exc.) in rats whose kidneys received a 20 Gy X-ray dose at weanling age (R) and in age-matched control rats (C) kept on a standard diet (standard) or a sodium poor diet (Na-poor) at several times after irradiation. No. of rats = number of rats in each group.

* Student's t test, $p < 0.05$.

From the 8th until the 16th week after irradiation, PRC tended to be lower in irradiated rats than in controls (Fig. 4). The rise in SBP from 16 to 24 weeks after irradiation was accompanied by a severe natriuresis which caused a negative salt balance. In the irradiated rats on a sodium-poor diet, weight loss and a decline in SBP occurred. The PRC was 6 to 8 times higher in irradiated rats than in controls. At that time the animals were in a very poor condition, suffering from end-stage renal failure, and had to be sacrificed.

The decline in urinary osmolality which had already started during the first weeks after irradiation was progressive. Urinary protein excretion was doubled by 8 weeks after irradiation, and increased to 9 to 20 times control values after 24 weeks (data not shown).

After 24 weeks, the GFR in irradiated rats declined to 43% and 21% of control values on the standard diet and the sodium-poor diet, respectively (Fig. 7). In these severely damaged kidneys, the reduction of the ERPF approached the decline in GFR. The filtration fraction was almost the same in irradiated rats and controls.

The SBP rose faster and fatal renal failure occurred earlier in irradiated rats kept on a sodium-restricted diet than in rats kept on a standard diet.

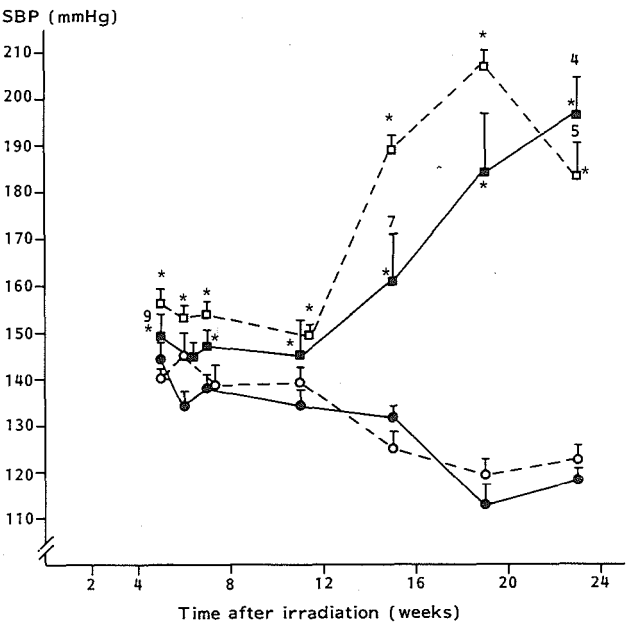


Fig 6
Longitudinal changes in SBP during 24 weeks in controls (●,○) or after a single X-ray dose of 20 Gy (■,□) to both kidneys in young rats, on a standard diet (solid lines) or a sodium restricted diet (dashed lines). Data represent means and SEM. Each group initially consisted of 10 rats. Figures indicate changes in group size due to death.
* $P<0.05$ vs controls.

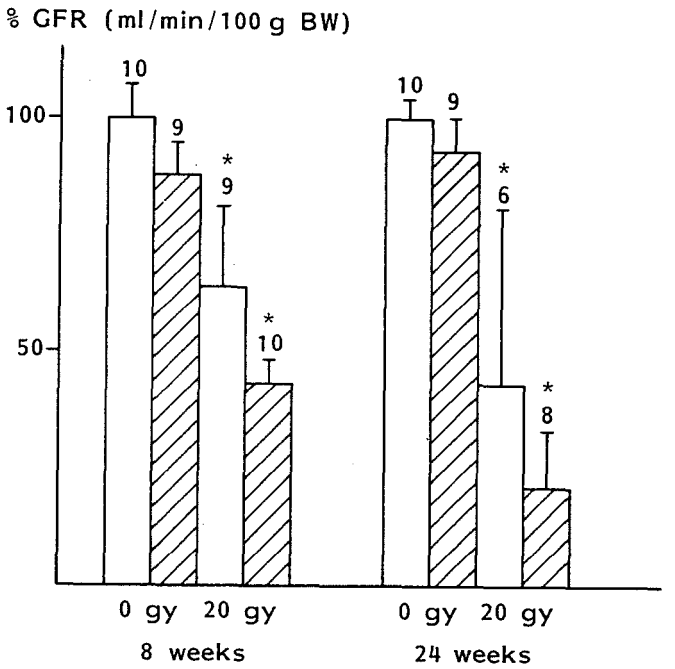


Fig 7
The GFR (ml/min/100g BW) expressed as a % of controls fed on a standard diet, determined 8 and 24 weeks after sham irradiation or a single X-ray dose of 20 Gy to both kidneys in young rats, on a standard diet (open bars) or a sodium restricted diet (hatched bars). Data represent means and SD. Figures indicate group size. * P<0.05 vs controls.

DISCUSSION

Blood pressure is determined by vascular resistance and circulating volume. These two factors reciprocally support hypertension. The magnitude by which each factor contributes to the elevation of blood pressure may vary

with the underlying cause. The relative contribution of these two factors to the development and maintenance of radiation hypertension is unclear.

Unlike radiation hypertension, renovascular hypertension has been the subject of extensive experimental research. Experimental studies on hypertension after unilateral renal artery constriction with or without nephrectomy of the contralateral kidney have made clear that the presence of a healthy, contralateral kidney is decisive for the way in which hypertension is mediated. Pathophysiologically, a fall in perfusion pressure, due to a constriction of the renal artery, initially induces sodium and fluid retention in that kidney. Sodium and fluid retention may cause an increase in circulating volume, leading to a blood pressure rise when the vascular resistance remains unchanged. When a healthy, contralateral kidney is present, however, sodium and fluid are excreted and the renin-angiotensin system becomes activated in the affected kidney (10). Thus, in the presence of a healthy, contralateral kidney, renal artery constriction induces a renin-mediated hypertension, whereas in the absence of a healthy kidney a sodium and fluid-dependent hypertension develops (10, 14).

Likewise, unilateral renal irradiation, in the presence of an intact contralateral kidney, may activate the renin-angiotensin system in the irradiated kidney. The resulting hypertension can be reversed by nephrectomy, as shown by clinical case reports (2, 3, 15, 16). However, these case reports concern seriously damaged, end-stage kidneys with an almost completely occluded renal artery. It was shown experimentally that after unilateral kidney irradiation, the development of radiation hypertension was postponed by the presence of a healthy contralateral kidney (17, 18). This suggests that volume retention might also be involved in the development of unilateral renal radiation hypertension.

In the present experiment we studied the role of the renin-angiotensin system and sodium retention in the development of hypertension after bilateral kidney irradiation in the rat. Our results show that the PRC tended to be lower in rats with radiation-induced hypertension than in normotensive controls. A low PRC is thought to be an indication for sodium retention (9). On the other hand, it can be argued that the PRC was high in relation to the SBP. Thus, a supportive role of the renin-angiotensin system in hypertension after bilateral kidney irradiation cannot be completely excluded. When the SBP passes the level of 180 mmHg, pressure natriuresis occurs (19) and the renin-angiotensin system becomes a dominating factor in sustaining the elevated blood pressure. The blood pressure regulation is taken over by the renin-angiotensin system and becomes malignant. Eventually, a negative salt balance, hypovolemia and vasoconstriction form a fatal vicious circle. Such a situation occurred 16 weeks after a X-ray dose of 20 Gy. The comparison of the changes in SBP and PRC after the low X-ray doses to both kidneys in young and adult rats showed that they were independent of age at the time of irradiation. Since this study was part of an investigation on the sensitivity of the young rat kidney to nephrotoxic insults, the second experiment concerned only young rats. Previously, we reported on differences in functional radiation damage between young and adult rats. In young rats renal function impairment developed more rapidly after irradiation compared to adult rats. Despite the earlier occurrence of radiation damage in the young rat kidney, the isoeffective doses for various renal function end points were the same in both age groups after 5 months (4).

To investigate the role of a sodium and fluid retention in radiation hypertension, dietary sodium content was lowered to 10% of the standard diet values immediately following a 20 Gy X-ray dose to weanling rat kidneys. In

agreement with the calculated sodium needs, the sodium intake on the sodium-poor diet was sufficient for normal body growth in control rats (12, 20). The observed increase of PRC due to dietary sodium restriction is a well-known phenomenon (8, 21, 22). We found a gradual decline of the PRC to normal values in control rats during prolonged sodium restriction. Studies on the changes in PRC during dietary sodium restriction are usually limited to short follow-up periods. A normalization of the PRC after 8 weeks of dietary sodium restriction in unilaterally nephrectomized rats, was previously reported from our laboratory (22). The reason for this fall in PRC is not clear. It was not accompanied by a change in either SBP or urinary sodium/potassium excretion ratio, suggesting a sensitization for one of the compounds of the renin-angiotensin system, as has been described for angiotensin II. (9).

Renin stimulates drinking behavior (23). As a consequence polydipsia, polyuria and low urine osmolality were present. The high PRC obtained in the first weeks after the start of the low sodium diet did not influence blood pressure in controls or irradiated rats. This can be explained by the decreased pressor response to angiotensin II (24). Concomitantly, aldosterone stimulation was found to be increased (24). Changes in the plasma aldosterone activity may be reflected by a change in the urinary sodium/potassium ratio. When the SBP started to rise at 12 weeks after a 20 Gy X-ray dose to both kidneys, this ratio was lower (although not statistically different) in irradiated rats than in controls, fed a standard diet. In irradiated (20 Gy) rats on a low sodium diet, the sodium/potassium ratio was significantly lower than control values at 8 and 12 weeks. After 12 weeks, the SBP started to rise. A lower urinary sodium/potassium ratio prior to the rapid increase of the SBP, coupled with a low PRC in irradiated rats compared to controls on the same diet, supports the premise that volume

retention is involved in the development of renal radiation hypertension.

Likewise, the rapidly increasing SBP following a 20 Gy X-ray dose to both kidneys of young rats seemed to coincide with sodium retention, again indicating a hypertension mediated by volume expansion. However, sodium retention leading to hypertension is difficult to trace by metabolic studies (25). The amount of daily sodium retention necessary to elevate blood pressure in the rat is very low, especially in gradually developing, mild hypertension. This might explain why sodium retention could not be demonstrated in gradually developing hypertension after a 12.5 Gy X-ray dose to both kidneys of young and adult rats in our first experiment.

Dietary sodium restriction neither postponed nor alleviated the rise in SBP after a high X-ray dose to both kidneys in young rats. Thus, bilateral kidney irradiation caused a low renin hypertension, irresponsive to dietary salt restriction. An explanation for this might be that the kidney damaged by irradiation is able to retain sodium very effectively.

The role of dietary sodium restriction in renal experimental hypertension is controversial. Unilateral renal artery constriction with an intact contralateral kidney was shown to be accompanied by volume retention (19). Dietary sodium restriction successfully lowered renal hypertension in these rats in some experiments (21), but not in others (17, 26). Similarly, hypertension due to unilateral renal artery constriction with contralateral nephrectomy was reported to be cured by dietary sodium restriction (27). Other investigators did not observe a change in blood pressure in the one-kidney-one-clip model after dietary sodium restriction (21, 28, 29). However, addition of a converting enzyme antagonist (28) or an angiotensin inhibitor (29) to the sodium-restricted diet did effectively lower blood pressure, suggesting that both sodium retention and renin mediated vasoconstriction were involved. After allogenic kidney transplantation in

the rat, dietary sodium restriction effectively attenuated the development of hypertension (22).

Hypertension is a late complication of renal radiation. Previous studies (4) and the present experiment already showed a marked reduction in renal function at the time that the SBP was only slightly increased in irradiated rats. Eight weeks after a single X-ray dose of 20 Gy to both kidneys of young rats, the GFR and urine osmolality were severely depressed, indicating severe glomerular and tubular damage. Morphologic vascular damage also occurs early after renal irradiation (30). The involvement of various tissue compartments in renal radiation injury argues for a complex pathogenesis of radiation hypertension. Although our experimental results favor tubular sodium and volume retention, renin production secondary to tubular sodium loss or hypoperfusion due to vascular damage may also have occurred. Concomitant renal function deterioration may stimulate a further blood pressure increase (31). The elevated blood pressure causes vascular damage, especially in the renal arteries and arterioles (26). Irradiation sensitizes the vessels to hypertensive injury (32), rendering the irradiated kidney especially prone to hypertensive vascular damage. Thus, hypertension as such causes a rapid progression of renal functional damage.

In conclusion, hypertension following bilateral renal irradiation was not primarily mediated by the renin-angiotensin system. Although balance studies indicated that sodium retention played a role in the development of hypertension after a high X-ray dose to both kidneys in young rats, dietary sodium restriction failed to protect against the rise in SBP. The hypertension was progressive. Using a high X-ray dose the SBP finally became malignant with high PRC.

References

1. P.B. Kunkler, R.F. Farr and R.W. Luxton, The limit of renal tolerance to X rays. An investigation into renal damage occurring following the treatment of tumours of the testis by abdominal baths. Brit. J. Radiol. 25, 190-201 (1952).

2. A.B. Crummy, S. Hellman, H.C. Stansel and P.B. Hukill, Renal hypertension secondary to unilateral radiation damage relieved by nephrectomy. Radiol. 84, 108-111 (1965).

3. D.K. Bloomfield, D.H. Schneider and V. Vertes, Renin and angiotensin II studies in malignant hypertension after X-irradiation for seminoma. Ann. Intern. Med. 68, 146-151 (1968).

4. H.T.M. Jongejan, A.J. van der Kogel, A.P. Provoost and J.C. Molenaar, Renal radiation damage in young and adult rats. Int. J. Rad. Oncol. Biol. Phys. 13, 225-232, (1987).

5. J.H. Dirks, J.F. Seely and M. Levy, Control of extracellular fluid volume and the pathophysiology of edema formation. In The Kidney (B.M. Brenner and F.C. Rector, Eds.) Vol 1, pp. 495-542. W.B. Saunders Company, Philadelphia, 1976.

6. G.M.C. Masson, The renin-angiotensin-aldosteron system. In Renal Hypertension (I.H. Page and J.W. McCubbin, Eds.) pp. 214-242. Year Book Medical Publishers, Inc, Chicago, 1968.

7. E.E. Muirhead, E.G. Daniels, E. Booth, W.A. Freyburger and J.W. Hinman, Renomedullary vasodepression and antihypertensive function. Arch. Path. 80, 43-49 (1965).

8. F. Gross, Renin, renal function, and hypertension. In Cardiovascular regulation in health disease. (Bartorelli and A. Zanchetti, Eds) IRC, Milano, 1970, pp. 273-293.

9. J.H. Laragh, J.E. Sealey, A.P. Niarchos and T.G. Pickering, The vasoconstriction-volume spectrum in normotension and in the pathogenesis of hypertension. Fed. Proc. 41: 2415-2423 (1982).

10. J.H. Laragh, The renin system for long-term control over vasoconstriction and sodium-volume homeostasis in the spectrum of hypertension: three new frontiers in research. Clin. Exp. Hypertension Part A 5, 921-939 (1983).

11. M.H. de Keyzer, A.P. Provoost and F.H.M. Derkx, Absence of activation in vitro of renin in rat plasma. Clin. Sci. 62, 435-437 (1982).

12. J.M. Brensilver, F.H. Daniels, G.S. Lefavour, R.M. Malseptic, J.A. Lorch, M.L. Ponte and S. Cortell, Effect of variations in dietary sodium intake on sodium excretion in mature rats. Kidney Int. 27, 497-502 (1985).

13. A.P. Provoost, M.H. de Keyzer, E.D. Wolff and J.C. Molenaar, Development of renal function in the rat. Renal Physiol. 6, 1-9 (1983).

14. J.D. Swales and J.D. Tange, The influence of acute sodium depletion on experimental hypertension in the rat. J. Lab. Clin. Med, 78, 369-379 (1971).

15. A.P. Shapiro, T. Cavallo, W Cooper, D. Lapenas, K. Bron and G. Berg, Hypertension in radiation nephritis. Arch. Intern. Med. 137, 848-851 (1977).

16. W.C. Hulbert, V.M. Anderson, L.J. Ettinger, T.C. Putnam, B.P. Wood and R. Rabinowitz, Hyperreninemic hypertension secondary to radiation nephritis in a child. Urol. 26, 153-156 (1985).

17. E.R. Fisher and H.R. Hellstrom, Pathogenesis of hypertension and pathologic changes in experimental renal irradiation. Lab. Invest. 19, 530-538, (1968).

18. A. Ljungqvist, G. Unge, C. Lagergren and G. Notter, The intrarenal vascular alterations in radiation nephritis and their relationship to the development of hypertension. Acta Path. Microbiol. Scand. A 79, 629-638 (1971).

19. J. Mohring, B. Mohring, H. Neumann, A. Philippi, E. Homsy, H. Orth, G. Dauda, S. Kazda and F. Gross, Salt and water balance and renin activity in renal hypertension of rats. Am. J. Physiol. 228: 22 1847-1855 (1975).

20. A. Aviv, T. Kobayashi, H. Higashino, J.W. Bauman and S.S. Yu, Chronic sodium deficit in the immature rat: Its effect on adaptation to sodium excess. Am. J. Physiol. 242, E241-247 (1982).

21. L.W. Miksche, U. Miksche and F. Gross, Effect of sodium restriction on renal hypertension and on renin activity in the rat. Circ. Res. 27, 973-984 (1970).

22. M.H. de Keyzer, A.P. Provoost, E.D. Wolff, W.J. Kort, I.M. Weyma, M van Aken and J.C. Molenaar, The effect of a reduced sodium intake on post-renal transplantation hypertension in rats. Clin Sci. 66, 269-276 (1984).

23. J.T. Fitzsimons, Thirst. Physiol. Rev. 52, 536-539 (1972).

24. T.F. Ferris, The kidney and hypertension. Arch. Intern. Med. 142, 1889-1895 (1982).

25. J. Biollaz, B. Waeber, J. Diezi, M. Burnier and H.R. Brunner, Lithium infusion to study sodium handling in unanesthetized hypertensive rats. Hypertension 8, 117-121 (1986).

26. L. Tobian and K. Coffee, Effect of thiazide drugs on renovascular hypertension in contrast to their effect on essential hypertension. Proc. Soc. Exp. Biol. Med. 115, 196-198 (1964).

27. L. Tobian, The electrolytes of arterial wall in experimental renal hypertension. Circ. Res. 4, 671-675 (1956).

28. A.A. Seymour, J.O. Davis, R.H. Freeman, J.M. DeForrest, B.P. Rowe, G.A. Stephens and G.M. Williams, Sodium and angiotensin in the pathogenesis of experimental renovascular hypertension. Am. J. Physiol. 240, H788-H792 (1981).

29. H. Gavras, H.B. Brunner, E.D. Vaughan and J.H. Laragh, Angiotensin-sodium interaction in blood-pressure maintenance of renal hypertensive and normotensive rats. Science 180, 1369-1372 (1973).

