

Amelioration of radiation nephropathy by acetylsalicylic acid

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Abstract. This investigation was carried out to assess the amelioration by two antithrombotic drugs of radiation nephropathy in mice. Mouse kidneys were given split-dose irradiation to total doses between 17 and 22 Gy. A first group of animals was given acetylsalicylic acid (ASA) in drinking water, a second received daltroban, a thromboxane A₂/prostaglandin H₂ receptor antagonist, and a third received normal tap water, serving as a control. Both antithrombotic drugs were started 1 week prior to the irradiation and were given throughout the whole follow-up period. Renal function was assessed every 4 weeks from 18 weeks after the start of irradiation onwards by measuring the [⁵¹Cr] EDTA retention and haematocrit. The dose of ASA (600 mg/kg/day) caused an inhibition of thromboxane A₂ and prostacyclin biosynthesis to 19 ± 10 (mean ± SEM) and 85 ± 22%, respectively, as assessed by the excretion of their urinary metabolites. A significant sparing effect on the renal function after irradiation was observed in the ASA-treated animals. Using the latency time to reach 4% residual plasma activity of [⁵¹Cr] EDTA, a dose-modifying factor of 1.19 was calculated. No effect was seen with daltroban (10 mg/kg/day). Histopathological analysis of the kidneys at 12 months after irradiation demonstrated a substantially lower level of damage in the ASA-treated mice compared with daltroban-treated and radiation-only animals. These data indicate that long-term treatment with ASA is effective in reducing renal functional impairment after irradiation.

1. Introduction

Radiation-induced effects on kidney function have been extensively investigated over the past 10 years (e.g. reviewed by Robbins and Hopewell 1987, Stewart and Williams 1991). A continuously progressive deterioration of renal function after moderate radiation doses has been observed both in man and in experimental animals (Stewart *et al.* 1988, Dewit *et al.* 1990). More recently, a distinct clinical picture, known as bone marrow transplantation (BMT) nephropathy, has been reported following total-body irradiation prior to BMT (Lawton *et al.* 1991, 1994, Lönnerholm *et al.* 1991).

BMT is predominantly applied in younger patients, with usually a high probability of long-term survival. Therefore, BMT nephropathy becomes increasingly important and ways to prevent it or reduce its severity are urgently needed.

Despite strong efforts from many investigators there is today still no consensus on the pathogenesis of radiation nephropathy. Some have argued that damage to the parenchymal cells, in particular the tubular epithelium, is the critical lesion (Withers *et al.* 1980, 1986, Michalowski 1981), while others have reported that glomerular and microvascular injury are more important (Rubin and Casarett 1968, Glatstein *et al.* 1977). In the microvasculature, the endothelial cell is considered to be one of the critical target cells for radiation injury in the kidney (Rubin and Casarett 1968, Phillips *et al.* 1972, Nelson *et al.* 1984, Reinhold *et al.* 1991, Jaenke *et al.* 1993). Although renal functional impairment after irradiation is expressed only after many months, it is likely that during the so-called latency period events occur, for instance in the microvasculature, which might be of great importance in the onset and progression of radiation damage in the kidney. Early histopathological changes in small renal blood vessels after irradiation are characterized by capillary endothelial cell swelling with leukocyte attachment, rounding up of endothelial cells and detachment from the basal membrane (Nelson *et al.* 1984, Reinhold *et al.* 1991, Jaenke *et al.* 1993). Later on, progressive endothelial cell loss occurs, initiating microthrombus formation, which might ultimately lead to vascular occlusion (Reinhold *et al.* 1991).

We have recently demonstrated enhanced platelet adhesion to the extracellular matrix of human endothelial cells after irradiation by an increase in von Willebrand factor release (Verheij *et al.* 1994a). In addition, a prolonged and radiation dose-dependent decrease in endothelial prostacyclin (PGI₂) formation *in vitro* and *in vivo* has been reported (Eldor *et al.* 1983, 1987, 1989, Hahn *et al.* 1983, Sinzinger *et al.* 1984, Verheij *et al.* 1994b), with no effect on platelet thromboxane (TXA₂) formation (Allen *et al.* 1981). PGI₂

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is a well-known potent vasodilator and platelet aggregation inhibitor with, in addition, very important homeostasis-maintaining properties in the kidney (Dunn 1979, Schor *et al.* 1981). Consequently, these adverse radiation effects on the microvasculature may have major implications on the kidney function.