30. B.W. Wachholz and G.W. Casarett, Radiation hypertension and nephrosclerosis. Rad. Res. 41, 39-56 (1970).

31. H. Tsuruda, S. Okuda, K. Onoyama, J. Oh and M. Fujishima, Effect of blood pressure on the progress of renal deterioration in rats with renal mass reduction. J. Lab. Clin. Med. 107, 43-50 (1986).

32. A.W. Asscher, C. Wilson, S.G. Anson, Sensitisation of blood-vessels to hypertensive damage by X-irradiation. Lancet 1, 580-583 (1961).

Acknowledgement; We thank the Radiobiological Institute TNO (Rijswijk, the Netherlands) (Head: Prof. D.W. van Bekkum) for the use of the X-ray facilities and the Laboratory for Internal Medicine (Dijkzigt Hospital, Rotterdam, the Netherlands) (Head: Prof. M.A.D.H. Schalekamp) for enabling us to perform renin-assays. The technical assistance of Mrs E. Fierret was appreciated. Mrs A. Ribbink-Goslinga gave valuable stylistic advice.

CHAPTER 4

NEPHROTOXICITY OF CIS-PLATIN COMPARING YOUNG AND ADULT RATS

H.T.M. Jongejan, A.P. Provoost, ED. Wolff, J.C. Molenaar.

Dept of Pediatric Surgery and Pediatrics, Erasmus University Medical School, Rotterdam, The Netherlands.

(This chapter has been published in: Ped Res 20:9-14, 1986)

SUMMARY

The effect of Cis-platin upon the glomerular filtration rate and effective renal plasma flow was determined using a radio-isotope clearance technique in young (3 weeks old) and adult (over 12 weeks old) rats. Cis-platin was administered i.v.in dosages ranging from 2.5 to 10 mg/kg body weight, either as a single dose or fractionated over 5 consecutive days. Following either dose regimen, identical total doses of Cis-platin caused less severe nephrotoxicity in young rats than in adult ones. In adult rats fractionated dosage significantly reduced nephrotoxicity. This was not observed in young rats. The difference in nephrotoxicity between young and adult rats was due to the renal handling of Cis-platin. After a single dose of 5 and 7.5 mg/kg body weight, platinum concentrations were measured in urine and renal tissue. During the first 2 days after Cis-platin administration, up to 60% of the amount of platinum injected was excreted in the urine of both age groups. There was a marked difference, however, in renal Pt concentration between the two groups. In young rats renal Pt concentration was only 63% and 49% of that in adult rats after 5 and 7.5 mg/kg body weight respectively. We believe that this is due to the comparatively larger renal mass in relation to body weight in the young animals. Relatively more renal tissue provides at least partial protection against nephrotoxic drugs in these young rats.

Abbreviations

BW	body weight
Cr-51 EDTA	Chromium-51 Ethylenediaminotetra-acetic acid
ERPF	effective renal plasma flow
GFR	glomerular filtration rate
i.v.	intravenous
Pt	platinum

INTRODUCTION

Cis-platin (Cis-diamminedichloroplatinum, abbreviated as CDDP) has been proven to be a valuable agent in adult oncologic therapy and is increasingly used in pediatric oncology (1,2,3). Animal(4,5) as well as clinical (6,7,8) studies have demonstrated that therapeutic dosage leads to nephrotoxicity. This side effect may in fact be dose limiting (4,9). The nephrotoxicity can be alleviated but not entirely prevented, by good hydration, forced diuresis and prolonged administration (7,8,10).

It has been suggested that the incidence of nephrotoxicity in children is less than in adults(11). Clinical and experimental data substantiating this statement are scarce. In the present study we compared glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) of young and adult rats after various doses of Cis-platin. We also evaluated the renal handling of this drug by measuring urinary excretion and renal accumulation of Pt.

MATERIALS AND METHODS

Experiments were carried out using male rats of an outbred wistar strain (WU/CPB,TNO Zeist,The Netherlands). The animals were selected according to body weight (BW) and age. The young rats were 3-4 weeks of age weighing 50-65 g, the adult rats were more than 12 weeks of age, weighing 270-340 g. Tap water and food were available at libitum.

Renal Function Determination

The GFR and the ERPF were determined by a plasma clearance technique, as described in detail elsewhere (12). This method involves i.v. injection of Cr-51 EDTA and I-125 Iodohippurate. (Amersham International, Amersham,

England) and a single timed blood sample collected 60 min. after the injection. The method allows for repeated use in the same animal.

Pt determination

The Pt content of urine and renal tissue was determined by atomic absorption spectrophotometry using a Perkin Elmer 2380 spectrophotometer. The urine samples were analyzed without pretreatment and read against a standard curve based on aqueous standards. The kidneys were weighed, freeze dried and digested in 65% HNO_3 at 200*C in 1.5-2 hours. Pt-standards were added to control kidneys which were similarly treated. The dry residue was then dissolved in 2 ml of distilled water, and analyzed.

Experimental protocol

Single dosage regimen;

Cis-platin (kindly supplied by Bristol Meyers BV, Weesp, The Netherlands) was diluted with distilled water to a final concentration of 1 mg Cis-platin, 9 mg NaCl and 10 mg mannitol per ml.

Cis-platin was administered i.v. in a dose of 2.5, 5 or 7.5 mg/kg BW in adult rats and 5, 7.5 or 10 mg/kg BW in the young animals. The highest dose was determined by the 100 % mortality rate for both age groups. Control animals were given i.v. injections of distilled water. At the start of the experiments the groups consisted of five to 15 rats.

Renal function was determined 4, 7, 14, 21, 42 and 105 days after drug administration.

Fractionated dose regimen;

In this regimen the total amount of Cis-platin was administered i.v. in equal doses over 5 successive days. The total dosage was the same for adult and young rats, viz 5x1, 5x1.5 or 5x2 mg/kg BW. Control animals were given 5 daily i.v. injections of distilled water. At the start of the experiment the groups consisted of five to 10 rats.

Renal function was determined 7, 10, 17, 24, 42 and 105 days after the first drug administration.

Renal Pt handling

In seven young and eight adult rats, the urinary excretion and the renal accumulation of Pt were determined after a single i.v. injection of Cis-platin in a dose of 5 or 7.5 mg/kg BW. Urine was collected for 2 days. Subsequently, the kidneys were removed and weighed. Both, urine and renal tissue were analyzed for Pt.

Statistics

Data are recorded as mean and SD. Within each age group differences in means of either GFR or ERPF comparing the various treatment groups and controls were established by one way analysis of variance. When a difference was indicated ($P<0.05$), the Newman-Keuls test was applied to see which pair(s) of groups were statistically different. Student's t-test was applied to establish significant differences between young and adult rats regarding renal Pt handling and the GFR after the same dosage regimens. Statistical difference was indicated by a P value <0.05.

RESULTS

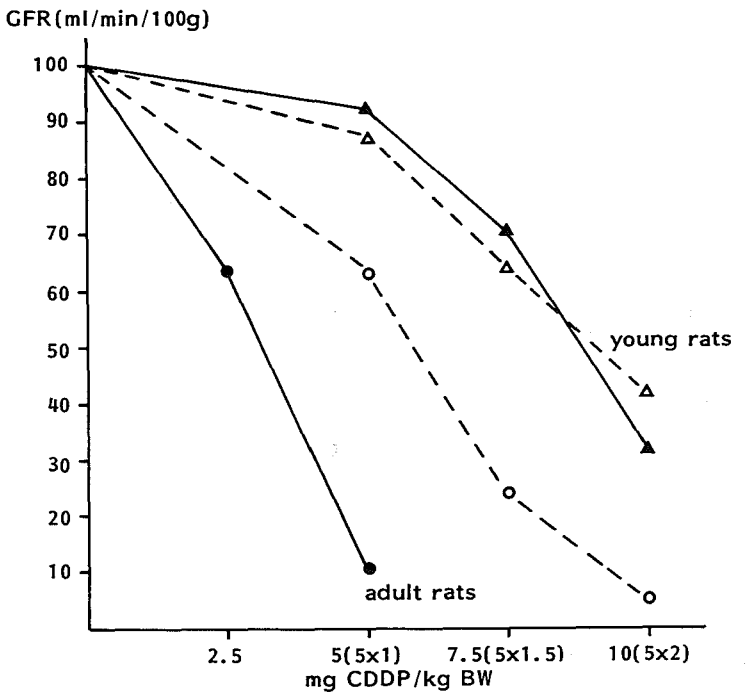
Single dosage regimen

Adult animals

The adult animals showed marked weight loss in the first days after Cis-platin administration (table 1). Particularly after doses amounting to 7.5 mg/kg BW, the rats suffered from anorexia and diarrhea. The animals receiving this dose all died on the fourth day after Cis-platin administration with little residual function. The lower doses of Cis-platin resulted in a dose dependent fall in both GFR and ERPF, the lowest value

being measured 4 days after drug administration. After this initial fall there was recovery during the next 3 weeks (table 1). Subsequently, renal function stabilized. With 5 mg per kg BW the GFR initially fell to 10 % of the control value and recovered to 50-60% of control after 15 weeks. After 2.5 mg/kg BW the initial fall in GFR was 40%, while subsequent recovery was complete.

FIG.1. Comparison of the initial changes in GFR of either young or adult rats treated with Cis-platin.



The GFR (ml/min/100 g BW) is given as a percentage of that of control rats, on day 4 after the first administration of Cis-platin for the single dose and day 7 after the first administration for the fractionated dose regimen. Solid lines and closed symbols represent single dosage, broken lines and open symbols represent fractionated dosage. Circles represent adult rats and triangles represent young rats.

TABLE 1. RENAL FUNCTION IN ADULT RATS AFTER SINGLE DOSAGE OF CIS-PLATIN *

	Day 0		Day 4			Day 7			Day 14			Day 21			Day 42			Day 105		
Dose	n	BW	n	BW	GFR	ERPF	n	BW	GFR	ERPF	n	BW	GFR	ERPF	n	BW	GFR	ERPF	n	BW	GFR	ERPF	n	BW	GFR	ERPF
0	5	298 8	5	305 7	2.38 0.18	5.38 0.34	5	308 9	2.26 0.14	5.19 0.18	5	316 9	2.57 0.26	5.34 0.33	5	326 13	2.63 0.1	4.91 1.00	5	329 39	2.53 0.28	5.21 0.43	5	408 25	2.85 0.16	5.49 0.07
2.5	5	302 24	5	291 32	1.45** 0.49	3.72** 0.50	5	298 33	1.86** 0.23	4.47** 0.39	5	318 40	2.43** 0.39	5.09 0.40	5	322 40	1.98** 0.5	4.13 1.58	5	362 59	2.28 0.30	4.99 0.29	5	426 73	2.84 0.42	5.17 0.41
5	5	297 21	5	273 27	0.23** 0.04	0.60** 0.16	5	265** 28	0.64** 0.14	2.09** 0.31	5	278 29	1.14** 0.26	2.98** 0.13	5	275** 26	1.06** 0.2	3.15 0.46	4	298 37	1.19** 0.36	3.51** 0.54	5	368 54	1.44** 0.48	4.44** 0.61
7.5	5	295	All	animals died																						

* All data are mean values and SD. The dose of Cis-platin is given in mg/kg BW; BW in g; GFR in ml/min; ERPF is in ml/min.
** p · 0.05 from control values.

TABLE 2. RENAL FUNCTION IN YOUNG RATS AFTER SINGLE DOSAGE OF CIS-PLATIN *

	Day 0		Day 4			Day 7			Day 14			Day 21			Day 42			Day 105		
Dose	n	BW	n	BW	GFR	ERPF	n	BW	GFR	ERPF	n	BW	GFR	ERPF	n	BW	GFR	ERPF	n	BW	GFR	ERPF	n	BW	GFR	ERPF
0	5	64 4	5	83 7	0.62 0.14	1.44 0.27	4	88 15	0.76 0.06	1.78 0.16	4	145 9	1.39 0.14	2.03 0.18	4	185 8	1.80 0.21	3.61 0.15	4	276 21	2.17 0.30	4.82 0.38	3	390 13	2.67 0.16	4.90 0.15
5	15	61 4	13	60** 8	0.39** 0.16	0.96** 0.35	12	71** 10	0.56** 0.27	1.35** 0.47	8	103** 15	0.83** 0.25	2.04** 0.38	6	150** 20	1.21** 0.29	2.87** 0.36	5	232** 31	1.82 0.24	4.08 0.44	4	364 49	2.62 0.29	5.35 0.42
7.5	10	60 3	10	50** 3	0.25** 0.12	0.76** 0.20	8	57** 6	0.43** 0.10	1.13** 0.29	7	71** 13	0.56** 0.15	1.47** 0.3	8	113** 15	0.77** 0.11	1.95** 0.25	7	209** 24	1.61 0.37	4.03 0.63	7	346 47	2.12 0.45	4.90 0.35
10	5	55 8	4	41** 2	0.09** 0.05	0.21** 0.11	All animals died																			

* All data are mean values and SD. The dose of Cis-platin is given in mg/kg BW; BW in g; GFR in ml/min; ERPF in ml/min.
** p · 0.05 from control values.

Young rats

The young rats failed to grow during the first days after drug administration but resumed gaining weight after 1 week. As a result dose-dependent differences in BW were observed and remained statistically different up to the 6th week (table 2). Young rats treated with 10 mg/kg BW all died in the 1st week following administration. When expressed in ml/min the GFR was significantly less in young rats treated with Cis-platin than in controls. As both GFR and BW were reduced, the GFR per 100g BW showed little difference in rats treated with 5 or 7.5 mg/kg BW when compared to the control rats.

Comparison between young and adult rats

In the single dose regimen, a dose of 5 and 7.5 mg/kg BW was administered to both young and adult rats. When the initial changes in renal function were compared (expressed as percentage of the controls), the fall in GFR was less in the young rats than in the adult ones. This was true not only when the GFR was related to BW (ml/min/100g BW) (fig 1) but also when absolute values (ml/min) were taken into consideration (table 1,2). Furthermore renal function remained permanently reduced in the adult rats, while there was complete recovery in the young ones.

Fractionated dosage regimen

Adult rats

The weight loss observed during the first 2 weeks after drug administration was comparable with the weight loss after corresponding single doses. However, fractionation did reduce the mortality rate and immediate nephrotoxicity (table 3). All animals survived after 5x1.5 mg/kg BW, while only 50% died during the first 3 weeks after a dose of 5x2 mg per kg BW. The initial impairment in renal function was less severe after 5x1 mg per kg BW than after 1x5 mg per kg BW. However, at the end of the 15

TABLE 3. RENAL FUNCTION IN ADULT RATS AFTER FRACTIONATED DOSAGE OF CIS-PLATIN *

	Day 0		Day 7			Day 10			Day 17			Day 24			Day 42			Day 105		
Dose	n	BW	n	BW	GFR	ERPF	n	BW	GFR	ERPF	n	BW	GFR	ERPF	n	BW	GFR	ERPF	n	BW	GFR	ERPF	n	BW	GFR	ERPF
5×0	5	319 17	5	325 21	2.17 0.28	4.67 0.57	5	327 22	2.41 0.14	5.22 0.29	4	339 24	2.50 0.23	4.37 0.22	4	345 28	2.46 0.21	4.96 0.18	4	379 44	3.15 0.34	5.84 0.31	4	434 54	2.90 0.37	5.42 0.13
5×1	5	292 15	5	261** 12	1.12** 0.32	3.05** 0.48	5	268** 14	1.32** 0.10	3.73** 0.44	5	278** 17	1.58** 0.24	4.38 0.32	5	291** 14	1.57** 0.26	4.36 0.34	4	320** 13	1.70** 0.38	4.15** 0.32	3	360** 11	2.09** 0.38	4.95 0.20
5×1.5	5	262 19	5	245** 19	0.39** 0.19	1.92** 0.55	5	237** 20	0.55** 0.34	2.00** 0.68	5	216** 26	0.76** 0.31	2.43** 0.8	5	254** 23	0.78** 0.29	3.16** 0.70	5	290** 30	0.90** 0.38	3.36** 0.86	5	330** 29	1.11** 0.44	3.91 1.13
5×2	10	287 19	9	249** 21	0.11** 0.11	0.74** 0.58	8	225** 18	0.13** 0.15	0.73** 0.66	5	243** 21	0.25** 0.20	1.01** 0.35	5	245** 35	0.32** 0.23	1.19** 0.91	5	278** 33	0.32** 0.22	1.37** 1.00	3	314** 35	0.31** 0.19	1.40** 0.86

* All data are mean values and SD. The dose of Cis-platin is given in mg/kg BW; BW in g; GFR in ml/min; ERPF in ml/min.
** $p < 0.05$ from control values.

TABLE 4. RENAL FUNCTION IN YOUNG RATS AFTER FRACTIONATED DOSAGE OF CIS-PLATIN *

	Day 0		Day 7			Day 10			Day 17			Day 24			Day 42			Day 105		
Dose	n	BW	n	BW	GFR	ERPF	n	BW	GFR	ERPF	n	BW	GFR	ERPF	n	BW	GFR	ERPF	n	BW	GFR	ERPF	n	BW	GFR	ERPF
5×0	5	53 3	5	79 4	0.72 0.10	1.74 0.12	5	92 6	0.77 0.13	1.90 0.05	5	135 9	1.22 0.12	2.33 0.25	5	167 11	1.69 0.23	3.53 0.15	5	257 21	2.11 0.24	4.64 0.26	4	356 37	2.53 0.41	5.50 0.45
5×1	10	54 4	8	57** 8	0.46** 0.17	1.09** 0.29	5	76** 5	0.77 0.15	1.72 0.21	9	103** 16	0.83** 0.30	1.86** 0.50	7	133 25	1.17** 0.37	2.63** 0.52	7	226 27	1.84 0.37	3.85 0.30	7	347 49	2.50 0.34	5.38 0.63
5×1.5	10	60 8	7	56** 6	0.33** 0.06	0.86** 0.23	7	66** 5	0.42** 0.11	1.28** 0.25	7	100** 7	0.54** 0.16	1.54** 0.24	7	127** 15	0.92** 0.30	2.39** 0.35	7	207** 22	1.35 0.59	3.66 0.81	7	322 40	2.00 0.52	5.00 0.80
5×2	10	57 3	5	45** 5	0.16** 0.05	0.41** 0.23	2	51** 13	0.34** 0.21	0.93** 0.38	3	64** 22	0.46** 0.28	1.26** 0.53	3	100** 28	0.53** 0.39	1.61** 0.77	2	195** 63	1.07** 1.09	2.99 2.03	All animals died			

* All data are mean values and SD. The dose of Cis-platin is given in mg/kg BW; BW in g; GFR in ml/min; ERPF in ml/min.
** $p < 0.05$ from control values.

weeks follow-up the renal impairment was similar after either dosage regimen.

Young rats.

Fractionation caused a growth retardation similar to that observed after a single dose when 5 mg/kg BW was administered. With 7.5 mg/kg BW, the growth reduction was less when given fractionated than after a single dose (table 2 and 4). The GFR (ml/min) of rats treated with Cis-platin was less than that of controls. However, the GFR relative to body weight (ml/min /100g) showed little difference from control values. After fractionated dosage of Cis-platin the GFR was generally comparable to the GFR after an identical single dose.

Comparison between young and adult rats.

In the young rats, renal function was less affected by the same dose of cis-platin given fractionated than in adult animals. The GFR per 100g BW, as percentage of the mean value in controls, was determined on day 4 after drug administration and is plotted against the total dose of Cis-platin. As shown in figure 1, the dose response curve for the adult rats is much steeper than for young rats. This difference remained at the end of the 15 week follow-up period (fig 2).

Renal Pt-handling

During the first 2 days after a single dose of 5 mg Cis-platin per kg BW about 60% of the administered amount of Pt was excreted in the urine of young as well as adult rats. After 7.5 mg Cis-platin per kg BW the young rats excreted relatively more Pt than the adult rats (table 5). Most of the Pt was excreted during the first day after administration. After 2 days both Cis-platin doses caused a similiar Pt accumulation in young and adult rats of 1.5-2% of the injected amount of Pt. However, the renal Pt concentration was significantly lower in the young rats compared to the adult rats(table 5). This was due to the larger renal mass relative to BW in the young rats.

As shown in Table 5 the wet kidney weight per 100g BW of young rats was 179% and 165% of that of adult rats in the 5 and 7.5 mg dose group respectively.

TABLE 5. RENAL HANDLING OF A SINGLE IV DOSE OF 5 AND 7.5 MG/KG BW OF CIS-PLATIN IN YOUNG AND ADULT RATS *

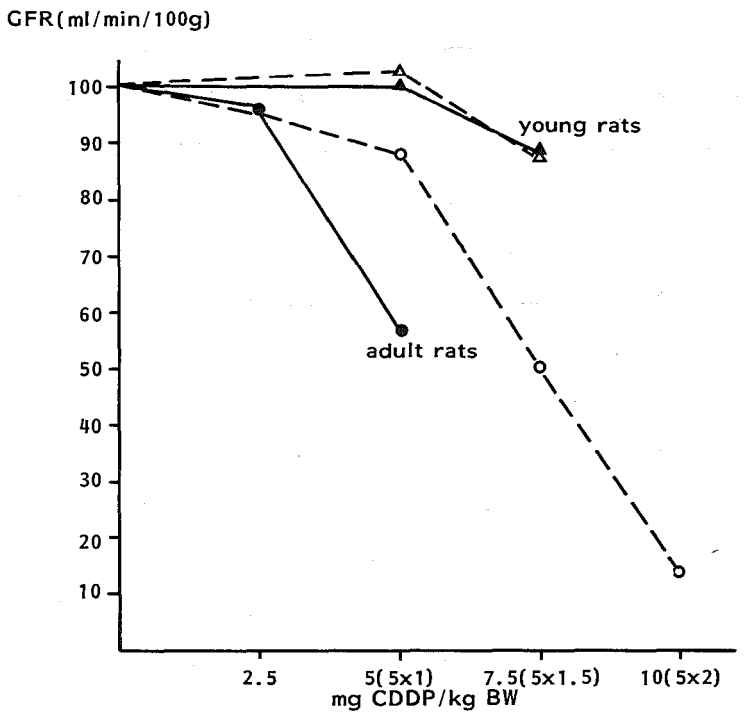
	Dose	n	BW (g)	48-H urinary Pt excretion (% of Pt injected)	Renal Pt accumulation	Kidney wt (g/100 g BW)	Renal Pt concentration (µg/gW kidney wt)
Adult	5	8	302 ± 11	57.4 ± 7.7	1.64 ± 0.50	0.38 ± 0.03	14.7 ± 70
	7.5	7	289 ± 8	46.3 ± 3.6	1.24 ± 0.45	0.31 ± 0.02	19.3 ± 7.3
Young	5	7	49 ± 5 **	59.3 ± 8.2	1.99 ± 0.90	0.68 ± 0.07**	9.2 ± 3.7**
	7.5	7	53 ± 2 **	69.3 ± 12.6**	1.00 ± 0.29	0.51 ± 0.06**	9.4 ± 2.5**

* All data mean values ± SD.
** $p < 0.05$ when compared with the same dose in adult rats.

DISCUSSION

Our study has shown that an identical dose of Cis-platin per kg BW administered to either young or adult rats, leads to a deterioration of renal function which is comparitively less severe in young rats. In both, young and adult rats the deterioration in renal function occurs during the initial days after Cis-platin administration with gradual recovery during the following weeks. Complete recovery is more likely to occur in young rats with a higher degree of permanent damage in adult rats. In young rats Cis-platin temporarily arrests body growth and renal development, but this does not affect the potential for growth and development. The initial impairment in renal function of young rats is almost proportionate to the retardation in growth. While the absolute GFR (ml/min) of the young rats under treatment dropped well below that of controls, the GFR in relation to BW only fell below control values when the highest dose of Cis-platin was administered. Fractionated dosage reduced the initial renal damage in the Fig 2. Comparison of the permanent changes in GFR of either young or adult

rats treated with Cis-platin.



The GFR (ml/min/100 g BW) is given as a percentage of that of control rats, 15 wk after the first administration of Cis-platin. For symbols see legend of Figure 1.

adult rats, allowing for higher doses. Fractionation did not have that effect on the young animals, while it had hardly any effect on the permanent renal damage in either age group.

Apart from changes in renal function, both young and adult rats suffered from gastro-intestinal toxicity immediately on drug administration. The animals became anorectic and paricularly the higher dosage led to hemorrhagic diarrhea. It is not clear to what extent gastro-intestinal toxicity and the resultant dehydration contributes to the deterioration in

renal function and subsequent mortality in young rats. In the young rats the gastro-intestinal complications rather than renal failure seemed to play a major part in the acute morbidity and mortality.

The nephrotoxicity of Cis-platin has been subject to extensive study (4, 5, 7, 8, 13, 14); the exact mechanism is not yet clear. The morphologic, biochemical and functional disarrangement is rather unspecific, and has also been observed after heavy metal poisoning (7, 15). Morphologically, renal damage is largely confined to the pars recta of the proximal tubule (4, 5, 6, 16). Despite the tubular location of the renal damage, the GFR still seems to be a rather sensitive parameter to quantitate the nephrotoxicity (14). It can not be excluded, however, that the measured GFR in fact underestimates the actual GFR, because of tubular backleak of the marker isotope (16). The decrease in GFR has also been attributed to the vasoconstriction of renal vessels by an activation of the renin-angiotensine system (7). Such an activation may be a direct effect of cis-platin or function indirectly as a protective feedback mechanism, secundary to the excessive salt and fluid losses.

The difference in nephrotoxicity of Cis-platin in either young or adult rats is based on the difference in renal Pt concentration. Our analysis of the renal handling of Pt indicated that there was hardly any difference in the renal accumulation of Pt when comparing young and adult rats. Renal Pt-excretion was also the same in young and adult rats after a dose of 5 mg/kg BW. After 7.5 mg/kg BW, the urinary Pt-excretion was less efficient in the adult rats compared to the young rats. This might be due to the severe decline in renal function in the adult rats. The concentration of Pt in renal tissue, however, was significantly lower in young rats than in adult ones. This difference is due to the larger amount of renal tissue in relation to BW in the young rats (12). The magnitude of the Pt levels in

renal tissue measured by us are comparable to those reported by others (9,17). If Cis-platin nephrotoxicity is related to the Pt concentration in the kidney, the lower Pt-levels found in the kidneys of young rats provide an explanation for the attenuated nephrotoxicity observed in these animals.

An attenuated nephrotoxicity in young rats is not a unique finding for Cis-platin. Similar results have been obtained, comparing the nephrotoxicity of the aminoglycosides gentamicin and amikacin in young and adult rats. Both compounds when administered in dosages relative to BW were found to be less nephrotoxic in young rats. At the same time, despite a similar renal uptake, tissue aminoglycoside concentrations were found to be lower in these rats (18). Consequently, we surmised that drug induced nephrotoxicity will generally be less in young than in adult rats, due to the larger amount of renal tissue relative to BW. In this respect it may be of importance that like in humans, kidney weight relative to BW decreases with age (19).

In conclusion, when Cis-platin is administered in doses relative to BW this drug is less nephrotoxic in young rats than in adult ones. This reduction in nephrotoxicity is the result of the comparatively larger amount of renal tissue relative to body weight present in young rats.

REFERENCES

1. Baum ES, Gaynon P, Greenberg L, Krivit W, Hammond D 1979 Phase II study of Cis-dichlorodiammine-platinum (II) in childhood osteosarcoma ; Children's Cancer Study Group Report. Cancer Treat Rep 63:1621-1626

2. Pratt CB, Hayes A, Green AA, Evans WE, Senzer N, Howarth CB, Ransom JL, Crom W 1981 Pharmacokinetic evaluation of Cis-platin in children with malignant solid tumors; a phase 2 study. Cancer Treat Rep 65:1021-1026

3. Vietti TJ, Nitschke R, Starling KA, van Eys J 1979 Evaluation of Cis-dichlorodiammineplatinum (II) in children with advanced malignant diseases; Southwest Oncology Group Studies. Cancer Treat Rep 63:1611-1614

4. Goldstein RS, Mayor GH 1983 The nephrotoxicity of Cis-platin. Life Sci 32:685-690

5. Ward JM, Fauvie KA 1976 The nephrotoxic effects of Cis-diamminedichloroplatinum (II) (NSC 119875) in male F344 rats. Toxicol Appl Pharmacol 38:535-547

6. Lippmann AJ, Helson C, Helson L, Krakhoff IH 1973 Clinical trials of Cis-diamminedichloroplatinum (NSC - 119875). Cancer Chemother Rep 57:191-200

7. Madias NE, Harrington JT 1978 Platinum nephrotoxicity. Am J Med 65:307-314

8. Prestayko AW, D'Aoust JC, Issell BF, Crooke ST 1979 Cis-platin (Cis-diamminedichloroplatinum II). Cancer Treat Rev 6:17-39

9. Harrison R, McAuliffe CA, Zaki A, Baer J, Sharma H, Smith A, Jackson H, Fox BW 1983 A comparative study of the distribution in the male rat of platinum labelled Cis-dichlorodiammine platinum (II), cis-trans-dichloro-dihydroxy-bis(Isopropylamine)platinum (II), and cis-dichloro-bis-Cyclopropylamine platinum (II). Cancer Chemother Pharmacol 10:90-95

10. Pera MF, Zook BC, Harder HC 1979 Effect of mannitol or furosemide diuresis on the nepohrotoxicity and physiological disposition of Cis-dichlorodiammineplatinum (2) in rats. Cancer Res 39:1269-1278

11. Kamalakar P, Freeman AI, Higby DJ, Wallace HJ, Sinks LF 1977 Clinical response and toxicity with Cis-dichlorodiammine platinum (II) in children. Cancer Treat Rep 61:835-839

12. Provoost AP, de Keijzer MH, Wolff ED, Molenaar JC 1983 Development of renal function of the rat. Renal Physiol 6:1-9

13. Guarino AM, Miller DS, Arnold ST, Pritchard JB, Davis RD, Urbanek MA, Miller TJ, Litterst CL 1979 Platinate toxicity: past, present and prospects. Cancer Treat Rep 63:1475-1483

14. Stark JJ, Howell SB 1978 Nephrotoxicity of Cis-platinum (II) dichlorodiammine. Clin Pharmacol Ther 23:461-466

15. Levi J, Jacobs C, Kalman MS, McTigue M, Weiner MW 1980 Mechanism of Cis-platin nephrotoxicity:1 Effect of sulfhydryl groups in rat kidneys. J Pharmacol Exp Therap 213:545-550

16. Chopra S, Kaufman JS, Jones TW, Hong WK, Gehr MK, Hamburger RJ, Flamenbaum W, Trump BF 1982 Cis-diammirnedichloroplatinum-induced acute renal failure in the rat. Kidney Int 21:54-64

17. Litterst CL, Torres LJ, Guarino AM 1976 Plasma levels and organ distribution of platinum in the rat, dog and dogfish shark following single intravenous administration of Cis-diamminedichloroplatinum (II). J Clin Hematol Oncol 7:169-179

18. Provoost AP, Adejuyeigbe O, Wolff ED 1985 Nephrotoxicity of aminoglycosides in young and adult rats. Ped Res 19:1191-1196

19. Chantler C 1979 The Kidney. In: Godfrey S, Baum JD (eds) Clinical Paediatric Physiology. Alden Press, Oxford, pp 356-398

ACKNOWLEDGEMENT This study was supported by a grant from the Dutch Cancer Foundation(Koningin Wilhelmina Fonds). We thank Mrs A. Ribbink-Goslinga for her stylistic help and Mrs T. Stehman for performing the Pt-analyses at the Pediatric Research Lab of Sophia's Childrens Hospital (head: Prof. Dr. H. J. Degenhart).

CHAPTER 5

INTERACTION OF cis-DIAMMINEDICHLOROPLATINUM AND RENAL IRRADIATION ON RENAL FUNCTION IN THE YOUNG AND ADULT RAT

H.T.M. Jongejan(1), A.J. van der Kogel(2), A.P. Provoost(1), J.C. Molenaar(1).

1. Dept of Paediatric Surgery, Erasmus University Rottrdam, Rotterdam, The Nethelands.
2. Los Alamos National Laboratory, Life Sciences Division, Los Alamos, N.M.87545, USA.

(This chapter has been published in: Radiother Oncol 10:49-57, 1987)

SUMMARY

The interaction between single low doses of Cis-diamminedichloro-platinum(II) (c-DDP) and renal irradiation (7.5, 10, 12.5 Gy) on renal function and systolic blood pressure (SBP) was investigated in young (3-4 weeks old, BW 45-65 g) and adult rats (over 12 weeks old, BW 230-290 g). The glomerular filtration rate (GFR) and effective renal plasma flow (ERPF), plasma creatinine, urea and SBP were measured over 24 weeks. Changes in ERPF,plasma creatinine and urea concentrations parallelled GFR changes, but tended to be less pronounced. In young rats, BW and GFR were 10-20% below control values after c-DDP administration (5 mg/kg BW). Irradiation caused a dose-dependent drop in GFR, starting 4 weeks after irradiation in young rats. When c-DDP was given immediately after irradiation to the young rats, the loss of renal function was more pronounced than after either treatment modality alone. Dose-effect curves for a >25% reduction of the GFR relative to controls (ml/min) after 24 weeks gave an ED50 of 9.8 Gy for irradiation alone and 4.6 Gy for irradiation followed by c-DDP. After correction for the drug effect, dose-effect curves were similar for renal irradiation given alone or followed by c-DDP administration in young rats. In adult rats, c-DDP (2.5 mg/kg BW) or irradiation alone did not significantly alter renal function during the follow-up period. Only 12.5 Gy in combination with c-DDP, caused a significant reduction in GFR after 16 weeks in adult rats. In adult rats data were too limited for probit analysis. A significant rise in systolic blood pressure was only observed in young rats 16 weeks after 12.5 Gy, when GFR had declined to 60% of control values. The SBP was not influenced by c-DDP. In young rats, the similarity in dose-response curves after irradiation alone or irradiation plus c-DDP, corrected for c-DDP-induced renal damage, indicated an additive interaction between a

single dose of c-DDP and irradiation in the young rat kidney. In the adult rat kidney, the low response in the combined therapy groups up till 24 weeks after irradiation, suggest at best an additive interaction. These results suggest that the target cells for renal damage to c-DDP and irradiation differ.

Key words: Cis-diamminedichloroplatinum, irradiation, renal function, rat, age.

INTRODUCTION

To achieve local and systemic tumour control in the oncological patient, radiotherapy and chemotherapy are combined with increasing frequency. Moreover, it has been shown experimentally that some chemotherapeutic drugs may interact with irradiation and potentiate the therapeutic effect achieved by irradiation (7,8). The therapeutic gain obtained by the combination of chemotherapy and irradiation depends on a differential effect on tumour and normal tissues. The susceptibility of the tissue for the separate therapies depends on its proliferative activity, as well as on the dosage schedule and time sequence of the therapies (9). Dosage and sequence are important for the interaction between drugs and irradiation. When drugs and irradiation are applied in close approximation, the effects on tumours and normal tissues seem most enhanced (9). In general, enhanced tissue effects are observed when the drug shows a specific tissue toxicity (9). This enhancement is usually additive (17). However, DNA-binding agents, such as Cis-diamminedichloroplatinum (c-DDP) could theoretically cause supra-additive tissue damage when combined with irradiation. In vitro studies indicate radiosensitization of tumour cells by c-DDP (3,7). A combination of c-DDP administration and irradiation enhanced the therapeutic effect on tumours in mice (8). Pilot clinical studies have shown the feasibility of a combined treatment with c-DDP and irradiation (13, 20, 22, 23). Evaluation of the therapeutic benefit of the combined treatment for human tumours should await the outcome of phase III trials in Europe and the U.S.A..

The therapeutic advantage of this combination depends on its toxicity for normal tissues. Experimentally, the effect of c-DDP and X-rays on normal tissues were mainly investigated in rapidly proliferating tissues such as

skin (6, 15) and gut (5, 14, 16). As nephrotoxicity is the dose-limiting side effect of c-DDP, the effect of combined c-DDP and X-ray treatment on the kidney is specifically interesting. Clinical data on this subject are not yet available. Experimental data on the effect of combined c-DDP and X-ray treatment on late responding tissues such as the kidney are scarce (24).

In previous experiments, we studied the separate effect of either irradiation (12) or c-DDP (11) on renal function in young and adult rats. Equal doses of c-DDP (in mg/kg BW) caused less renal functional damage in young rats than in adult rats. This could be explained by the larger kidney weight relative to BW in young rats compared to adult rats. Thus, the same dose of c-DDP caused a lower renal platinum concentration in young rats compared to adult rats (11). After bilateral kidney irradiation in young and adult rats, functional renal damage was found to occur earlier in young rats (12).

In the present study we have investigated the age-dependence of renal injury following the combined administration of c-DDP and irradiation. Single doses of c-DDP and radiation, causing a limited renal function decline on their own, were applied in combination. Following treatment, renal function and systolic blood pressure (SBP) were measured for 6 months.

MATERIALS AND METHODS

Inbred male Wistar rats were used for this study (Wag/Rij). The young rats were just weaned, 3-4 weeks old (BW 45-65 g), the adult rats were over 12 weeks old (BW 230-290 g). Each treatment group initially consisted of 10 young or 10 adult rats. Water and food were available ad libitum.

Both kidneys were palpated, fixed in a circular radiation field and

irradiated while the rat was under ethrane anaesthesia. A Philips-Muller X-ray generator was used at 300 kV and 10 mA (3 Gy/min) with a 1 mm Cu filter. The focus-skin distance was 182 mm. The radiation dose was calculated from the midplane of the kidney. The diameter of the radiation field was 3 cm.

c-DDP (kindly provided by Bristol-Myers BV, Weesp, The Netherlands) was dissolved in distilled water and the resultant solution contained 1 mg c-DDP, 9 mg NaCl and 10 mg Mannitol per ml. c-DDP was administered i.v. (sublingual vein in young rats; dorsal vein of the penis in adult rats) as a single dose, 2.5 mg /kg BW for the adult rats and 5 mg /kg BW for the young rats, 0.5-2 hours after irradiation. These doses were based on equal levels of toxicity as determined in previous experiments (11). Controls were sham irradiated and injected i.v. with normal saline. Time references in the text relate to the time of irradiation.