We therefore became interested in investigating the eventual protective effect of certain antithrombotic drugs, which interact with prostaglandin metabolism and with platelet aggregation, on radiation-induced renal functional impairment in mouse. The drug of first choice was acetylsalicylic acid (ASA, aspirin) because of its well-documented high efficacy as a thrombosis-preventing agent in the clinic (Willard *et al.* 1992, Patrono 1994). This particular mode of activity of ASA is attributed to a dose-dependent inhibition of the enzyme cyclooxygenase, which catalyses the formation of PGI₂ in endothelial cells and TXA₂ in platelets. In the appropriate dose, ASA selectively causes major inhibition of platelet TXA₂ formation, with only a moderate reduction of endothelial cell PGI₂ production (Oates *et al.* 1988, Patrono 1994). A theoretically even more attractive approach is to use a thromboxane receptor antagonist such as daltroban. This drug has the potential advantage of blocking only the effects of TXA₂, while leaving the production of PGI₂ unaffected (Lefer and Darius 1987, Smith *et al.* 1989, Patscheke 1990). To test the efficacy of these agents in ameliorating radiation nephropathy, a mouse kidney model, which is well established in our laboratory, was used (Stewart *et al.* 1984, 1986, 1988).

2. Materials and methods

2.1. Experimental design and irradiation

Experiments were carried out in accordance with the national regulations for animal experimentation. Experimental protocols were approved by the local animal welfare committee before the start of these studies. Female C3H/Nu mice, aged 12–14 weeks and weighing 24–29 g at the start of treatment, were randomly allocated to the various treatment groups (six mice per dose point, 12 mice in the control group). A total of 78 animals was used. Both kidneys of unanaesthetized mice were irradiated via lateral tangential fields as previously described (Stewart *et al.* 1986). Briefly, irradiation was performed with 250-kV X-rays, operating at 15 mA and filtered with 0.5-mm Cu. The dose-rate at the position of the kidneys was 2.35 Gy/min and mice were rotated through 180° half-way through each irradiation in order to maximize dose uniformity.

Irradiation was given in two equal fractions, separated by 2 weeks. One week before irradiation one-third of the animals were provided acetylsalicylic acid (ASA, 600 mg/kg/day; Sigma, St Louis, MO, USA) in acidified drinking water, one-third received daltroban in their drinking water (10 mg/kg/day; batch no. 820 422 59 H; Boehringer Mannheim GmbH, Mannheim, Germany), and one-third had access to normal tap water, all *ad libitum*. The dose of ASA was chosen to obtain maximal inhibition of thromboxane A₂ synthesis with only a moderate effect on prostacyclin production. Hereto, concentrations of the urinary metabolites TXB₂ and 6-keto-PGF_{1α} were measured in 24-h collected urine samples 3 days after the start of ASA administration (see below). Occasionally, serum levels of salicylic acid were also measured, using a fluorescence polarisation immuno-assay (FPIA), from 50-μl blood samples taken from the retro-orbital plexus. The FPIA was run on the Abboth TDx-Flx™ according to the procedures described in the TDx Assays Manual. The sensitivity was determined to be 5.0 μg/ml.

The dose of daltroban was chosen in accordance with the manufacturer's advice, based upon its proven effectiveness in reducing atherosclerotic lesions in rabbits (Pill *et al.* 1990). This dose is approximately 10% of the maximal tolerated dose after chronic administration of daltroban in various animal species (SmithKline Beecham and Boehringer Mannheim 1990). In addition, this dose causes a strong and long-lasting inhibition of thromboxane receptor-mediated platelet aggregation *ex vivo* (Pill *et al.* 1990).

The drug concentrations in drinking water were calculated based on weekly measurements of water intake and body weights. The concentration of ASA was measured spectrophotometrically, being 0.28–0.30 mg/ml. The drugs were given continuously throughout the entire follow-up period. Sham-irradiated animals for each drug regimen served as controls. Prior to and 6 months after the start of administration of the drugs 24-h urine production was collected for prostaglandin metabolite analysis. Renal function was measured from 18 weeks after the first dose of irradiation at monthly intervals until the mice became ill from uraemia or until killing 12 months after irradiation. After killing the kidneys were removed and prepared for histological examination.

2.2. Measurement of renal function

Kidney function was assessed by measuring the haematocrit and the plasma retention of [⁵¹Cr] EDTA using the single sample method as previously described (Stewart *et al.* 1986, 1988). Briefly, intraperitoneal injections of [⁵¹Cr] EDTA (specific activity 36–74 MBq

per mg Cr; Amersham International, Amersham, UK) were given at a dose of 0.37 MBq in 0.1 ml per mouse. After 30 min, 50- μ l blood samples were taken from the retro-orbital plexus, collected in heparinized capillary tubes and centrifuged at 12 000 *g* for 3 min. The haematocrit (Hct) was measured as the percentage of packed cell volume. A 20- μ l plasma sample was taken for counting in an autogamma counter. Results are expressed as residual radioactivity (percentage of injected dose) per ml plasma. A reduction in Hct and an increase in residual [^{51}Cr] EDTA reflect a decrease in renal function.