For 24 weeks after irradiation and drug administration several measurements were performed repeatedly in the same rats. Renal function was measured using a radio-isotope technique, permitting repeated measurements in the same animal (19). The radio-activity remaining in the plasma one hour after the i.v. injection of Cr-51-EDTA and I-125-hippuran was used to calculate the glomerular filtration rate (GFR) and effective renal plasma flow (ERPF), respectively. Simultaneously with blood sampling for renal function measurements, plasma was obtained for the determination of urea and creatinine concentrations, using standard methods. To prevent excessive loss of blood in young rats, these additional measurements were started 8 weeks after irradiation. Renal function measurements were performed 2, 4, 8, 12, 16 and 24 weeks after irradiation.

The SBP was measured plethysmographically using the tail-cuff method in the unanaesthetized rat (2). After a training period of 6 weeks following

irradiation, the SBP was measured three times a week for the remainder of the follow up period.

Statistics

One way analysis of variance was applied to reveal differences between groups. When a difference was indicated ($p<0.05$) the Newman-Keuls test was used to reveal which groups differed. In young rats, renal function results permitted probit-analysis after 8 weeks. In adult rats, the expression of renal damage was too limited to construct dose-effect curves for probit analysis.

RESULTS

Renal function in young rats

In young rats, the GFR (ml/min) increased rapidly during the first month of the study, as is shown for the control and the 7.5 Gy group of animals (Fig. 1a). Because of these renal function changes in young rats, findings concerning renal function in the treatment groups will be related to age-matched controls.

After 2 weeks, the increase in GFR (ml/min) (Fig. 1a,b) and BW was reduced in young rats treated with c-DDP in comparison with age matched control rats that did not receive c-DDP. When the GFR was expressed in ml/min/100 g there were no significant differences between the two groups, 2 weeks after treatment (Table I). In young rats that received c-DDP only, the GFR (ml/min) stabilized at 80% of control values after 8 weeks. However related to BW, the GFR in rats receiving c-DDP was only slightly below control levels. This difference was only significant after 4 weeks (Table I).

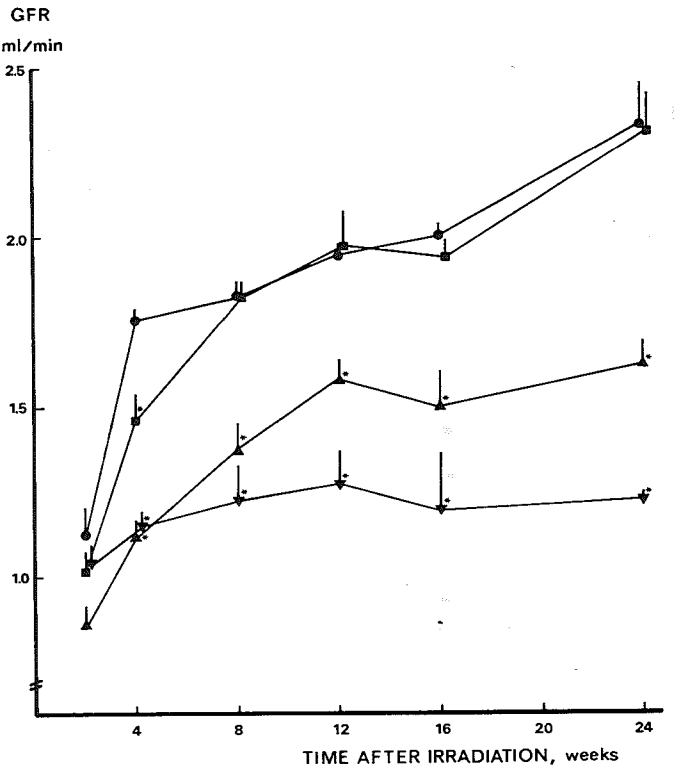


Fig 1a. Time related changes in GFR (ml/min) during 24 weeks after a radiation dose of 7.5 (■), 10 (▲) or 12.5 (▼) Gy to both kidneys of young rats and age-matched controls (●). Each group contained 9-10 rats. Data are means ± SEM. *, Significantly different from controls.

Four weeks after irradiation, the GFR changes depended on the X-ray dose. The 7.5 Gy dose had no effect on GFR. After 10 and 12.5 Gy, the normal developmental increase of the GFR (ml/min) was reduced compared with control rats (Fig. 1a). When irradiation was combined with c-DDP, the normal increase in GFR was affected more severely than after either treatment modality alone (Fig. 1b, Table I). Probit analysis was carried out

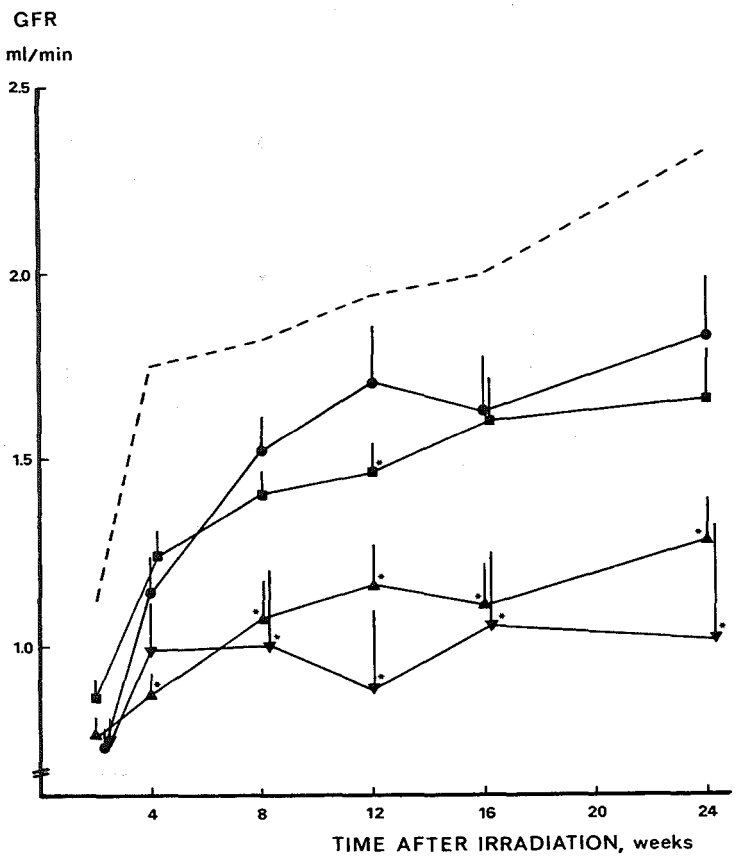


Fig 1b. Time related changes in GFR (ml/min) during 24 weeks, in controls (-) and after i.v. c-DDP (5mg/kg BW) either given separately (●) or combined with a radiation dose of 7.5 (■), 10(▲) or 12.5(▼) Gy to both kidneys of young rats. All groups contained 8-10 rats, except the 12.5 Gy group that contained 5-7 rats depending on the time of assay. Data are means ± SEM. *,Significantly different from controls.

using a reduction in the GFR (ml/min) of at least 25% of that of age-matched controls as an end point. This decline in GFR clearly indicated functional renal damage and was outside the control range. Until 8 weeks after irradiation, the proportion of animals showing this level of damage was too limited for the analysis. In the period 12-24 weeks after irradiation the

ED50 values (dose that causes a 25% or more reduction of the control GFR in 50% of the animals) declined gradually. Figure 2 shows the dose-effect curves obtained by probit analysis 24 weeks after treatment. The ED50 value was 9.8 Gy in rats that were irradiated only and 4.6 Gy for the combined therapy group. To study the effect of irradiation on renal function, excluding the drug effect, rats that only underwent irradiation were compared with controls whereas the combined therapy groups were compared with rats that received c-DDP only. After this correction for the drug effect, the ED50, calculated by probit analysis, was 9.8 Gy (S.D.=0.7) for irradiation only and 9.7 Gy (S.D.=1.8) for combined c-DDP/irradiation

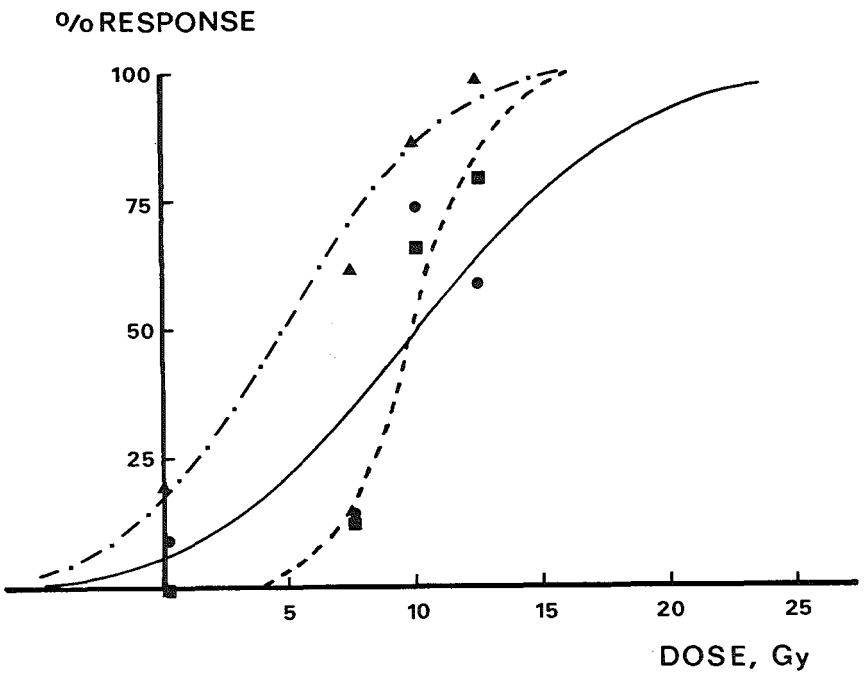


Fig. 2. Dose-effect curves giving the proportion of animals showing a ≥25% reduction in GFR (ml/min) in young rats 24 weeks after irradiation. ■-■, Kidney irradiation (p=0.58, slope=0.38, LD50=9.8 Gy). ▲-▲, Kidney irradiation followed by c-DDP, not corrected for c-DDP toxicity (p=0.70, slope=0.20, LD50=4.6 Gy). ●-●, Kidney irradiation followed by c-DDP, corrected for c-DDP toxicity (p=0.13, slope=0.15, LD50=9.7 Gy).

treatment 24 weeks after treatment.

GFR was linearly related to the reciprocal values of plasma urea and creatinine concentrations (18). At the time of the first measurement, 8 weeks after irradiation, the reciprocal values of plasma urea differed significantly from controls in all treatment groups except for the 7.5 Gy groups. This was also true for the reciprocal values of the plasma creatinine concentrations.

The effects of c-DDP and irradiation on the ERPF were less pronounced than the effects on the GFR. A significant reduction of the ERPF was only found in the 12.5 Gy groups starting 2 months after irradiation.

TABLE 1. TIME RELATED CHANGES IN GFR (ML/MIN PER 100 G) AFTER RENAL IRRADIATION AND/OR c-DDP (5 MG/KG BW) ADMINISTRATION IN YOUNG RATS

	Time after irradiation (weeks)											
	2		4		8		12		16		24	
Group	n	GFR	n	GFR	n	GFR	n	GFR	n	GFR	n	GFR
C	10	1.051	10	1.032	10	0.779	10	0.724	10	0.670	9	0.697
		0.125		0.111		0.071		0.035		0.045		0.122
7.5 Gy	10	0.973	10	0.933	10	0.824	10	0.754	10	0.683	10	0.708
		0.102		0.095		0.073		0.115		0.059		0.087
10 Gy	10	0.887	8	0.782[a]	8	0.692	9	0.668	9	0.569	9	0.536
		0.188		0.070		0.124		0.094		0.104		0.081
12.5 Gy	9	0.981	10	0.771[a]	10	0.595[a]	9	0.516[a]	10	0.452[a]	10	0.407
		0.145		0.114		0.128		0.096		0.193		0.213
c-DDP	7	0.862	9	0.814[a]	10	0.732	10	0.690	10	0.590	10	0.582
		0.181		0.153		0.113		1.187		0.155		0.149
7.5 Gy	8	0.950	6	0.886[a]	8	0.687	8	0.593[a]	7	0.593	8	0.535
+ c-DDP		0.106		0.168		0.060		0.071		0.089		0.113
10 Gy	8	0.952	8	0.695[a]	8	0.588[a]	8	0.535[a]	8	0.448[a]	8	0.466[a]
+ c-DDP		0.167		0.077		0.115		0.118		0.109		0.113
12.5 Gy	7	0.937	5	0.710[a]	5	0.520[a]	4	0.385[a]	4	0.398[a]	5	0.335[a]
+ c-DDP		0.102		0.144		0.166		0.165		0.127		0.219

[a] Significantly different from controls (C), $p < 0.05$.
n = Number of rats in each group.
Data are means ± S.D.

Renal function in adult rats

In adult rats, c-DDP or irradiation alone did not significantly reduce the GFR (Table II) or the ERPF (in ml/min and ml/min/100g) compared with control values during the whole follow-up period. Of the combined therapy groups only the 12.5 Gy plus c-DDP showed a progressive decline of the GFR and ERPF starting 12 weeks after irradiation. After 24 weeks the value differed significantly from controls. At this time, the reciprocal of the plasma urea concentration was significantly depressed. However, plasma creatinine concentrations remained normal.

TABLE 2. TIME RELATED CHANGES IN GFR (ML/MIN PER 100 G) AFTER RENAL IRRADIATION AND/OR c-DDP (2.5 MG/KG BW) ADMINISTRATION IN ADULT RATS

	Time after irradiation (weeks)											
	2		4		8		12		16		24	
Group	n	GFR	n	GFR	n	GFR	n	GFR	n	GFR	n	GFR
C	10	0.661	10	0.661	10	0.705	10	0.632	10	0.606	10	0.649
		0.056		0.088		0.048		0.057		0.039		0.057
7.5 Gy	10	0.739	10	0.703	10	0.663	10	0.653	10	0.684	9	0.640
		0.077		0.073		0.039		0.051		0.080		0.100
10 Gy	10	0.754	9	0.750	9	0.588	9	0.715	9	0.615	9	0.684
		0.060		0.065		0.176		0.045		0.077		0.200
12.5 Gy	9	0.852	9	0.721	10	0.646	10	0.672	10	0.637	10	0.590
		0.387		0.060		0.079		0.047		0.075		0.114
c-DDP	9	0.690	8	0.662	9	0.630	9	0.698	9	0.651	9	0.616
		0.114		0.075		0.084		0.064		0.055		0.042
7.5 Gy +	9	0.738	10	0.702	10	0.683	10	0.677	10	0.657	10	0.594
c-DDP		0.056		0.160		0.044		0.050		0.070		0.019
10 Gy +	10	0.707	10	0.657	8	0.694	9	0.684	9	0.602	9	0.583
c-DDP		0.055		0.210		0.087		0.060		0.105		0.083
12.5 Gy +	10	0.733	10	0.729	10	0.650	10	0.649	10	0.566	10	0.497
c-DDP		0.115		0.072		0.089		0.062		0.048		0.048

[a] Significantly different from control (C), $p < 0.05$.
n = Number of rats in each group.
Data are means ± S.D.

Systolic Blood Pressure

Blood pressure was first measured after a 6-week training period, and continued until 23 weeks following irradiation. The mean SBP in young and adult control rats varied from 120-140 mmHg. Radiation doses of 10 Gy or less, either alone or combined with c-DDP did not change the SBP in either age group. In young rats, both the 12.5 Gy group and the 12.5 Gy plus c-DDP group showed a rise in SBP, starting 12 weeks after irradiation. By that time, the GFR of these rats had declined to 65% of control values. After 20 weeks, the mean SBP in these groups approached 160 mmHg (Fig 3).

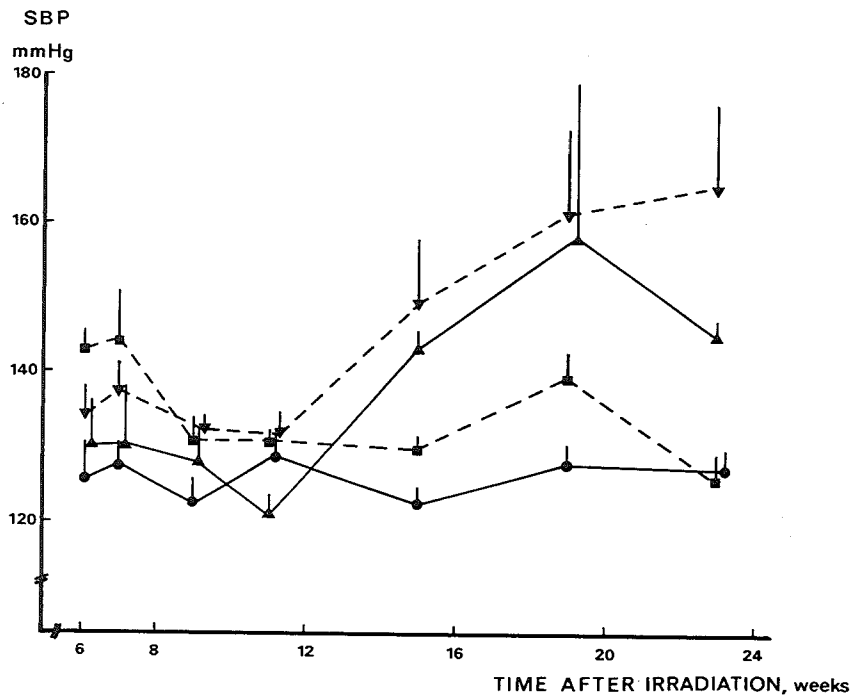


Fig 3. Time related changes in SBP (mmHg) in young rats during 23 weeks in controls (■), after 5 mg c-DDP/kg BW (●) and after a radiation dose of 12.5 Gy to both kidneys either given alone (▼) or combined with 5 mg c-DDP/kg BW (▲). Data are means ± SEM.

The apparent decline of the mean SBP in young rats, 24 weeks after 12.5 Gy +c-DDP, was due to death of one hypertensive rat in an already small group (Fig. 3). In adult rats, there was only a tendency to increase to a mean value of 135 mmHg after 24 weeks in the 12.5 Gy plus c-DDP group.