2.3. Prostaglandin analysis

Animals were housed in individual cages with wire mesh floors for a 24-h urine collection. To evaluate eventual stress of the mice, water and food intake were carefully monitored and did not appear to differ greatly from the normal nutritional habits. In addition, urine volumes were produced as expected by the animals, suggesting no major strain on them by the change of housing. Urine volumes were measured and samples were taken for measurement of 6-keto-PGF $_{1\alpha}$ and TXB $_2$, which are the stable products of PGI $_2$ and TXA $_2$, respectively, using a specific radioimmunoassay (Thomas *et al.* 1978). The tritium-labelled tracers were purchased from NEN Dupont (Dreieich, Germany). Antiserum against 6-keto-PGF $_{1\alpha}$ was purchased from Biotecx Laboratories (Houston, TX, USA) and anti-serum against TXB $_2$ from Chemicon International (Temecula, CA, USA). The unlabelled standard prostanooids were supplied by Paesal and Lorei (Frankfurt, Germany). Cross reactivity between the main PGs, that is 6-keto-PGF $_{1\alpha}$, PGF $_{2\alpha}$, PGE $_2$ and TXB $_2$, was < 5%. A 50% cross reactivity existed, however, between TXB $_2$ and its β -oxidation product 2,3-dinor-TXB $_2$, whereas that with its enzymatic degradation product 11-dehydro-TXB $_2$ was < 5% (de Waart *et al.* 1994). TXB $_2$ is thought to represent renal TXA $_2$ production, whereas 2,3-dinor-TXB $_2$ and 11-dehydro-

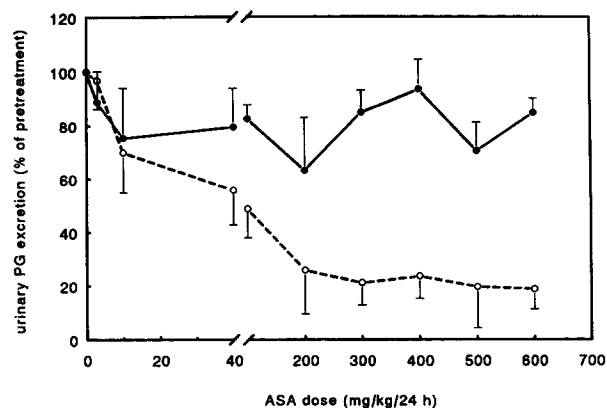


Figure 1. Inhibitory effect of increasing doses of ASA on urinary excretion of 6-keto-PGF $_{1\alpha}$ (●) and TXB $_2$ (○). Both metabolites were measured in a 24-h urine sample produced 3 days after the start of ASA administration. Data are expressed as percentages of the pretreatment value (mean \pm SEM; six mice per dose group).

TXB $_2$ probably reflect extrarenal TXA $_2$ biosynthesis (FitzGerald *et al.* 1983).

2.4. Histopathological analysis

At the time of killing, kidneys were removed, fixed in buffered formalin and embedded in paraffin. Sections (5 μ m) were made and stained with haematoxylin & eosin. The sections were examined and scored in a semiquantitative way by two independent observers without prior knowledge of the treatment of the various mouse groups. The severity of the lesions was graded as none (0), mild (1), moderate (2), moderate to severe (3), or severe (4) changes. Representative specimens were photographed.

2.5. Statistical analysis

Statistical analyses of the data were performed by standard procedures using a paired Student's *t*-tests. For

Table 1. Effect of ASA on urinary excretion of prostaglandin metabolites.

	Pretreatment		6 months post-treatment	
	6-Keto-PGF $_{1\alpha}$ (ng/24 h)	TXB $_2$	6-keto-PGF $_{1\alpha}$ (ng/24 h)	TXB $_2$
XRT* only (<i>n</i> = 24)	1.47 \pm 0.68	11.1 \pm 3.2	1.37 \pm 0.57	11.5 \pm 4.2
XRT + ASA (<i>n</i> = 24)	1.12 \pm 0.60	8.9 \pm 3.0	0.92 \pm 0.39	1.82 \pm 0.77**

Data are expressed as mean \pm SD.

*Radiation treatment.

***p* < 0.0005 compared with pretreatment value.

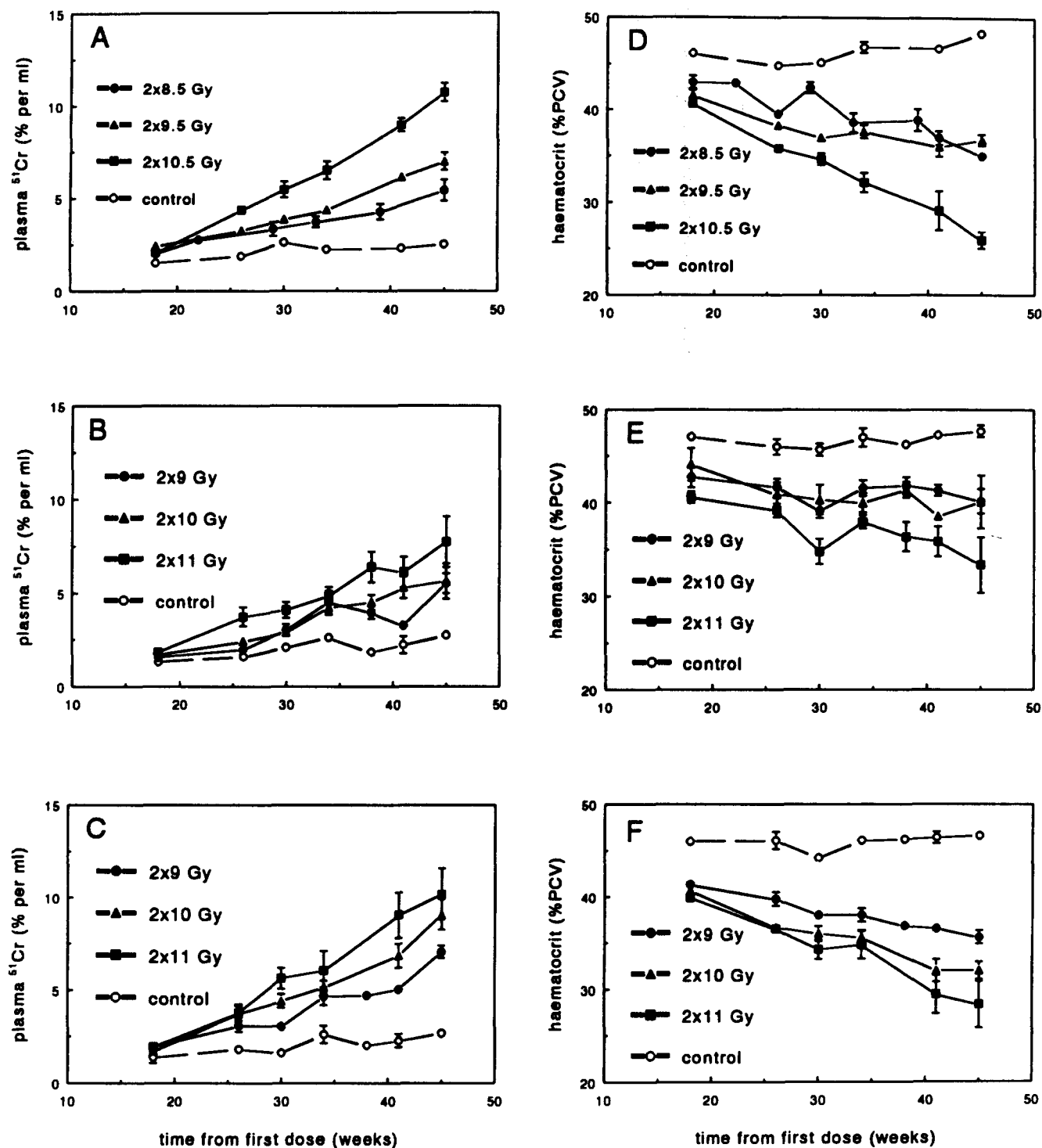


Figure 2. Time course of development of renal damage after graded X-ray doses given in two equal fractions in 2 weeks. Renal function was measured every 4 weeks from 18 weeks after the first fraction onwards by assessing [^{51}Cr] EDTA residual activity in plasma 30 min after injection (left) and haematocrit (right). (A and D) X-rays only; (B and E) X-rays + ASA; and (C and F) X-rays + daltroban. Each data point represents the mean \pm SEM of a group of 4–6 mice.

the latency times data were analysed using an Analysis of Covariance, including a test for linearity. The test of Levene (1960) was used to test equality of SDs between

the different treatment groups. Shapiro and Wilk's test (1965) was used to evaluate further Normality. Differences were considered significant when $p < 0.05$.