DISCUSSION

The nephrotoxicity of single doses of c-DDP and irradiation in young and adult rats was investigated. c-DDP and radiation were administered in doses known to cause a limited decline in renal function (11, 12). Both c-DDP and irradiation caused renal function damage in young rats. When used in combination, the reduction in renal function compared to age matched controls, was more pronounced than after either treatment modality alone. The similar dose-effect curves obtained after correction for c-DDP induced renal damage suggest that renal radiation damage is only additional to the damage caused by c-DDP. Thus, c-DDP induced renal damage as well as sub sequent regeneration and irradiation induced damage occur independently in the young rat kidney. In adult rats, c-DDP alone or irradiation alone did not significantly affect the GFR during the follow-up period. Only the highest radiation dose (12.5 Gy) in combination with c-DDP caused a slowly progressive decline in GFR, starting after 12 weeks. The changes in the reciprocal values of plasma urea and creatinine concentrations were similar to the GFR changes. The changes in ERPF were less pronounced.

Systolic hypertension (>140 mmHg) was only observed in young rats, 12-16 weeks after 12.5Gy, given alone or in combination with c-DDP. A rise in SBP occurred only after the GFR had dropped to 65% of the control GFR. The SBP was only elevated in irradiated rats. c-DDP, given alone or in combination with irradiation, did not by itself influence the SBP. Although the SBP

started to rise when renal functional damage was well established, its appearance might accelerate a further renal function decline. The damaged kidney is particularly prone to hypertensive injury (25) and irradiation might sensitize the blood vessels to hypertensive damage (1). The mechanisms of the rise in blood pressure following irradiation have been the subject of a more extensive study (12a).

The early expression of radiation nephropathy in young rats compared to adult rats is in agreement with our earlier findings (12). Normally, the GFR (ml/min) quadruples during the 8 weeks between the age of 3 and 11 weeks. Kidney irradiation with doses of 10 Gy or more at the age of 3 weeks, diminished this rapid increase in renal function. After 8 weeks, the increase in renal function slowed down and was similar in control and irradiated rats. The early manifestation of radiation nephropathy might be due to the higher proliferation rate in the young rat kidney, leading to a more rapid expression of renal damage. This initial inhibition of the increase in renal function in young rats, was followed by a further decline, starting earlier at higher doses. In adult rats, the same X-ray doses did not cause an effect on renal function until 8-12 weeks after irradiation. After that time, renal function declined dose-dependently and progressively. Twenty weeks after irradiation, dose-effect curves in young and adult rats were quite similar (12). The higher proliferation rate in the young rat kidney compared to the adult rat kidney, might be an explanation for the early expression of renal radiation damage in the young rat kidney. Autoradiographic studies of kidneys of 3-weeks and 4-month-old rats showed that the percentage of mitotic cells was almost 3 times higher in the renal cortex of young rats (0.39%) compared to that of adult rats (0.14%). In the renal medulla, the mitotic activity was similar (0.04-0.05%) for young andadult rats (21).

The experiments concerning c-DDP nephrotoxicity in young and adult rats showed that the same dose of c-DDP (mg/kg BW) caused less renal damage in young rats than in adult rats. This could be explained by the larger kidney weight relative to body weight in young rats (11) compared to adult rats. Similar levels of renal function were obtained in young and adult rats when the young rats received twice the adult dose (in mg/kg BW). In agreement with earlier findings in young rats, c-DDP (5 mg/kg BW) inhibited body growth and renal function development. When renal function was related to BW (GFR in ml/min/100g) no significant differences from control values were found 2 weeks after drug administration in young rats. In adult rats, c-DDP (2.5 mg/kg BW) had no effect on GFR, when measured at least 2 weeks after drug administration.

Renal injury caused by c-DDP mainly affects the proximal tubule. Four days after single drug administration in rats, this damage is most pronounced showing cell swelling and necrosis (10). The subsequent renal regeneration was accompanied by an extensive proliferation in the straight portion of the proximal tubule, where damage was most pronounced (4). Renal function was restored rapidly (11). Provided the target cells for c-DDP and irradiation are the same, the attempted proliferation following c-DDP-induced damage would be expected to accelerate the expression of radiation-induced damage. At the first renal function measurement, 2 weeks after c-DDP administration in young rats, the GFR was 80-90% of control values in irradiated as well as in nonirradiated rats. At this time, irradiation did not show an effect on renal function. The similarity in renal function after c-DDP administration, irrespective of renal irradiation, suggests an undisturbed regeneration of the c-DDP-induced renal damage when this treatment is combined with irradiation. Functional renal damage due to irradiation manifested itself in young rats four weeks after

treatment. After correction for drug toxicity, radiation damage was the same in c-DDP treated rats as in saline treated rats, as was shown by the ED50 values obtained by probit-analysis. Thus, our data indicate only additive toxicity of irradiation and c-DDP in the young rat kidney. This suggests that c-DDP and irradiation have different target cells in the kidney. Another explanation for the no more than additive interaction between irradiation and c-DDP might be that the low doses of c-DDP used in this study damaged, but did not destroy, the proximal tubular cells. Functional renal recovery occurred without cell proliferation and was not inhibited by irradiation. Experimentally, the effect of combined radiation and c-DDP treatment was studied in rapid proliferating tissues such as skin (15) and gut (14, 16). In these studies, c-DDP enhanced the radiation effect. An unequivocal influence of time sequencing of c-DDP administration and irradiation on normal tissue damage did not emerge from the literature (5, 6, 15, 16). However, tissue damage seems to be most pronounced when drug administration and irradiation are applied in close proximity (9). When c-DDP was given after irradiation, small variations in time interval between irradiation and c-DDP administration did not seem to influence the response of the kidney (personal communication; E. van Rongen). Likewise, Stewart and coworkers recently found that renal toxicity in mice was most pronounced when c-DDP was given 0.5 hour before to 1 day after irradiation (Dose modifying factor=1.3, at 9 months after irradiation, not corrected for drug toxicity)(24). As the enhancement of renal radiation damage did not depend on c-DDP and X-ray sequencing, this group considered direct X-ray sensitization to be unlikely. This leaves additive toxicity and inhibition of radiation repair (7) as the explanation for the enhanced renal damage by combined treatment with c-DDP and irradiation.

It must be emphasized that the present study only deals with the effect

of the combination of single doses of c-DDP and radiation. In vitro studies indicate that the enhancement of radiation damage by c-DDP may be more pronounced after fractionated irradiation than after single dose irradiation. This is due to inhibition of the repair of sublethal radiation damage (7).

In conclusion, the present study in young rats shows that the renal damage caused by c-DDP is additive to the damage caused by a single dose of radiation to the kidneys. The additivity of the renal damage in young rats indicates that the irradiated kidney is still capable of restoring the initial renal damage induced by c-DDP, to the same extent as a non-irradiated kidney. Thus, despite inhibition of renal growth and development in the young rat by irradiation, the capacity for repair of c-DDP-induced damage remained relatively intact. Functional renal damage was reflected by the GFR as well as plasma creatinine and urea concentrations. The SBP only increased after a change had occurred in these parameters.

References

1. Asscher, A.W., Wilson, C., Anson, S.G. Sensitisation of blood-vessels to hypertensive damage by X-irradiation. Lancet 1: 580-583, 1961.

2. Bunag, R.D. Validation in awake rats of a tail cuff method for measuring systolic blood pressure. J. Appl. Physiol. 34: 279-282, 1973.

3. Carde, P., Laval, F. Effect of cis-dichlorodiammine platinum II and X rays on mammalian cell survival. Int. J. Radiat. Oncol. Biol. Phys. 7:929-933, 1981.

4. Dobyan, D.C., Levi, J., Jacobs, C., Kosek, J., Weiner, M.W. Mechanism of cis-platinum nephrotoxicity: II Morphologic observations J. Pharmacol. Exp.Ther. 213: 551-556, 1980.

5. Dewit, L., Oussoren, Y., Bartelink, H. Dose and time effects of cis-diamminedichloroplatinum(II) and radiation on mouse duodenal crypts. Radiother. Oncol. 4: 363-371, 1985.

6. Douple, E.B., Eaton, W.L., Tulloh, M.E. Skin radiosensitization studies using combined cis-dichlorodiammineplatinum (II) and radiation. Int. J.Radiat. Oncol. Biol. Phys. 5: 1383-1385, 1979.

7. Douple, E.B., Richmond, R.C. Radiosensitization of hypoxic tomour cells by cis- and trans-dichlorodiammineplatinum (II). Int. J. Radiat. Oncol. Biol. Phys. 5: 1369-1372, 1979.

8. Douple, E.B., Richmond, R.C. Enhancement of the potentiation of radiotherapy by platinum drugs in a mouse tumor. Int. J. Radiat. Oncol. Biol. Phys. 8: 501-503, 1982.

9. Fu, K.K. Biological basis for the interaction of chemotherapeutic agents and radiation therapy. Cancer 55: 2123-2130, 1985.

10. Goldstein, R.S., Mayor, G.H. The nephrotoxicity of cisplatin. Life Sci. 32: 685-690, 1983.

11. Jongejan, H.T.M., Provoost, A.P., Wolff, E.D., Molenaar, J.C. Nephrotoxicity of Cis-platin in young and adult rats. Pediat. Res. 20: 9-14,1986.

12. Jongejan, H.T.M., Kogel, van der A.J., Provoost, A.P., Molenaar, J.C. Radiation nephropathy in young and adult rats. Int. J. Rad. Rad. Oncol. Biol. Phys. 13: 225-232, 1987.

12a. Jongejan, H.T.M., Kogel van der, AJ, Provoost, A.P., Molenaar, J.C. Hypertension after bilateral kidney irradiation in young and adult rats. Rad. Res. 111: 474-487, 1987.

13. Leipzig, B., Wetmore, S.J., Putzeys, R., Suen, J.Y., Snyderman, N.L. Cis-platin potentiation of radiotherapy. Arch. Otolaryngol. 111: 114-118,1985.

14. Luk, K.H., Ross, G.Y., Phillips, T.L., Goldstein, L.S. The interaction of radiation and Cis-diamminedichloroplatinum (II) in intestinal crypt cells. Int. J. Radiat. Oncol. Biol. Phys. 5: 1417-1420, 1979.

15. Maase, von der, H. Effect of cancer chemotherapeutic drugs on the radiation-induced skin reactions in mouse feet. Br. J. Radiol. 57: 697-707,1984.

16. Maase, von der, H. Interactions of radiation and adriamycin, bleomycin, mitomycin C or cis-diamminedichloroplatinum II in intestinal crypt cells. Br. J. Cancer 49: 779-786, 1984.

17. Phillips, T.L. Chemical modification of radiation effects. Cancer 39:987-999, 1977.

18. Provoost, A.P., Molenaar, J.C. Changes in the glomerular filtration rate after unilateral nephrectomy in rats. Pflugers Arch. 385: 161-165, 1980.

19. Provoost, A.P., de Keyzer, M.H., Wolff, E.D., Molenaar, J.C. Development of renal function in the rat. Renal Physiol. 6: 1-9, 1983.

20. Reimer, R.R., Gahbauer, R., Bukowski, R.M., Hewlett, J.S., Groppe, C.W., Weick, J.K., Antunez, A.R. Simultaneous treatment with cisplatin and radiation therapy for advanced solid tumors: a pilot study. Cancer Treat. Rep. 65: 219-222, 1981.

21. Reiter, R.J., McCreight, C.E., Sulkin, N.M. Age differences in cellular proliferation in rat kidneys. J. Geront. 19: 485-489, 1964.

22. Shipley, W.U., Coombs, L.J., Einstein, A.B., Soloway, M.S., Wasjman, Z., Prout, G.R., National Bladder Cancer Collaborative Group. Cis-platin and full dose irradiation for patients withinvasive bladder carcinoma: a preliminary report of tolerance and local response. J. Urol. 132: 899-903, 1984.

23. Soloway, M.S., Ikard, M., Scheinberg, M., Evans, J. Concurrent radiation and cisplatin in the treatment of advanced bladder cancer: a preliminary report. J. Urol. 128: 1031-1033, 1982.

24. Stewart, F., Bohlken, S.B., Begg, A.B., Bartelink, H. Renal damage in mice after treatment with cisplatinum alone or in combination with X-irradiation. Int. J. Rad. Oncol. Biol. Phys. 12: 927-933, 1986.

25. Tsuruda, H., Okuda, S., Onoyama, K., Oh,Y., Fujishima, M. Effect of blood pressure on the progress of renal deterioration in rats with renal mass reduction. J. Lab. Clin. Med. 107: 43-50, 1986.

This work was supported by the Dutch Cancer Fund (Koningin Wilhelmina Fonds). We thank Dr D. van Bekkum, Radiobiologic Laboratory, TNO, Rijswijk) for putting the X-ray apparatus at our disposal. The technical assistance of Mrs E. Fierret and stylistic advice of Mrs A Ribbink-Goslinga were greatly appreciated.

CHAPTER 6

POTENTIATED NEPHROTOXICITY OF CIS-PLATIN WHEN COMBINED WITH AMIKACIN, COMPARING YOUNG AND ADULT RATS

Hermina.T.M. Jongejan, Abraham P. Provoost, Jan C. Molenaar.

Department of Pediatric Surgery, Erasmus University, Rotterdam, The Netherlands.

(This chapter has been submitted for publication)

ABSTRACT

We compared the nephrotoxic interaction between Cis-platin (Cp) and Amikacin (Am) in young and adult rats, using different dosage combinations. Following a single i.v. dose of Cp, Am was administered s.c. for 14 days. The dose of Cp was chosen to cause a moderate fall in the glomerular filtration rate (GFR), while a dose of Am was chosen that had only a minimal effect on GFR. Such a non-toxic course of Am seriously aggravated the Cp induced fall in GFR in adult rats. Only a limited recovery was observed during a 15 week follow-up period. In young rats, a non-toxic Am course did not aggravate the Cp induced impairment in GFR. However, when the dose of Am was increased to a nephrotoxic level, the Cp induced change in GFR was potentiated. As in adult rats, there was only a partial recovery of the GFR. In conclusion, in both adult and young rats, an Am course following a single injection of Cp results in a potentiation of the Cp-induced nephrotoxicity.

KEY WORDS: Cis-platin; Amikacin; Nephrotoxicity; GFR; ERPF; Rats

INTRODUCTION

Previously, we have compared in young and adult rats the nephrotoxicity of two aminoglycosides, gentamicin and amikacin (Am) (1), as well as that of chemotherapeutic drug Cis-platin (Cp) (2). For all three drugs, young rats required a higher dosage relative to BW to achieve a comparable impairment in the renal function. As many chemotherapeutic agents also suppress the immune system, anticancer treatment is often complicated by serious infections. This may require specific antibiotic treatment, in which aminoglycosides play a crucial role to tackle gram-negative bacterial infections. Owing to the nephrotoxicity of both Cp and aminoglycosides, extra attention is indicated when these drugs are used in combination (3,4).

The renal effects of aminoglycosides upon an already damaged kidney may be different from the effect upon an intact kidney. Experimental studies in rats showed a reduction in aminoglycoside nephrotoxicity in kidneys predamaged by either potassium-dichromate (5) or mercuric-chloride (6). Furthermore, a transient insensitivity to aminoglycoside nephrotoxicity is known to develop during prolonged treatment. Renal function recovers despite continuation of the aminoglycoside administration (7). However, studies dealing specifically with the nephrotoxic interaction between Cp and aminoglycosides showed that a short course, with a non-toxic dose, of tobramycin potentiated the Cp-induced acute renal damage (8,9).

In order to establish age related differences in the nephrotoxicity of a combined treatment with Cp and an aminoglycoside, we studied the effect of a single dose of Cp immediately followed by a course of Am during 14 days, in young and adult rats. Am was chosen as it was found to be less nephrotoxic than gentamicin (1).

MATERIALS AND METHODS

Male Wistar rats of an outbred strain were used (Zentral Institut fuür Versuchstierzucht, Hannover FRG). Food and tap water were available ad libitum.

Renal function determination.

The glomerular filtration rate (GFR) and the effective renal plasma flow (ERPF) were determined by a plasma clearance technique, as described in detail elsewhere (10). In short, this method involves an intravenous (i.v). injection of Cr-51 EDTA and I-125 Iodohippurate (Amersham International, Amersham, England) and a single, timed blood sample collected 60 min after the injection. The method allows for repeated use in the same animal.

Experimental protocol.

At the start of the experiment, the animals were divided into four groups of adult rats and seven groups of young rats, each group consisting of 8-10 animals. The adult rats were over 12 weeks old, with a body weight (BW) of over 250 grams; the young rats were 3-4 weeks old with a BW of 45-70 grams. The codes for the various experimental groups, indicating types of treatment and dosage are presented in Table 1 for adult rats and in Table 2 for young rats.

The Cp (kindly provided by Bristol-Myers BV, Weesp, The Netherlands) was administered in a single i.v. injection in a solution containing 0.5 mg Cp and 9 mg NaCl per ml of distilled water. The Am (kindly provided by Bristol-Myers BV, Weesp, The Netherlands) was administered subcutaneously (s.c.), daily for 2 weeks, dissolved in 0.9% NaCl in a concentration of 50 mg/ml. Controls received equal volumes of saline i.v. and/or s.c. When the drugs were given in combination, the Am was started immediately following

TABLE 1.

Group-codes, initial body weight, and treatment schedules for the various groups of adult rats.

ADULT	(n)	BW (gram)	Cp (mg/kg BW)	Am
A-C	(8)	311±19	-	-
A-Cp5	(9)	300±38	5	-
A-Am60	(8)	325±30	-	60
A-Cp5Am60	(10)	315±30	5	60

Data on BW are Mean±S.D.
The Cp was administered as a single i.v.dose; Am was administered s.c. daily during 14 days.

TABLE 2.

Group-codes, initial body weight, and treatment schedules for the various groups of young rats.

YOUNG	(n)	BW (gram)	Cp (mg/kg BW)	Am
Y-C	(8)	58±6	-	-
Y-Cp7.5	(9)	57±5	7.5	-
Y-Am180	(8)	59±7	-	180
Y-Cp7.5Am180	(8)	58±5	7.5	180
Y-Cp5	(9)	58±8	5	-
Y-Am540	(8)	59±7	-	540
Y-Cp5Am540	(8)	58±5	5	540

Data on BW are Mean±S.D.
The Cp was administered as a single i.v.dose; Am was administered s.c. daily during 14 days.

the Cp injection.

As equal doses (mg/kg BW) of Am (1) or Cp (2) being less nephrotoxic in young than in adult rats, the administered dose was higher in young rats to inflict a comparable degree of renal functional impairment (Table 1 and 2).

Renal function was measured at weeks 2, 4, 6, 9, and 15 after the first administration.

Statistics.

Statistical differences between the means of renal function parameters were assessed by oneway analysis of variance. In these analyses various groups of either adult or young rats treated with a single drug or receiving the combined treatment and the control group were clustered together. In case of statistical significant differences, i.e. an F value indicating $p<0.05$, the Newman-Keuls test was applied to detect which pair(s) of means were different.

The analyses were performed with commercially available software, SPSS/PC+ (SPSS Inc. Chicago, Ill), on a personal computer.

RESULTS

Adult rats

Data on the longitudinal changes in BW, GFR and ERPF of adult rats following the various treatments are presented in Table 3.

A single Cp injection of 5 mg/kg BW was fatal for three out of nine rats. In the remaining six animals Cp caused a significant fall in GFR and ERPF, measured 2 weeks after the injection. Only a slight recovery of the GFR was observed during the follow-up. The ERPF recovered to a level below that of A-C rats, although the difference was no longer statistically significant. The GFR and ERPF of adult rats, treated with Am in a dose of 60

TABLE 3.