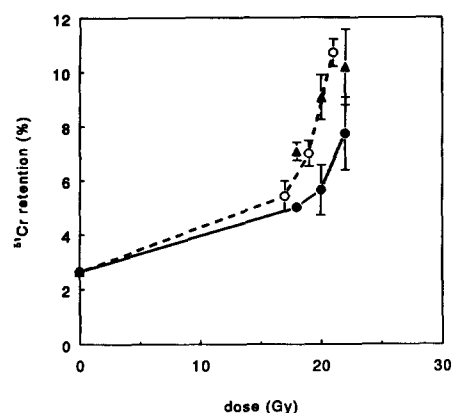


Figure 3. Dose-response curves for renal damage (residual [^{51}Cr] EDTA activity in plasma 30 min after injection) at 45 weeks from the start of irradiation. Irradiation was given in two equal fractions in 2 weeks. (●) X-rays + ASA; (▲) X-rays + daltroban; and (○) X-rays only. Data are expressed as mean \pm SEM for groups of 4–6 mice.

3. Results

3.1. Effect of antithrombotic medication on prostaglandin metabolism

Urinary TXB_2 and 6-keto- $\text{PGF}_{1\alpha}$ production were differentially suppressed by increasing doses of ASA (Figure 1). The maximal inhibition of TXB_2 , down to $19 \pm 10\%$ (mean \pm SEM), was obtained with a dose of 600 mg/kg/day ASA, with a reduced production of 6-keto- $\text{PGF}_{1\alpha}$ to only $85 \pm 22\%$. This dose was therefore chosen for the radiation experiments. As a check, urinary prostaglandin metabolite production was measured in the radiation experiment after 6 months of drug therapy. Approximately the same extent of inhibition of

prostanoid synthesis was observed (Table 1). Serum levels of salicylic acid were nearly always < 10 mg/l, irrespective of the dose of ASA (data not shown). Neither radiation nor daltroban had a significant effect on the prostaglandin metabolism (data not shown).

3.2. Renal function

The mouse renal function was analysed from 18 to 45 weeks after the first fraction. A progressive and radiation dose-dependent decrease in kidney function was observed after irradiation alone (Figure 2A and D). In the irradiated mice treated with ASA the rate of development of renal injury was substantially reduced over the entire follow-up period (Figure 2B and E). At 45 weeks a small shift of the dose-response curve towards higher doses for the ASA-treated animals was observed, by a factor of approximately 1.15 (Figure 3). Using the latency time to reach a certain level of damage (4% residual [^{51}Cr] EDTA activity), a significant increase in the time to reach this level of damage for a given radiation dose was seen in the ASA-treated mice compared with controls (Figure 4; $p < 0.0001$). A dose-modifying factor of 1.19 was derived from these data for the ASA-treated animals. For the mice given daltroban (Figure 2C and F) no significant difference in the amount of renal damage or in the rate of development of damage was detected compared with irradiated controls. Animals treated with only ASA (Figure 2B and E) or daltroban (Figure 2C and F) showed no renal functional impairment.

3.3. Histopathological studies

Most animals were killed for histopathological analysis of the kidneys after 12 months of follow-up (only four were prematurely killed due to illness). Glomerular changes ranged from endothelial cell swelling and increased intracapillary cellularity to capillary collapse with tuft shrinkage, capillary obliteration due to deposition of fibrillary material and fibrinoid necrosis with nuclear fragmentation. The glomerular capillary walls showed double contours of the glomerular basement membrane with network-like formations due to endothelial swelling and proliferation. The tubulo-interstitial compartment showed cystic dilatation of tubules with flattening of the epithelium and focal interstitial fibrosis with inconspicuous round cell infiltrates. No evident pathology was observed in the endothelium or media of large blood vessels.

The histopathological scoring for glomerular and tubular changes was performed in 80% of the treated animals (Figure 5). Mice irradiated in combination with ASA showed a much lower degree of renal damage

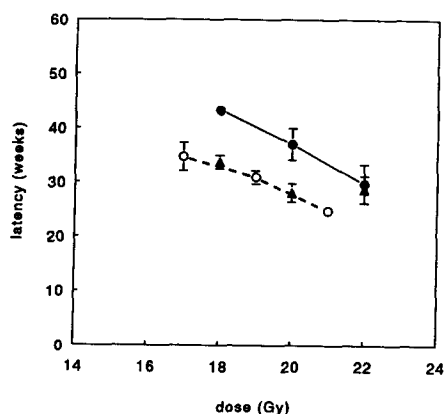


Figure 4. Latency times for development of renal damage (4% residual [^{51}Cr] EDTA activity level). The latency time was calculated from the start of irradiation (two equal fractions in 2 weeks). (●) X-rays + ASA; (▲) X-rays + daltroban; and (○) X-rays only. Data are expressed as mean \pm SEM. The difference in latency times between X-rays + ASA, and X-rays alone is statistically highly significant ($p < 0.0001$).

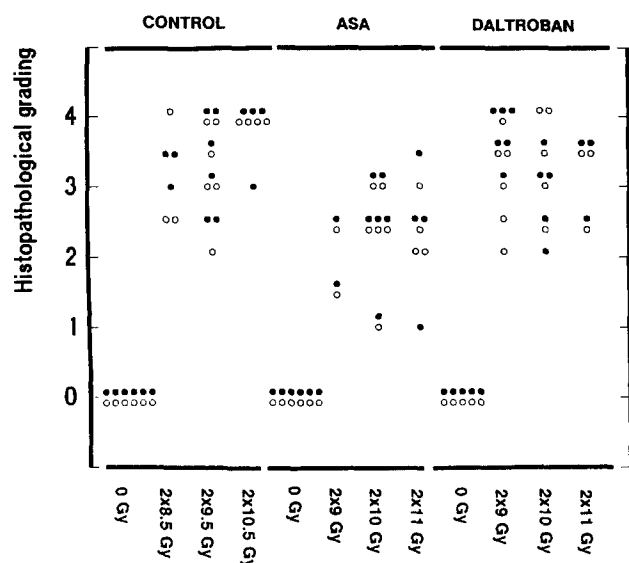


Figure 5. Histopathological grading of radiation-induced glomerular (●) and tubular (○) changes 12 months after the start of drug treatment. The severity of the lesions in the specimens was scored as none (0), mild (1), moderate (2), moderate to severe (3), or severe (4).

compared with irradiated-alone and daltroban-treated animals: only 25% of the former exhibited a histopathological grade ≥ 3 , whereas in the latter two this was 81 and 75%, respectively. Examples of typical histopathological findings are shown in Figure 6. No clear relationship was found between the radiation dose and the degree of renal injury, which is probably the result of the fact that the kidneys were isolated at the end of the follow-up period when renal damage had progressed towards a common end stage of the disease. No evidence of renal damage was found in the unirradiated mice given long-term ASA or daltroban.

4. Discussion

In this study we aimed at investigating whether protection of the vascular compartment of the kidney would result in a sparing effect on functional radiation nephropathy. ASA (aspirin) is known as one of the most effective drugs in secondary prevention of cardiovascular occlusive disease (Patrono 1994). Assuming that such chronic vascular disease and radiation-induced microvascular injury share in their pathogenesis a common pathway in which platelet adhesion plays an important role, antiplatelet agents are obvious candidates to be tested. ASA is the drug of first choice because of its proven effectiveness in the clinic (Willard *et al.* 1992, Patrono 1994). A theoretically even better drug is a thromboxane A_2 /prostaglandin H_2 (TXA_2 /PGH $_2$) receptor antagonist, such as daltroban, which does not

interfere with the prostaglandin metabolism but exerts its effect exclusively at the platelet receptor level. Both drugs were administered before the start of the irradiation and continued throughout the entire follow-up period. The reason for this approach is that potentially important biological processes occur within hours after irradiation of the kidney and continue to take place over weeks and months thereafter. *In vitro* studies suggest that the early events are primarily biochemical in nature, such as a reduced antithrombotic state of the endothelial cell (diminished PGI $_2$ production, increased von Willebrand factor release) (Eldor *et al.* 1984, 1989, Sinzinger *et al.* 1984, Sporn *et al.* 1984, Verheij *et al.* 1994a, b), a compromised cell membrane integrity (Konings *et al.* 1978, Ward *et al.* 1991) and an altered cytokine and growth factor release (Weichselbaum *et al.* 1993). Later on, radiation-induced endothelial cell loss occurs with subsequent platelet adhesion to exposed subendothelium. As glomerular endothelial cells have a relatively high cell turnover (Pabst *et al.* 1983), exposure to subendothelium with platelet adherence and microthrombus formation very likely occurs within weeks after irradiation. Hence, to increase the probability of being effective, an antithrombotic drug such as ASA needs to be present over the entire period of development of radiation nephropathy.

Using this approach, we were able to show a distinct reduction in the rate of development of radiation nephropathy with ASA in a dose which reduced the platelet TXA_2 production by approximately 80% (Figures 1 and 2). Taking the latency time to reach 4% residual activity of [^{51}Cr] EDTA in the serum, a dose-modifying factor of 1.19 was calculated. No significant amelioration by daltroban of radiation-induced renal functional impairment was observed. Consistent with these findings is the fact that the histopathological analyses of the irradiated kidneys after 12 months also revealed a lower degree of renal injury in the ASA-treated mice, whereas no such effect was seen in the animals given daltroban.