LONGITUDINAL CHANGES IN BW (gram), GFR AND ERPF (ml/min) OF THE VARIOUS GROUPS OF ADULT RATS

	A-C	A-Cp5	A-Am60	A-Cp5Am60
Week 2	(8)	(6)	(8)	(6)
BW	343 ± 26	304 ± 26**	358 ± 40	302 ± 43**
GFR	2.53 ± 0.26	1.31 ± 0.50**	2.80 ± 0.32	0.68 ± 0.67***
ERPF	6.05 ± 0.36	4.05 ± 1.19**	6.04 ± 0.32	2.30 ± 2.20***
Week 4	(8)	(6)	(8)	(5)
BW	372 ± 26	332 ± 39	392 ± 40	327 ± 59
GFR	2.77 ± 0.25	1.75 ± 0.58**	3.01 ± 0.36	0.96 ± 0.81***
ERPF	6.06 ± 0.31	5.41 ± 1.97	6.12 ± 0.32	2.92 ± 2.26***
Week 6	(8)	(6)	(8)	(5)
BW	390 ± 35	347 ± 37	413 ± 46	335 ± 76
GFR	2.64 ± 0.34	1.60 ± 0.58**	2.81 ± 0.37	0.93 ± 0.96*
ERPF	5.69 ± 0.41	4.47 ± 1.18	5.53 ± 0.57	2.56 ± 2.64***
Week 9	(8)	(6)	(8)	(4)
BW	424 ± 34	379 ± 35	445 ± 43	378 ± 93
GFR	2.72 ± 0.11	1.75 ± 0.69**	2.94 ± 0.42	1.29 ± 1.15*
ERPF	5.62 ± 0.30	4.42 ± 1.44	5.95 ± 0.49	3.15 ± 2.58*
Week 15	(8)	(6)	(8)	(3)
BW	460 ± 41	424 ± 32	490 ± 45	424 ± 97
GFR	2.93 ± 0.30	1.91 ± 0.63**	3.01 ± 0.29	1.04 ± 1.11***
ERPF	5.88 ± 0.30	5.05 ± 0.93	5.85 ± 0.38	2.72 ± 1.98***

All data are mean ± S.D. ** $p < 0.05$ vs A-C; * $p < 0.05$ vs A-C and A-Am60; *** $p < 0.05$ vs A-C, Am60, and A-Cp5;

Statistically significant differences between A-Cp5 and A-Am60 are not presented here.

mg/kg BW for 14 days, did not differ significantly from that of A-C rats.

The combined treatment of Cp5 and Am60 was fatal for four out of ten adult rats. The GFR and ERPF of the remaining six rats, measured at week 2, was significantly less not just from that of A-C rats, but also from rats treated with either Am60 or Cp5 alone. During the follow-up, another three rats that received the combined treatment, died with signs of renal failure. Neither GFR or ERPF, showed much further recovery, and both levels remained below that of the three other treatment groups.

Since the BW of rats treated with Cp5, either alone or in combination with a 14 days-course of Am60, remained significantly less than that of A-C and A-Am60 treated rats, the results of the GFR determinations are also

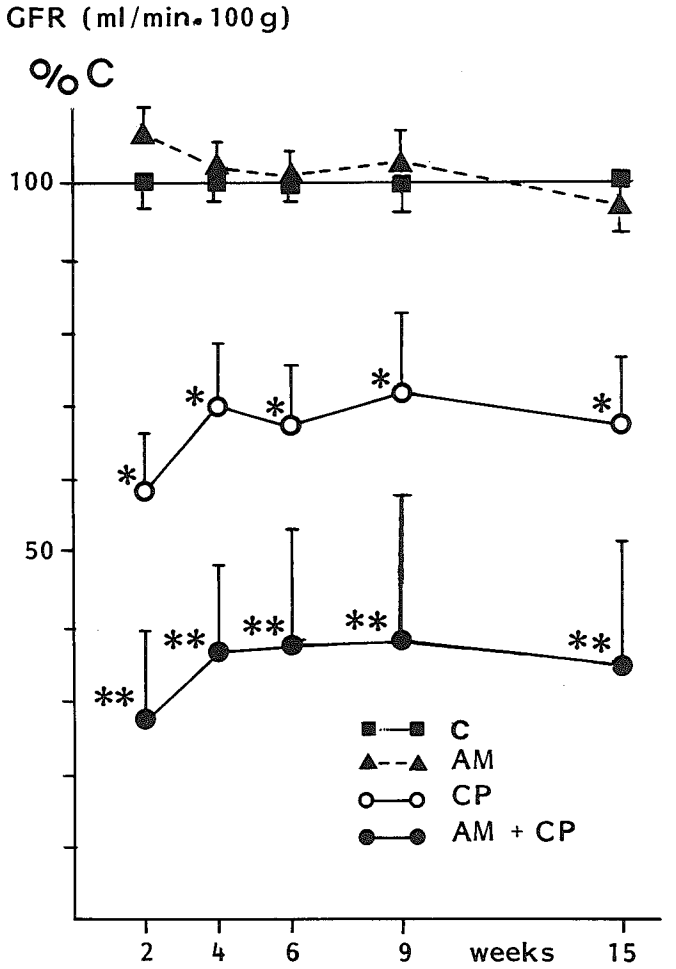


Fig 1: Longitudinal changes in GFR (ml/min/100 g BW) in adult rats.
Data are mean±S.D., expressed as a percentage of A-C control values.
Number of rats are as indicated in Table 3. For the A-Cp5Am60 rats, only data from the three surviving rats measured at all time points were included allowing for a better comparison.
* $p<0.05$ vs A-C; ** $p<0.05$ vs A-C, A-Am60 and A-Cp5.
Statistical differences between A-Cp5 and A-Am60 are not presented.

presented per 100 g BW (Figure 1). In this way, we can separate the effects of the various types of treatment on GFR from the overall effects. It is clear that a course of Am60 which is not nephrotoxic when given alone did potentiate the impairment in GFR caused by a single injection of Cp5. On

average, the effect of Cp alone was a 30% reduction of the GFR, while effect of the combined treatment amounted to a reduction of 60% in the surviving rats.

Young rats

Data on the longitudinal changes in BW, GFR and ERPF of young rats following the various types of treatment are presented in Table 4. Next to Y-C rats, two clusters of three treatment groups each can be distinguished. Firstly, there are the three groups of rats treated with either Cp7.5, or Am180 or the combination Cp7.5Am180. Secondly, there are the three groups of rats treated with either Cp5, or Am540, or the combination Cp5Am540.

TABLE 4.

LONGITUDINAL CHANGES IN BW (gram), GFR AND ERPF (ml/min) OF THE VARIOUS GROUPS OF YOUNG RATS

	Y-C	Y-Cp7.5	Y-Am180	Y-Cp7.5 +Am180	Y-Cp5	Y-Am540	Y-Cp5 + Am540
Week 2	(8)	(7)	(8)	(8)	(8)	(8)	(4)
BW	140 ± 12	102 ± 11	140 ± 13	91 ± 16	116 ± 21**	132 ± 10	76 ± 15***
GFR	1.61 ± 0.26	0.67 ± 0.26**	1.51 ± 0.20	0.56 ± 0.20*	1.09 ± 0.43**	1.20 ± 0.29**	0.30 ± 0.20***
ERPF	3.33 ± 0.39	1.85 ± 0.38**	3.35 ± 0.40	1.86 ± 0.45*	2.72 ± 0.71	3.11 ± 0.42	1.00 ± 0.79***
Week 4	(8)	(7)	(8)	(8)	(8)	(8)	(4)
BW	212 ± 28	161 ± 16**	223 ± 19	152 ± 26*	188 ± 36	214 ± 16	130 ± 18***
GFR	2.34 ± 0.39	1.19 ± 0.50**	2.37 ± 0.29	1.03 ± 0.46*	1.73 ± 0.48**	2.46 ± 0.28	0.66 ± 0.31***
ERPF	4.26 ± 0.45	2.92 ± 0.74**	4.41 ± 0.40	2.82 ± 0.94*	3.61 ± 0.92	4.51 ± 0.57	2.02 ± 0.78***
Week 6	(8)	(7)	(8)	(8)	(8)	(8)	(4)
BW	278 ± 26	218 ± 19**	282 ± 20	207 ± 20*	248 ± 48	275 ± 17	175 ± 15***
GFR	2.40 ± 0.25	1.27 ± 0.53**	2.49 ± 0.34	1.22 ± 0.48*	1.85 ± 0.60**	2.61 ± 0.45	0.68 ± 0.27***
ERPF	4.95 ± 0.45	3.48 ± 0.80**	5.12 ± 0.27	3.47 ± 0.82*	4.06 ± 0.97**	5.25 ± 0.21	2.33 ± 0.78***
Week 9	(8)	(7)	(8)	(8)	(8)	(8)	(4)
BW	345 ± 32	280 ± 26**	357 ± 22	276 ± 22*	320 ± 64	341 ± 25	229 ± 80***
GFR	2.65 ± 0.37	1.50 ± 0.40**	2.79 ± 0.32	1.43 ± 0.54*	2.17 ± 0.63**	2.95 ± 0.25	0.89 ± 0.41***
ERPF	5.52 ± 0.37	4.54 ± 0.43**	5.43 ± 0.89	4.26 ± 0.79*	5.13 ± 0.76	5.78 ± 0.41	2.86 ± 1.10***
Week 15	(8)	(7)	(8)	(8)	(8)	(7)	(4)
BW	422 ± 50	360 ± 41**	439 ± 31	352 ± 30*	349 ± 81	420 ± 42	296 ± 14***
GFR	2.79 ± 0.34	1.85 ± 0.51**	2.84 ± 0.17	1.52 ± 0.66*	2.31 ± 0.63**	2.98 ± 0.19	1.06 ± 0.50***
ERPF	5.82 ± 0.31	5.10 ± 0.36**	5.98 ± 0.29	4.56 ± 1.08*	5.41 ± 0.79	5.75 ± 0.42	3.57 ± 1.01***

All data are Mean ± S.D. ** $p < 0.05$ vs Y-C; * $p < 0.05$ vs Y-C and Y-Am180; *** $p < 0.05$ vs Y-C, Y-Am540, and Y-Cp5; Statistical differences between the groups of rats treated with either Cp or Am alone are not presented here.

A single Cp injection of 7.5 mg/kg BW was fatal for two out of nine

rats. The GFR and ERPF of the surviving rats were significantly below those of Y-C rats during the whole follow-up period. When compared with Y-C rats, a 14 day course of Am180 affected neither GFR nor ERPF.

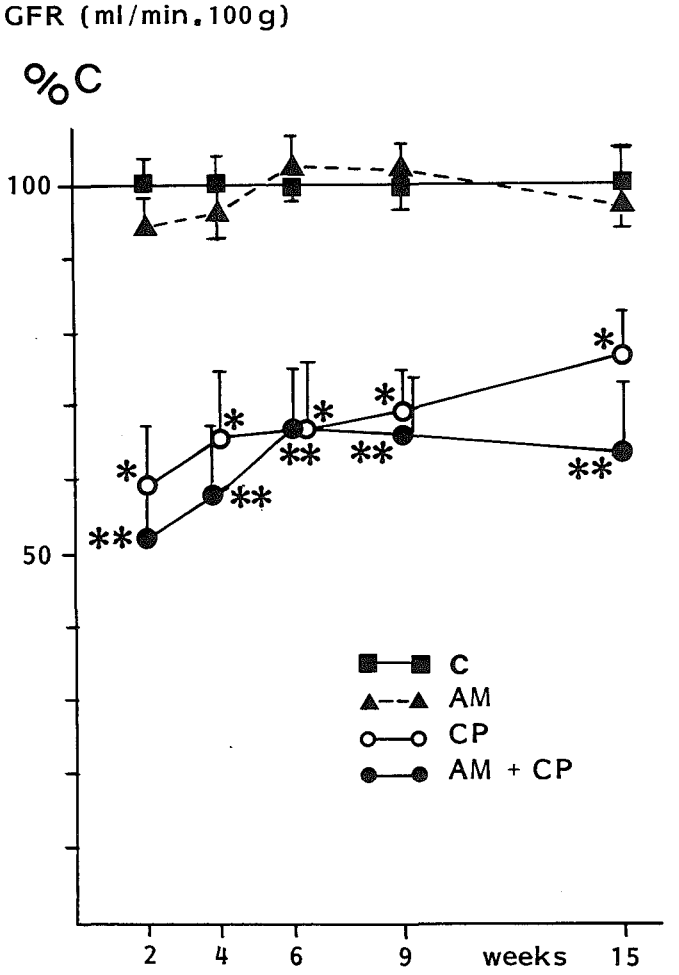


Fig. 2: Longitudinal changes in GFR (ml/min/100 g BW) in Y-C, Y-Cp7.5, Y-Am180, and Y-Cp7.5Am180.
Data are mean±S.D., expressed as a percentage of Y-C control values. Number of rats are as indicated in Table 4. * $p<0.05$ vs Y-C; ** $p<0.05$ vs Y-C and Y-Am180. Statistical differences between Y-Cp7.5 and Y-Am180 are not presented.

The combined treatment of young rats with Cp7.5 and Am180 resulted in

GFR and ERPF levels that were significantly less than those of Y-C and Y-Am180 rats. However, both the GFR and the ERPF of Y-Cp7.5Am180 rats at no point diverged significantly from the GFR and ERPF of rats treated with Cp7.5 alone.

Since the treatment of young rats with Cp7.5, either alone or followed by a course of Am180, reduced body growth when compared with Y-C and Y-Am180 treated rats, the GFR data are given per 100 g BW (Figure 2). It is clear that a course of Am180 did not aggravate the impairment in GFR caused by a single injection of Cp7.5.

A single Cp injection of 5 mg/kg BW was fatal for one out of eight rats. The GFR of the surviving rats was significantly less than that of Y-C rats during the whole follow-up period. Although the ERPF of Y-Cp5 rats was somewhat less that that of Y-C rats, this difference was only statistically significant at week 6. When compared with Y-C rats, the GFR was significantly less at the end of a 14 day course of Am540, while the ERPF remained the same. During the follow-up, the GFR recovered to a level not significantly different from that of Y-C rats.

The combined treatment of young rats with Cp5 and Am540 was fatal for four out of eight rats. In the surviving animals this treatment resulted in GFR and ERPF levels that were significantly less that those of Y-C, Y-Am540 and Y-Cp5 rats.

In comparison with Y-C and Y-Am540, the body growth of young rats appeared affected by Cp5 treatment, particularly when followed by a course of Am540. Consequently, the GFR data are also presented per 100 g BW (Figure 3). It is clear that a course of Am540, that is only slightly nephrotoxic when given alone, potentiated the impairment in GFR caused by a single injection of Cp5. On average, the effect of Cp alone was a 15% reduction in GFR, while the GFR of the rats that survived the combined treatment was

reduced by 55%.

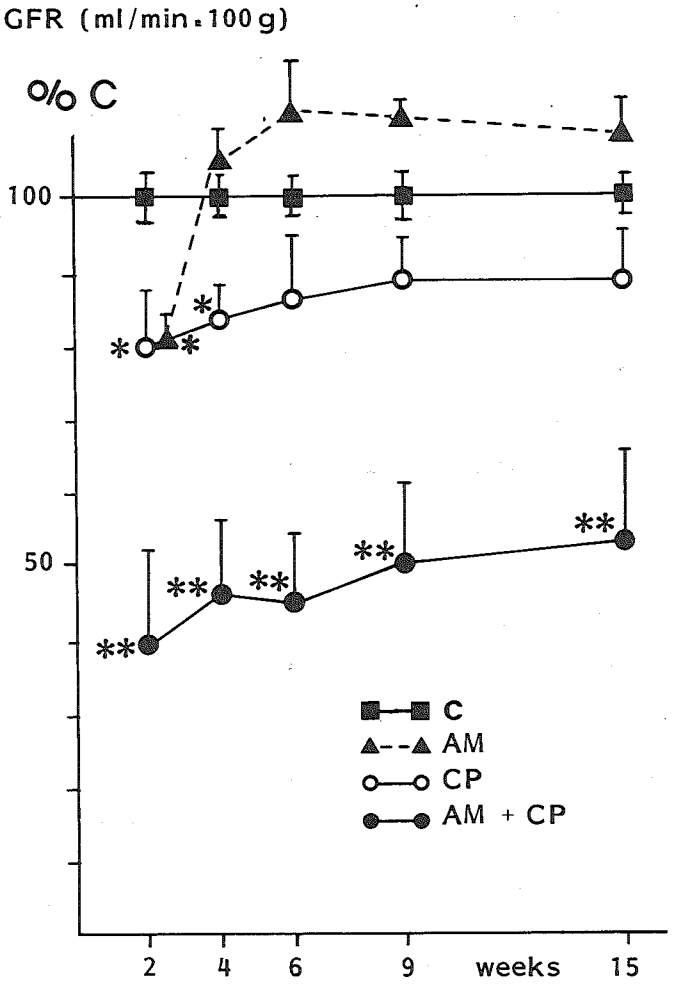


Fig 3.: Longitudinal changes in GFR(ml/min/100 gBW) in Y-C, Y-Cp5, Y-Am540 and Y-Cp5Am540 rats.
Data are mean±S.D., expressed as a percentage of Y-C control values. Number of rats are as indicated in Table 4. * $p<0.05$ vs Y-C; ** $p<0.05$ vs Y-C, Y-Am540 and Y-Cp5.Statistical differences between Y-Cp5 and Y-Am540 are not presented.

The results of the present experiments show that age-related differences exist between young and adult rats, with regard to the nephrotoxic interaction of a non or low nephrotoxic course of an aminoglycoside antibiotic given immediately after a moderately toxic single dose of Cp. The results indicate that depending on combined dosage, Am may seriously aggravate the GFR impairment induced by Cp in both adult and young rats.

Based on our previous research, comparing the nephrotoxicity of either aminoglycosides (1) or Cp (2) between young and adult rats, we used dosages that would induce similar changes in GFR in both age groups. As expected, the GFR was in the order of 60% of controls after 5 mg/kg BW in adults and after 7.5 mg/kg BW in young rats. When combined with a non-nephrotoxic 14 day course of Am, amounting to 60 mg/kg BW per day in adults and to 180 mg/kg BW per day in young rats, only adult rats showed a potentiation of the renal function impairment. In young rats, such a potentiation could be revealed by increasing the daily dose of Am to 540 mg/kg day, which in itself caused a 20% reduction in GFR per 100 g BW at the end of the treatment period. Consequently, we reduced the Cp dose to 5 mg/kg BW, which caused less impairment in the GFR than a dose of 7.5 mg/kg BW.