A point of concern is whether the effects of the two drugs can be fairly compared. First, the oral dose of ASA was quite high, close to what was maximally tolerated by the mice. Yet, in this dose range serum levels of salicylic acid were mostly < 10 mg/l, which is in the subtherapeutic range for a meaningful anti-inflammatory effect (data not shown). Second, the dose of daltroban was chosen primarily based on its toxicity (approximately 10% of the maximal tolerated dose) (SmithKline Beecham and Boehringer Mannheim 1990). Nevertheless, this dose has been shown to cause a strong and long-lasting inhibition of thromboxane receptor-mediated platelet aggregation *ex vivo* in various animal species (SmithKline Beecham and Boehringer

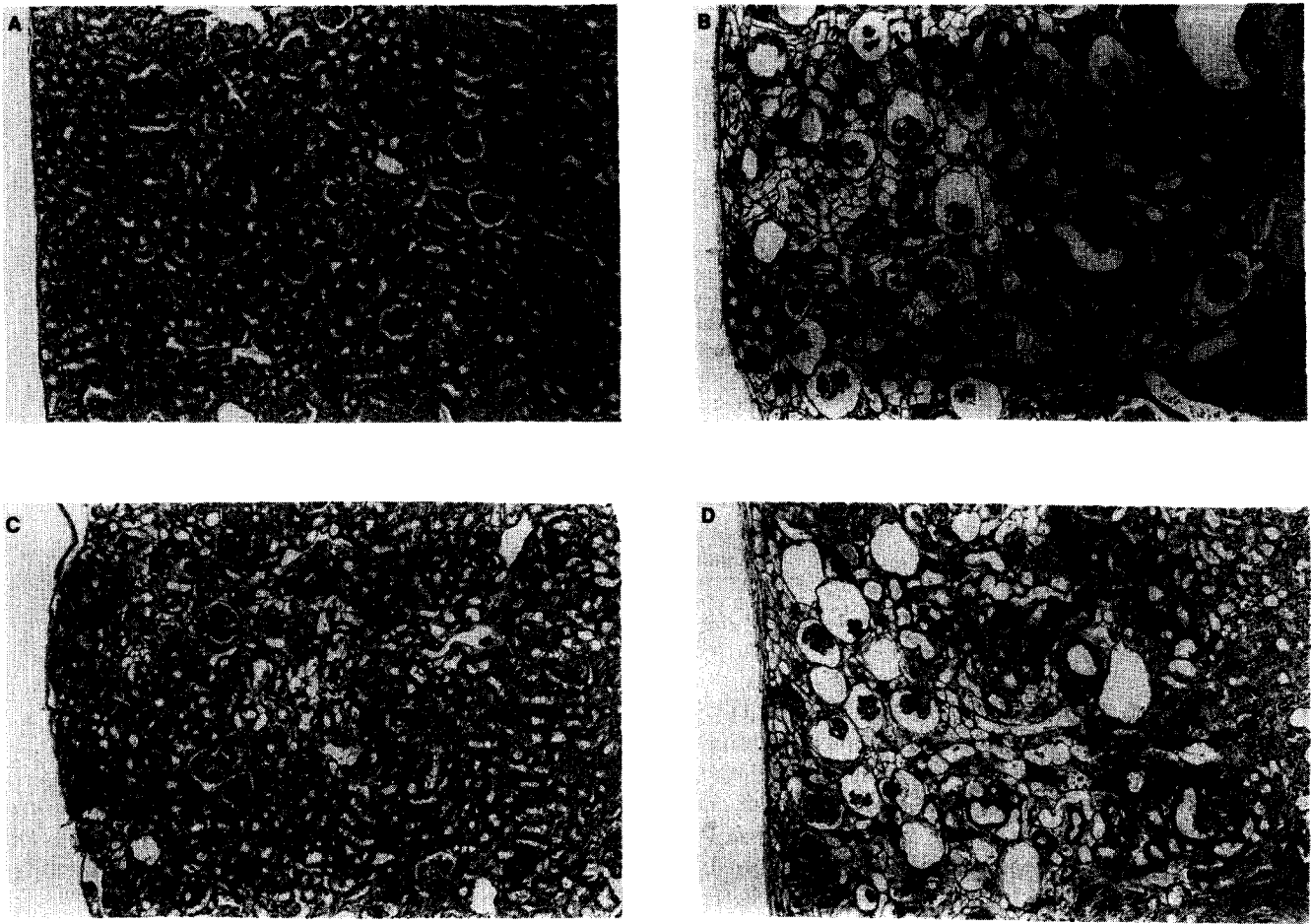


Figure 6. Representative photographs of renal histological specimens of mice killed at 12 months after treatment. (A) Sham-irradiated kidney (histopathological score = 0); (B) kidney irradiated to 21 Gy (score = 4); (C) kidney given ASA and irradiated to 22 Gy (score = 2); and (D) kidney given daltroban and irradiated to 22 Gy (score = 3–4). Sham-irradiated kidney was normal, whereas in the irradiation-only and daltroban-treated irradiated kidney extensive glomerular and tubular changes were observed. The ASA-treated irradiated kidney showed substantially less damage. Original magnification 100x, H&E.

Mannheim 1990). It is also known to reduce the progression of atherosclerotic lesions *in vivo* in the hypercholesteremic rabbit (Pill *et al.* 1990). Unfortunately, clinical phase III trials comparing the effectiveness of these two agents are presently lacking.

Several other drugs have been investigated for their eventual protective effect on radiation nephropathy, such as steroids (Caldwell 1971, Geraci *et al.* 1993) aminothiols (Williams and Denekamp 1983, Donaldson *et al.* 1984) and angiotensin-converting enzyme inhibitors (Robbins and Hopewell 1986, Cohen *et al.* 1992, 1994, Moulder *et al.* 1993a). Dose-modifying factors reported by these investigators were in the same order of magnitude as in the present study (Table 2). Sometimes, vasoactive agents are given fairly late after irradiation, at the time of expression of gross organ failure, when much of the irreversible radiation damage has already developed (Cohen *et al.* 1992, Moulder *et al.* 1993b). Since the pathogenesis of radiation vasculopa-

thy is very likely a continuum of various events, largely overlapping in time, we think a vasoprotective agent has the highest chance of being effective when it is given over a prolonged period of time, starting from the time of irradiation onwards. For some drugs, such as steroids, however, prolonged administration is not without serious risk, due to their side effects. In this respect, ASA is a very attractive drug, particularly in patients, in whom only very low doses are needed for an antithrombotic effect (Patrono 1994). At these doses of ASA, serious side effects have almost never been observed.

Based upon the present observations, it is tempting to speculate on the critical target tissue for radiation damage in the kidney. Clearly, one can conclude from these data that radiation injury to the vascular compartment contributes to renal functional impairment after irradiation. The extent of this contribution, or its relative importance, in the pathogenesis of radiation nephropathy are, however, difficult

Table 2. Dose-modifying factors in experimental radiation nephropathy.

Species	Drug	DMF*	Renal functional parameter	Reference
Mouse	WR-2721 (Aminothioli)	1.3	[⁵¹ Cr] EDTA retention urination frequency renal weight	Williams and Denekamp (1983)
	WR-2721 (aminothioli)	1.2	renal weight	Donaldson <i>et al.</i> (1984)
Rat	dexamethasone	1.2–1.3	[⁵¹ Cr] EDTA retention azotaemia	Geraci <i>et al.</i> (1993)
	captopril	1.2	azotaemia	Moulder <i>et al.</i> (1993a)
		1.4	proteinuria	
	captopril	1.2	creatinine clearance	Cohen <i>et al.</i> (1994)
		1.5	proteinuria	
		1.5	systolic blood pressure	
	enalapril	1.2	creatinine clearance	
		1.2	proteinuria	
		1.4	systolic blood pressure	

*Dose-modifying factor

to discern. First, the sensitivity of the [⁵¹Cr] EDTA retention assay is quite low, which prohibits a quantitative assessment of the radiation-modifying effect of ASA over the first 30 weeks after irradiation. Second, the histopathological scoring was done only at the end-stage of the renal functional impairment and allows only for a semiquantitative analysis. In order to obtain a better insight in the pathogenesis of radiation nephropathy, more sensitive markers for the various cell types in the kidney are needed. To monitor the extent of acute tubular cell damage, for instance, we performed serial measurements of specific tubular enzymes in patients with irradiated kidney and found no changes (Dewit *et al.* 1990). The activity of specific vascular growth factors, such as transforming growth factor β or basic fibroblast growth factor, is worthwhile investigating, but requires serial renal biopsies, since these factors have predominantly auto- and paracrine effects. Intervention-type studies such as the present one are ultimately needed to reveal the pathophysiological importance of each of these processes.

In summary, long-term administration of ASA at 600 mg/kg/day causes a significant reduction in the development and progression of radiation nephropathy in mouse. This protective effect of ASA was demonstrated both functionally over a prolonged time period and histomorphologically at the end stage. No protective effect was observed with daltroban, a TXA₂/PGH₂ receptor antagonist, at 10 mg/kg/day. These data clearly indicate a contributing role of the vascular compartment in the pathogenesis of radiation nephropathy.

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