Although both Cp and aminoglycosides are nephrotoxic, the mode of action as well as the time course of the renal changes are different. A single dose of Cp already induces nephrotoxic changes (2). The morphological changes consist of dose dependent cell damage, confined mainly to the straight portion of the proximal tubule, predominantly in the juxtamedullary zone (11,12). The drug is thought to be transported by the tubular cells (12) and concentrated intracellularly (13). Morphologically (14) as well as functionally (2,12) Cp-induced changes reach their peak 3 to 7 days after a single injection. There is a partial recovery of the renal function, occurring predominantly in the following 1-2 weeks, but some impairment of

renal function remains (2).

Aminoglycosides are concentrated mainly in the convoluted part of the proximal tubule by active reabsorption (15). Dose-dependent functional as well as structural changes develop during a course of aminoglycosides. The structural changes are most pronounced after 1 week, while the GFR falls mainly during the second week of drug administration (1,5,7,16). Subsequently, the GFR may even increase despite continuation of the aminoglycoside administration, and the kidney becomes temporarily insensitive to aminoglycoside toxicity (7).

Two explanations are possible for the interaction between Cp and Am. It may be seen as either a potentiation of the Cp-nephrotoxicity by Am or an increased sensitivity to Am of the kidney predamaged by Cp. The combined treatment causes a permanent impairment in GFR, but at a lower level than induced by treatment with the same dose Cp only. Thus, to our opinion the changes induced by the combined treatment can best be explained as an Am induced potentiation of Cp-nephrotoxicity.

Our present findings are in agreement with two previous reports (8,9), that a short non-toxic course of the amino glycoside tobramycin, given during or just after Cp treatment, was more nephrotoxic than Cp administered alone. Without further investigation, we can only speculate about the mechanism of this type of aminoglycoside-induced potentiation of Cp-nephrotoxicity. It has been reported that histopathological changes induced by the combined Cp and tobramycin treatment were more severe than after Cp alone (9). A possible explanation could be an interference of the aminoglycosides with the recovery of the initial Cp-induced renal damage.

The effects of aminoglycosides after Cp are at variance with those observed after the combined administration of the aminoglycoside, gentamicin, and other nephrotoxins, like potassium-dichromate (5) or

mercuric-chloride (6). For both compounds it has been reported pretreatment attenuated the nephrotoxic effect of a subsequent course of gentamicin. In case of potassium-dichromate, the gentamicin was administered after a one week interval. During that period, the initial damage had been partly repaired. In the combination mercuric-chloride and gentamicin, the drugs were administered together. Gentamicin did not interfere with the recovery from renal changes induced by mercuric chloride. Thus, the nephrotoxic mechanisms of Cp and mercuric-chloride are probably different.

In conclusion, in both adult and young rats, the administration of non- or low-toxic course of amikacin immediately following a single dose of Cp, may cause severe aggravation of the renal impairment caused by Cp alone. Clinicians should be aware of this potential consequence when Cp and aminoglycosides have to be used in combination.

REFERENCES

1.Provoost AP, Adejuyigbe O, Wolff ED (1985) Nephrotoxicity of aminoglycosides in young and adult rats. Pediatr Res 19:1191-1196

2. Jongejan HIM, Provoost AP, Wolff ED, Molenaar JC (1986) Nephrotoxicity of Cis-platin comparing young and adult rats. Pediatr Res 20:9-14

3. Haas A, Anderson L, Lad T (1983) The influence of aminoglycosides on the nephrotoxicity of Cis-diamminedichloroplatinum in cancer patients. J Infect Dis 147:363

4. Gonzalez-Vitale JC, Hayes DM, Cvitkovic E, Sternberg SS (1978) Acute renal failure after cis-Dichlorodiammine platinum (II) and gentamicin-cephalothin therapies. Cancer Treat Rep 62:693-698

5. Elliott WC, Houghton DC, Gilbert DN, Baines-Hunter J, Bennett WM (1982) Gentamicin nephrotoxicity. II. Definition of conditions necessary to induce acquired insensitivity. J Lab Clin Med 100:513-525

6. Luft FC, Yum MN, Kleit SA (1977) The effect of concomitant mercuric chloride and gentamicin on kidney function and structure in the rat. J Lab Clin Med 89:622-631

7. Elliott WC, Houghton DC, Gilbert DN, Baines-Hunter J, Bennett WM (1982) Gentamicin nephrotoxicity. I. Degree and permanence of acquired insensitivity. J Lab Clin Med 100:501-512

8.Kawamura J, Soeda A, Yoshida O (1981) Nephrotoxicity of Cis-diamminechloroplatinum (II) (Cis-platinum) and the additive effect of antibiotics: morphological and functional observation in rats. Toxicol Appl Pharmacol 58:475-482

9. Bregman CL, Williams PD (1986) Comparative nephrotoxicity of carboplatin and cisplatin in combination with tobramycin. Cancer Chemother Pharmacol 18:117-123

10. Provoost AP, De Keijzer MH, Wolff ED, Molenaar JC (1983) Development of renal function in the rat. Renal Physiol 6:1-9

11. Dobyan DC, Levi J, Jacobs C, Kosek J, Weiner MW (1980) Mechanism of Cis-platinum nephrotoxicity: II. Morphologic observations. J Pharm Exp Therap 213:551-556

12. Chopra S, Kaufman JS, Jones TW, Hong WK, Gehr MK, Hamburger RJ, Flamenbaum W, Trump BF (1982) Cis-diamminedichloroplatinum-induced acute renal failure in the rat. Kidney Int 21:54-64

13. Choie DD, Longnecker DS, Del Campo AA (1981) Acute and chronic Cisplatin nephropathy in rats. Lab Invest 44:397-402

14. Goldstein RS, Mayor GH (1983) The nephrotoxicity of Cisplatin. Life Sci 32:685-690

15. Whelton A (1982) Renal tubular transport and intrarenal aminoglycoside

distribution. In: Whelton A, Neu HC (eds) The aminoglycosides. Marcel Dekker, New York, pp:191-222

16. Gilbert DN, Houghton DC, Bennett WM, Plamp CE, Reger K, Porter GA (1979) Reversibility of gentamicin nephrotoxicity in rats: recovery during continuous drug administration. Proc Soc Exp Biol Med 160:99-103

ACKNOWLEDGEMENT

This study was supported by a grant from the Dutch Cancer Foundation (Koningin Wilhelmina Fonds).

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

7.1 METHODOLOGICAL ASPECTS.

The aim of the study was to compare the susceptibility of the young and the adult rat kidney to various treatment modalities as used in clinical oncology. Clinical and experimental information on this subject is scant and the findings are not well understood.

The kidneys of young individuals are still growing, and show a variable response to toxic stimuli corresponding to the stage of development and as such differing from the response of adult kidneys. The questions defined in the Introduction were all investigated in a rat model. Despite differences between the rat and the human kidney, this animal has been shown to provide a good model for studying the nephrotoxicity of drugs and the effects of other renal insults. Similarities in renal growth and development between human and weanling rat kidneys warrant the clinical relevance of the nephrotoxicity studies in weanling rat kidneys. However, extrapolation of our experimental findings in the rat to the human situation, albeit challenging, needs further clinical verification.

Drugs and radiation dosages that were known to be nephrotoxic in adult animals, were applied to young rats. Using various renal function parameters, renal injury in young and adult rats was compared. In all our experiments, the changes in the GFR and ERPF were used as the main indicators of a change in renal function. Despite similar changes in renal function, the mechanisms underlying the decline in GFR and ERPF may be different for irradiation and nephrotoxic drugs. A decline in GFR and ERPF may be attributed to vascular, glomerular or tubular effects. Vascular injury such as renal artery stenosis or thrombosis will result in occlusive changes leading to reduced renal blood flow and a subsequent reduction in GFR and ERPF to equal degree. Glomerular damage, causing a reduction in the

glomerular filtering area or changes in the capillary pressure, will induce changes in the GFR that will be disproportional to changes in ERPF. Tubular damage may result in a reduction in the GFR, due to either a rise in tubular hydraulic pressure secondary to tubular obstruction with necrotic cells or a reduction in renal perfusion by the tubulo-glomerular feedback mechanism (Wright and Briggs, 1979). According to this mechanism, tubular flow and/or urinary electrolyte concentration at the macula densa region of the distal tubule, influence renal perfusion. An increase in urine flow and electrolyte losses, due to proximal tubular damage, leads to vasoconstriction of the afferent arterioles and concomittant vasodilatation of the efferent arterioles. Thus, glomerular capillary hydraulic pressure is reduced. An intrarenal renin-angiotensin system is believed to be the prime hormonal mediator of this feedback mechanism.

7.2 RENAL FUNCTION AFTER KIDNEY IRRADIATION.

It was not until 1952 that the kidney was recognized as one of the most radiosensitive organs in the abdomen and dose-response correlations were made (Künkler et al., 1952). Based on these results, safety limits were set. For adults, 23 Gy (2300 rad) to both kidneys in five weeks or 20 Gy (2000 rad) for one kidney in two weeks is considered to be safe. For children, the safety limits were set a little lower: 15 Gy (1500 rad) to one kidney in two weeks (Moss et al., 1979). Information on the radiosensitivity of the growing kidney being limited, we studied renal functional damage after renal irradiation in young rats in comparison with adult rats.

In our study a single radiation dose to both kidneys in young and adult rats caused a dose dependent decline in glomerular as well as tubular renal function. The time it took for renal radiation damage to become manifest was

related to radiation dosage and the age at the time of renal irradiation. Higher radiation dosage shortened the latency time after which renal function damage occurred and renal radiation damage occurred sooner in young rats than in adult rats after the same radiation dose.

The early inhibition of renal function development following bilateral kidney irradiation in young rats can be explained by an inhibition of renal growth as shown by Donaldson et al. (1978) in weanling mouse kidneys. The inhibition of renal function development in young rats was most pronounced during the phase of rapid renal function development, the first two months after irradiation. The time it takes for renal radiation damage to appear depends on the proliferative activity in the kidney (Rubin et al., 1982), on the radiation dose and on the sensitivity of the method of measurement. Rapidly proliferating tissues such as skin and gut are known to respond quickly to radiation damage. The higher proliferation rate of the growing young rat kidney, compared with the adult rat kidney (Reiter et al., 1964), thus explains the accelerated manifestation of renal radiation damage in young rats compared with adult rats.

Two to four months after irradiation, the GFR stabilizes in young rats. This stabilization of renal function might indicate regeneration of the renal parenchym. As the regenerative ability of the kidney decreases with age (Reiter et al., 1964), no such delay in renal function decline occurred in adult rats. Six months after irradiation, the dose-response curves constructed for young and adult rats were similar, indicating an equal radiosensitivity of the kidney in both age-groups. It must be emphasized that the resemblance in radiosensitivity was only shown after a single radiation dose to the kidney. Fractionation of the radiation dose has been shown to be beneficial in reducing radiation damage in the kidney. This tissue sparing effect obtained by fractionation seemed inversely related to

the proliferative activity of the tissue (Stewart et al., 1987). Thus, the young rat kidney with its higher proliferation rate might yield less advantage from dose fractionation. Additional fractionated radiation studies in young animals should be performed to test this hypothesis.

The renal function parameters affected by renal irradiation were similar for young and adult rats. Of the renal function parameters measured, the GFR and urine osmolality showed the earliest changes. The decline in GFR results from damage to the glomerular membrane and a reduction of the glomerular perfusion. The reduction in glomerular perfusion may result from the tubulo-glomerular feedback mechanism secondary to tubular damage. The decline in urine osmolality most likely reflects tubular damage. The rise in blood pressure occurred when renal functional deterioration was well established and reflects vascular injury.

Within each age group, renal radiation damage presented earlier when higher doses were used. This has been widely recognized experimentally (Chauser et al., 1976; Madrazo et al., 1976; Robbins et al., 1986; Stewart et al., 1984; Williams et al., 1984). Clinically, an acute and late phase are distinguished in radiation nephropathy for adult patients. The acute syndrome occurring weeks to months after irradiation, is more serious and has a worse prognosis than the late syndrome, occurring years after irradiation (Greenberger et al., 1982). Both the severity of the radiation damage and the latency time are known to depend on the radiation dose. Therefore, the clinical distinction between an acute and a late syndrome seems to reflect gradual differences that are determined by the radiation dose received by the kidney.

7.3 SYSTOLIC BLOOD PRESSURE AFTER KIDNEY IRRADIATION.

In young and adult rats, the SBP rose when a marked decline in renal function was already present. The rise in SBP was more severe and presented earlier the higher the dose of radiation. A rise in blood pressure following kidney irradiation is well known. The exact role of kidney damage is not clear, as Fisher et al. (1968) reported that hypertension also developed after whole body irradiation with shielded kidneys. Thus, radiation hypertension may occur with intact kidneys. Conversely, our studies of the effect of cDDP and renal irradiation on SBP show that renal parenchymal damage caused by cDDP alone did not lead to a rise in blood pressure.

Vascular damage, due to irradiation, plays a key role in the development of systemic hypertension (Wachholz and Casarett, 1970). Systemic hypertension further damages kidney vessels and causes ischemic parenchymal lesions (Raij, 1986). The sensitization of irradiated blood vessels to hypertensive damage accelerates renal vascular damage even more (Asscher et al., 1961).

Although vascular damage seems to be decisive for the development of renal radiation hypertension, the mechanisms underlying the rise in blood pressure are not clear. The inverse correlation we found between PRC and SBP following bilateral kidney irradiation in the rat, suggested a subordinate role of the renin-angiotensin system in the development of renal radiation hypertension. Our sodium balance studies suggest that sodium and fluid retention is involved in the development of renal radiation hypertension. However, dietary sodium restriction did not influence SBP favourably.

The role of other factors involved in blood pressure regulation, such as the prostaglandin-kalikrein system and the natriuretic factor, in the development of renal radiation hypertension remains to be elucidated.

7.4 RENAL FUNCTION AFTER cDDP.

Our studies on cDDP nephrotoxicity showed that in comparison with adult rats, the same dose of cDDP (in mg/kg BW) resulted in lower renal concentrations and less nephrotoxicity in young rats. This was due to a larger kidney weight relative to body weight in the young animals. Similar results were found previously in our laboratory for two aminoglycosides (Provoost et al., 1985). Age-dependent differences in cDDP and aminoglycoside nephrotoxicity was reported by others (Braunlich et al., 1977; Kamalakar et al., 1977).

Studying drug toxicity in various animal species, Freireich et al. (1966) found comparable degrees of toxicity with identical drug dosage per body surface area (BSA). Up till now there was no sound explanation for these findings. Based on these findings, the dosage for anticancer drugs is usually in mg/m2 BSA whereas the majority of other drugs are dosed as mg/kg BW. For nephrotoxic drugs that are concentrated in and excreted by the kidney, whereby the renal concentration is related to toxicity, such as aminoglycosides (De Broe et al., 1986; Provoost et al., 1985) and cDDP (Jacobs et al., 1980; Litterst et al., 1976; Prestayko et al., 1979) dosage per gram kidney weight seems rational. As kidney weight is linearly related to BSA in man (Risdom, 1975) dosage per m2 BSA causes equal drug doses per gram kidney weight and thus equal toxicity. Consequently, nephrotoxic drugs that are excreted by the kidney and accumulate in this organ should be administered in doses relative to BSA.

Renal drug uptake, indicated by the renal drug content, expressed as a percentage of the administered total dose was comparable in young and adult rats after the same dose of cDDP or Amikacin. Thus, renal drug handling

seems to be the same in both age-groups. The clearance of aminoglycosides was found to be higher in children than in adults (Vogelstein et al., 1977). The authors felt the higher GFR values (ml/min per sq m BSA) in children compared with adults accounted for this. As the GFR-values (ml/min per 100 g BW) are comparable in weanling and adult rats (Provoost et al., 1983), a difference in aminoglycoside clearance is not expected between young and adult rats.

When renal drug injury occurred in young rats, its functional manifestation did not differ from that in adult rats. This indicates that the toxic mechanisms are independent of the age or developmental stage of the kidney.

7.5 LATE EFFECTS ON RENAL FUNCTION OF RADIATION OR cDDP.

Although the regenerative capacity of the kidney is considerable, all acute nephrotoxic insults may result in chronic renal damage. We observed a remarkable difference in the capacity to repair damage from renal irradiation and drug-induced nephrotoxicity. Renal irradiation caused a slowly progressive deterioration in renal function. Except for a period of stable renal function in young rats, the renal function after renal irradiation showed no signs of recovery. After cDDP administration, a considerable degree of recovery was observed, although chronic renal damage ensued after high doses.

Differences in primary renal injury site may explain divergent injury patterns after renal irradiation and drug administration. In cDDP-induced nephrotoxicity, renal damage is confined to the parenchym. Regeneration occurs by surviving cells and possibly pluripotent stem cells. The vascular damage caused by irradiation will result in tissue hypoperfusion and thus

lead to progressive, non-recoverable injury. When renal parenchymal regeneration is incomplete, the number of functioning nephrons will be reduced. Adaptation of the microcirculation of the remnant glomeruli will increase the single nephron filtration rate. The glomerular hyperfiltration causes an increase in glomerular capillary pressure and blood flow. The glomerular hypertension will eventually cause vascular damage and progressive glomerulosclerosis. Dietary protein restriction lowers glomerular hyperfiltration and postpones further renal deterioration (Brenner, 1983; Hostetter et al., 1981; Jamison, 1983). The progressive renal damage we observed after bilateral kidney irradiation in the rat can partly be explained by hyperfiltration. The delay in development of renal radiation damage by dietary protein restriction supports this hypothesis (Mahler et al., 1982; 1987). The chronic renal damage ensuing after cDDP administration may be due to a reduced number of functioning nephrons. Glomerular hyperfiltration will then cause a slowly progressive deterioration of renal function. Our follow-up was too short to evaluate the long term course of chronic drug-induced nephrotoxicity.

7.5 RENAL FUNCTION AFTER COMBINED TREATMENTS.

To improve therapeutic results, chemotherapy and radiotherapy are combined more and more. The combined administration of radiotherapy and cDDP seems to be effective therapeutically. Both therapeutic modalities are damaging to the kidney as described in this thesis.

A single dose of cDDP, given immediately after renal irradiation, aggravated renal functional impairment. However, taking into account the separate effects on renal function of cDDP and irradiation, the combined administration of cDDP and radiation caused no more renal damage than the

sum of both. Thus, cDDP and renal irradiation resulted in additive toxicity only.

Another combined treatment that was studied consisted of a combination of the aminoglycoside, Amikacin, immediately following a single injection of cDDP. It has been reported that aminoglycoside nephrotoxicity was attenuated after a prior nephrotoxic insult by either potassium dichromate (Elliott et al., 1982) or mercuric chloride (Luft et al., 1977). The mechanism by which previous renal damage protects against subsequent damage due to aminoglycosides remains unexplained. When two agents independently damage the kidney, additive toxicity is to be expected. Due to the functional relationship between several parts of the kidney, a synergystic interaction may ensue, especially when both nephrotoxic insults occur in close time approximation.

Our study on the combined effects of cDDP and Amikacin on renal function showed a serious functional deterioration after the combined administration of both drugs, whereas administration of Amikacin alone caused no or only very limited renal function damage. This effect was observed in young as well as adult rats. In young rats, however Amikacin had to be administered in much higher doses than in adult rats to increase the cDDP induced renal damage.

7.6 CONCLUSIONS

The following conclusions were drawn from the experimental results described in previous chapters:

- Bilateral renal irradiation causes similar renal function damage in young and adult rats, although the time course of the development of renal damage differs between both age-groups.

- In young rats, renal radiation damage following bilateral kidney irradiation becomes manifest sooner but progresses less rapidly than in adult rats. Renal function determined six months after irradiation, will show similar radiosensitivity of the young and the adult rat kidney.

- Systolic hypertension following bilateral kidney irradiation in rats developes when renal functional damage is well established.

- Although renal sodium and volume retention may be involved in the generation of renal radiation hypertension, dietary sodium restriction will not prevent the development of hypertension. Except for the final stage of malignant hypertension, the renin-angiotensin system plays no more than a subordinate role in the development and maintenance of renal radiation hypertension.

- The same quantity of cDDP given in a dosage related to BW and administered either as a single i.v. dose or as five subsequent doses,

causes less functional renal impairment in young rats than in adult rats.

- The lower nephrotoxicity of cDDP in young rats compared with adult rats can best be explained by the lower renal Pt-concentration due to the larger kidney weight relative to BW in young rats.

- The dose-limiting organ toxicity of cDDP may vary with age. In young rats gastro-intestinal toxicity seems the dose-limiting factor, as compared with nephrotoxicity in adult rats.

- A single dose of cDDP, administered immediately following a single radiation dose to both kidneys in young rats, causes renal function deterioration that will not exceed the expected deterioration calculated from the sum of the separate effects. Although data are limited, this also seems to be true for adult rats.

- In adult rats, an Amikacin course which is not toxic when given alone, will seriously aggravate cDDP-induced renal impairment when given immediately following cDDP. In young rats, this will only occur if the dose of Amikacin is raised to the nephrotoxic range.

7.7 REFERENCES

Asscher AW, Wilson C, Anson SG: Sensitisation of blood vessels to hypertensive damage by X-irradiation.
Lancet 1:580-583, 1961

Braunlich H, Kuo CH, Slocombe R, Hook JB: Cis-dichlorodiammineplatinum nephrotoxicity in rats of different ages.
Res Comm Chem Pathol Pharmacol 44: 279-291, 1984

Brenner BM: Hemodynamically mediated glomerular injury and the progressive nature of kidney disease.
Kidney Int 23:647-655, 1983

Chauser BM, Hudson FR, Law MP: Renal function in the rat following irradiation.
Rad Res 67:86-97, 1976

De Broe ME, Giuliano RA, Verpooten GA: Choice of drug dosage regimen. Two important risk factors for Aminoglycoside nephrotoxicity.
Am J Med 80:115-118, 1986

Donaldson SS, Moskowitz PS, Canty EL, Efron B: Radiation-induced inhibition of compensatory renal growth in the weanling mouse kidney.
Radiol 128:491-495, 1978

Elliott WC, Houghton DC, Gilbert DN, Baines-Hunter J, Bennett WM: Gentamicin nephrotoxicity II Definition of conditions necessary to induce acquired insensitivity.
J Lab Clin Med 100:513-525, 1982

Fisher ER, Hellstrom HR: Pathogenesis of hypertension and pathologic changes in experimental renal irradiation.
Lab Invest 19:530-538, 1968

Freireich EJ, Gehan EA, Rall DP, Schmidt LH, Skipper HE: Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey and man.
Cancer Chemother Rep 50:219-245, 1966

Greenberger JS, Weichselbaum RR, Cassady JR: Radiation nephropathy.
In: RE Rieselbach, MB Garnick (eds.), Cancer and the Kidney, Lea Febiger, Philadelphia, 1982, pp. 814-823

Hostetter MH, Olson JL, Rennke HG, Venkatachalam MA, Brenner BM: Hyperfiltration in remnant nephrons :a potentially adverse respons to renal ablation.
Am J Physiol 241:F85-93, 1981

Jacobs C, Kalman SM, Tretton M, Weiner W: Renal handling of cis-diamminedichloroplatinum (II).
Cancer Treat Rep 64:1223-1226, 1980

Jamison RL: Dietary protein, glomerular hyperemia and progressive renal

failure.
Annals Intern Med 99:849-851, 1983

Kamalakar P, Freeman AI, Higby DJ, Wallace HJ, Sinks LF: Clinical response and toxicity with Cis-dichlorodiammineplatinum (II) in children.
Cancer Treat Rep 61:835-839, 1977

Kunkler PB, Farr RF, Luxton RW: The limit of renal tolerance to X-rays: investigation into renal damage ocurring following the treatment of tumours of the testis by abdominal baths.
Br J Radiol 25:190-201, 1952

Litterst CL, Torres LJ, Guarino AM: Plasma levels and organ distribution of platinum in the rat, dog and dogfish shark following single intravenous administration of cis-dichlorodiammineplatinum (II).
J Clin Hematol Oncol 7:169-179, 1976

Luft FC, Nam Yum M, Kleit SA: The effect of concomitant mercuric chloride and gentamicin on kidney function and structure in the rat.
J Lab Clin Med 69:622-631, 1977

Madrazo AA, Churg J: Radiation Nephritis. Chronic changes following moderate doses of radiation.
Lab Invest 34:283-290, 1976

Mahler PA, Oberley TD, Yatvin MB: Histologic examination of the influence of dietary protein on rat radiation nephropathy.
Rad Res 89:546-558, 1982

Mahler PA, Rasey JS, Yatvin MB; Influence of protein nutrition on dose-survival relationship following rat kidney irradiation.
Rad Res 109:238-244, 1987

Moss WT, Brand WN, Batifora H: Radiation oncology- rationale, technique and results. 5th ed, CV Mosby, St Louis, 1979, Chapter 13, The Kidney.

Prestayko AW, D'Auost JC, Issell BF, Crooke ST: Cisplatin (cis-diamminedichloroplatinum (II).
Cancer Treat Rev 6:17-39, 1979

Provoost AP, Adejuyigbe O, Wolff ED: Nephrotoxicity of aminoglycosides in young and adult rats.
Ped Res 19:1191-1196, 1985

Provoost AP, De Keijzer MH, Wolff ED, Molenaar JC: Development of renal function in the rat.
Renal Physiol 6:1-9, 1983

Raij L: Role of hypertension in progressive glomerular injury in glomerulonephritis.
Hypertension 8:I30-I33, 1986

Reiter RJ, McCreight CE, Sulkin NM: Age differences in cellular proliferation in rat kidneys.
J Geront 19:485-489, 1964

Risdom RA: Gross anatomy of the kidney.
In: MI Rubin, TM Barratt (eds.), Pediatric Nephrology, Williams and Wilkins Comp., Baltimore, 1975, pp. 1-9

Robbins MEC, Hopewell JW: Physiological factors affecting renal irradiation tolerance: a guide to the treatment of late effects.
Br J Cancer 53:265-267, 1986

Rubin P, Van Houtte P, Constine L: Radiation sensitivity and organ tolerances in pediatric oncology: A new hypothesis.
Front Rad Ther Oncol 16:62-82, 1982

Stewart FA, Oussoren Y, Luts A, Begg AC, Dewit L, Lebesque J, Bartelink H: Repair of sublethal radiation injury after multiple small doses in mouse kidney: An estimate of flexure dose.
Int J Rad Oncol Biol Phys 13:765-772, 1987

Stewart FA, Soranson JA, Alpen EL, Williams MV, Denekamp J: Radiation-induced renal damage: the effects of hyperfractionation.
Rad Res 98:407-420, 1984

Vogelstein B, Kowarski AA, Lietman PS: The pharmacokinetics of amikacin in children.
J Ped 91:333-339, 1977

Wachholz BW, Casarett GW: Radiation hypertension and nephrosclerosis.
Rad Res 41:39-56, 1970

Williams MV, Denekamp J: Radiation induced renal damage in mice: influence of fraction size.
Int J Rad Oncol Biol Phys 10:885-893, 1984

Wright FS, Briggs JP: Feedback control of glomerular blood flow, pressure and filtration rate.
Physiol Rev 54:958-1006, 1979

SUMMARY

SAMENVATTING

SUMMARY

Chemotherapy, radiotherapy, and surgery all play a crucial role in modern oncology. The therapeutic advantage that can be achieved with chemotherapy and radiotherapy is subject to the side effects of these therapeutic modalities on normal organs and tissues. Toxicity studies in humans and animals are generally related to adult organisms. The lack of fundamental studies of the acute and chronic affects of chemotherapy and radiation on young kidneys provided the incentive for the present study.

The intoduction of this dissertation (Chapter 1) defines the questions that constituted the conceptual basis for the experiments:

1. What are the effects of radiation on the kidney of a young rat in comparison with the adult rat kidney?

2. What is the process of the so-called radiation hypertension and what causes it?

3. What is the functional damage in the young rat kidney in comparison with the adult rat kidney, after diverse doses of cDDP?

4. What is the effect of the administration of a single dose of cDDP, hardly toxic by itself, in combination with irradiation on renal function of the young or adult rat?

5. What is the effect of the administration of a single dose of cDDP in combination with a subsequent 14-day-course of Amikacin, hardly toxic by itself, on renal function of the young or adult rat?

re 1 - The acute and chronic effects of a single dose of radiation to both kidneys on renal function and blood pressure of young and adult rats are described in Chapter 2. The glomerular filtration and urine osmolality first show changes following irradiation. In both young and adult rats these changes occurred sooner and were more serious the higher the dose of radiation. The renal function of young rats began to deteriorate sooner than that of adult rats following and identical dose of radiation. Because the deterioration of renal function parameters proceeded more slowly in young rats than in adult ones, the deterioration in renal function found six months after an identical dose of radiation in either age group is comparable.

re 2 - Serious deterioration of renal function following bilateral kidney irradiation in rats, is associated with a rise in systolic blood pressure. The course of the systolic blood pressure in young and adult rats following kidney irradiation, is described in Chapter 3. The rise in systolic blood pressure is progressive in both age groups.

The pathogenesis of radiation hypertension was studied by evaluating sodium balance and plasma renin concentration following bilateral kidney irradiation of young rats on either a normal or a sodium restricted diet. There appears to be a negative correlation between systolic blood pressure elevation and plasma renin concentration. There were signs of sodium retention preceding the rise in systolic blood pressure in rats on a sodium restricted diet, which could not be found in rats on a normal diet. The development of systolic hypertension in young rats following bilateral kidney irradiation was not influenced by restricting sodium intake.

The renin-angiotensin system has a supportive role only in the development of systolic hypertension. Persistent, serious hypertension will

lead to a rise in plasma renin concentration and the development of malignant hypertension

re 3 - Chapter 4 deals with the effects of diverse doses of cDDP on renal function in young and adult rats. The dosage (in mg/kg BW) that will cause a certain decline in the glomerular filtration rate is 50- 100% higher in young rats than in adult ones. Judging from the platinum concentrations in kidney and urine, it appears that the percentual absorption and excretion of cDDP by the kidney is the same for both age groups. The difference in toxicity can be accounted for by the larger mass of renal tissue per kg body weight in young rats compared with adult rats.

re 4 and 5 - Chapters 5 and 6 describe the deterioration in renal function of young and adult rats following the combined administration of cDDP with, respectively, kidney irradiation or Amikacin administration.

The administration of cDDP following bilateral kidney irradiation in young or adult rats results in a deterioration of renal function, which is no greater than what may be expected from the combined total of the individual nephrotoxicity. The administration of cDDP had no effect on the course of radiation hypertension.

A single dose of CDDP, followed by a course of Amikacin that is not or minimally toxic by itself, may lead to a serious deterioration of renal function. In young rats this potentiation of renal damaged occurred only after a higher dose of Amikacin than in adult rats.

In the discussion (Chapter 7) the experimental results are compared with data from the literature. Information concerning young individulas being scant, there was little room for comparison. Consequently, the conclusions drawn were based mainly on our own research.

SAMENVATTING

In de moderne oncologische therapie spelen chemotherapie, radiotherapie en chirurgie een centrale rol. De therapeutische winst die behaald kan worden met chemotherapie en radiotherapie wordt mede bepaald door de bijwerkingen van deze therapieen op normale organen en weefsels. Toxiciteits-studies bij mens en dier hebben meestal betrekking op volwassen organismen. Gebrek aan fundamenteel onderzoek naar de acute en chronische effecten van chemotherapie en bestraling op jonge nieren vormden de aanleiding tot deze studie.

In de inleiding (Hoofdstuk 1) worden de vragen die ten grondslag liggen aan de experimenten geformuleerd.

1. Wat zijn de effecten van bestraling op de nier van een jonge rat, vergeleken met de volwassen rat?

2. Hoe is het beloop van de zogenaamde bestalingshypertensie en hoe wordt deze veroorzaakt?

3. Hoe is de functionele nierschade in de jonge rattenier in vergelijking tot de volwassen rattenier na verschillende doseringen cDDP?

4. Welke invloed heeft de gecombineerde toediening van een enkele, op zichzelf nauwelijks toxische dosering cDDP en bestraling van de nier op de nierfunctie van de jonge en volwassen rat?

5. Welke invloed heeft de gecombineerde toediening van een enkele dosis cDDP gevolgd door een 14 daagse toediening van Amikacin op de nier-

functie van de jonge en volwassen rat?

ad 1 - De acute en chronische effecten van een eenmalige bestraling van beide nieren op nierfunctie en bloeddruk van jonge en volwassen ratten worden beschreven in hoofdstuk 2. De glomerulaire filtratie en urine osmolaliteit tonen na bestraling het eerst veranderingen. Bij jonge zowel als volwassen ratten treden deze veranderingen eerder op en zijn zij ernstiger naarmate de bestralingsdosering hoger is. Na eenzelfde bestralingsdosis, toont de nierfunctie van jonge ratten eerder achteruitgang dan de nierfunctie van volwassen ratten. Doordat de achteruitgang van de nierfunctieparameters bij jonge ratten trager verloopt dan bij volwassen ratten, is de nierfunctie achteruitgang zes maanden na eenzelfde bestralingsdosis vergelijkbaar in beide leeftijdsgroepen.

ad 2 - Ernstige nierfunctie achteruitgang na dubbelzijdige nierbestraling in ratten gaat gepaard met een stijging van de systolische bloeddruk. Het beloop van de systolische bloeddruk na nierbestaling bij jonge en volwassen ratten wordt beschreven in hoofdstuk 3. In beide leeftijdsgroepen verloopt deze systolische bloeddrukstijging progressief.

De pathogenese van de bestralingshypertensie werd bestudeerd aan de hand van de natrium-balans en plasma-renine concentraties bij een normaal en natrium-beperkt dieet na dubbelzijdige nierbestraling van jonge ratten. Er bestaat een negatieve correlatie tussen de hoogte van de systolische bloeddruk en de plasma-renine concentratie. Er zijn aanwijzingen voor natrium-retentie voorafgaand aan de stijging in de systolische bloeddruk bij ratten gevoed met een natrium-arm dieet. Dit kon niet worden aangetoond bij ratten op een normaal dieet. De ontwikkeling van systolische hypertensie na dubbelzijdige nierbestraling in jonge ratten kan niet worden beinvloed door middel van een natrium-arm dieet.

Het renine-angiotensine systeem speelt niet meer dan een ondergeschikte rol tijdens de ontwikkeling van de systolische hypertensie. Bij lang bestaande, ernstige hypertensie treedt een stijging van de plasma-renine concentratie op en ontwikkelt zich een maligne hypertensie.

ad 3 - In Hoofdstuk 4 worden de effecten van verschillende doseringen cDDP op de nierfunctie in jonge en volwassen ratten beschreven. De dosering (in mg/kg lichaamsgewicht) die een bepaalde daling in de glomerulaire filtratie veroorzaakt is in jonge ratten 50-100% hoger dan in volwassen ratten. Gemeten aan de platina-concentraties in nier en urine blijkt de procentuele opname en uitscheiding van cDDP door de nier in beide leeftijdsgroepen gelijk te zijn. Het verschil in toxiciteit kan verklaard worden door de grotere hoeveelheid nierweefsel per kg lichaamsgewicht in jonge ratten vergeleken met volwassen ratten.

ad 4 en 5 - In Hoofdstuk 5 en 6 worden de gevolgen beschreven op de nierfunctie van jonge en volwassen ratten na de gecombineerde toediening van cDDP met respectievelijk nierbestraling en Amikacin. Toediening van cDDP na dubbelzijdige nierbestraling in jonge en volwassen ratten veroorzaakt een nierfunctie-achteruitgang die niet groter is dan te verwachten op grond van de som van afzonderlijke nefrotoxiciteiten. Toediening van cDDP heeft geen invloed op het beloop van de bestralings hypertensie.

Wordt een eenmalige cDDP dosering gevolgd door een Amikacine-kuur, die op zichzelf niet of nauwelijks nefrotoxisch is, dan kan dit tot een ernstige achteruitgang in nierfunctie leiden. Deze potentiering van nierbeschadiging trad bij jonge ratten op bij een hogere Amikacine dosering dan bij volwassen ratten.

In de Discussie (Hoofdstuk 7) worden de resultaten van de verschillende experimenten nader vergeleken met gegevens uit de literatuur. Mede gezien het geringe aantal literatuurgegevens was er weinig vergelijkingsmateriaal

en werden de de conclusies hoofdzakelijk geformuleerd op basis van het eigen onderzoek.

Acknowledgement

Een ieder, die bijdroeg tot het tot stand komen van dit proefschrift, wil ik hartelijk danken.

Curriculum Vitae

De schrijfster van dit proefschrift werd 25-9-1956 te Zwanenburg geboren. Na het VWO-A eindexamen aan het Keizer Karel College te Amstelveen (1975) begon zij in 1976, na een colloquiim doctum te hebben afgelegd, de Geneeskunde studie aan de Vrije Universiteit te Amsterdam. Tijdens deze studie werkte zij als student-assistente bij de afdeling Histologie (Hoofd: Prof. Langevoort). Na het arts-examen in 1982 werkte zij bij de afdeling Kinderheelkunde (Hoofd: Prof J.C.Molenaar) onde leiding van Dr A.P. Provoost aan een door het Koningin Wilhelmina Fonds gesubsidieerd onderzoek. Dit onderzoek verliep in samenwerking met de afdeling Radiobiologie van TNO (Rijswijk) (Hoofd: Prof D.W. van Bekkum) en leidde tot het tot stand komen van dit proefschrift.

Bestraling

ik maak grapjes
alsof ik vrolijk ben
koude schone handen
boetseren mijn bange lichaam
in de juiste vorm
ze lacht omdat ik vrolijk ben
maar in deze kleine ruimte
is de oorlog begonnen

Als ze mij alleen laat
tel ik de dodelijke seconden
die ze achter de stenen
schermen laat verstrijken
en huilend stort het
vernielend gif zich
onzichtbaar op mijn
bevroren lichaam

de seconden branden
zich door mijn huid
naar de plaats waar
ik bezig ben te sterven
en ze blazen zich op tot
dodelijke minuten
en als ze me lachend bevrijd
zeg ik gewoon: tot morgen

Uit: de Heiligen van
Slootervaart
Rienk Ratsma