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ORIGINAL ARTICLE

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The formation and persistence of carboplatin-DNA adducts in rats

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Abstract The formation and persistence of platinum-DNA adducts were studied with immuno(cyto)chemical methods in male and female Sprague-Dawley rats treated with a single i.p. dose of carboplatin. Linear dose-effect curves were observed for kidney and liver with an immunocytochemical assay using NKI-A59 antiserum that recognizes intrastrand cross-links. With this method, no staining of the nuclei due to platinum-DNA damage could be observed in the spleen, testis, uterus, or ovary after administration of up to 80 mg/kg carboplatin. A homogeneous staining of the nuclei in the liver was observed. The nuclear staining in the kidney was somewhat more intense but less homogeneous, with small groups of intensely stained nuclei occasionally being seen in the outer cortex. An approximately 15 to 20-times lower dose of cisplatin than of carboplatin was needed to reach equal staining levels in the liver and kidney. Plateau staining levels in both tissues were reached at between approximately 8 and 48 h after administration of the carboplatin. This was followed by a significant reduction in the kidney samples, whereas the staining levels in the liver section seemed to be more persistent. No major difference was observed between male and female rats in the formation and removal of DNA damage in these tissues. The levels of the various DNA adducts were measured with a competitive ELISA in liver, kidney, spleen, testis, and combined ovary/uterus samples collected at 8 and 48 h after carboplatin administration. At both 8 and 48 h, the highest platination levels were observed in the kidney, followed—in decreasing order—by the liver, combined uterus and ovary samples, spleen, and testis. At 8 h after administration of carboplatin, the relative occurrence of the bifunctional adducts Pt-GG (34%), Pt-AG (27%), and G-Pt-G (32%), was similar in all tissues. The same held for the monoadducts that amounted to about 7% of the total DNA platination. These data indicate that in the first few hours after carboplatin treatment, no preference for the formation of Pt-GG adducts was observed, which confirms our earlier observations obtained with cultured cells. When the total DNA-platination levels (calculated from the sum of the adducts) seen at 8 and 48 h after treatment were compared, a substantial decrease in DNA platination was observed in the kidney (37%), liver (30%) and ovary/uterus (39%), whereas the repair levels in the testis (9%) and, probably, the spleen (18%) were substantially lower. In all tissues studied, only the relative occurrence of the Pt-GG adducts increased between 8 and 48 h, and as a result, at 48 h, after carboplatin administration the Pt-GG adduct was the major adduct persisting in the DNA samples.

Key words Carboplatin · DNA adducts · ELISA · Immunocytochemistry · Rats

Abbreviations: *AAS* Atomic absorption spectroscopy · *ELISA* enzyme-linked immunosorbent assay · *Pt-GG*, *cis*-$Pt(NH_3)_2$d(pGpG) · *Pt-AG*, *cis*-$Pt(NH_3)_2$ d(pApG) · G-Pt-G *cis*-$Pt(NH_3)_2$d$(GMP)_2$ · *Pt-G* *cis*-$Pt(NH_3)_3$ dGMP

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Introduction

Cisplatin and carboplatin are two of the most effective chemotherapeutic drugs currently available for the treatment of ovarian, testicular, and several other types of cancer [4, 15]. The antitumor effect of these drugs is thought to be due to their interaction with DNA.

Although carboplatin and cisplatin have a large overlap in their cytotoxic effects and tumors show significant cross-resistance against both drugs, they differ, e.g., in their toxic side effects.

Cisplatin is mainly nephrotoxic and neurotoxic, whereas the dose limiting toxicity of carboplatin appears to be bone marrow toxicity [4, 5]. With respect to antitumor activity, it has been reported in clinical studies that tumors that are refractory to cisplatin, can be treated effectively with high doses of carboplatin in combination with hematological support [23]. Therefore, carboplatin can serve as an alternative for patients highly sensitive to cisplatin toxicity or can be used in combination with cisplatin to expand the therapeutic armamentaium [13]. Although carboplatin and cisplatin give rise to the same bifunctional DNA adducts, recent results suggest that carboplatin reacts according to a different mechanism. Cisplatin is reported to have a 200-fold higher DNA-binding activity than does carboplatin in vitro, whereas differences in the DNA-binding capacity and effective therapeutic dose between cisplatin and carboplatin are much smaller [6–20 times] in cultured cells and patients [2, 3, 17, 18, 28].

In cultured cells we recently discovered a difference also in DNA-adduct formation by these drugs [3]; although carboplatin forms the same bifunctional adducts as does cisplatin, their formation is much slower and their relative amounts are different from those seen after cisplatin treatment. The preference of cisplatin for pGpG sequences (in vitro and in vivo), resulting in relatively high levels of Pt-GG adducts (50–60%) as compared with the other bifunctional adducts Pt-AG (15–20%) and G-Pt-G (15–20%) [9], was not found for carboplatin in cells during the first 24 h after treatment. In carboplatin-treated cells, G-Pt-G (40%) and Pt-GG (30%) were the major adducts, followed by the Pt-AG adduct (16%). The relevance and general validity of this observation is not yet clear. Therefore, we studied the formation and persistence of DNA damage in tissues from carboplatin-treated rats at the cellular level by immunocytochemistry and investigated the presence of the various adducts in isolated and digested DNA samples with a competitive ELISA.

Materials and methods

Animals and treatments

Inbred male (320–449 g) and female (219–286 g) Sprague-Dawley rats aged 3–4 months and obtained from the specific pathogen-free breeding colony of the Netherlands Cancer Institute received a single i.p. dose of carboplatin. In a dose-response experiment, 0, 20, 40, or 60 mg/kg carboplatin was given to one male and one female per dose. The animals were killed at 20 h after the start of the experiment. For the time experiment (one male and one female rat for each time point), 80 mg/kg carboplatin was given and rats were killed at 1, 2, 4, 8, 24, 48, 72, 96, and 120 h after injection. For comparison of the effects induced by cisplatin, 0, 2, 4, 6, or 8 mg/kg cisplatin was given i.p. to female rats, which were killed 4 h later. Cisplatin was given in a solution of 0.14 M NaCl adjusted to pH 2–3 (Platinol, Bristol-Meyers, Weesp, The Netherlands), and carboplatin (Paraplatin, Bristol-Meyers; each vial contained 150 mg carboplatin and 150 mg mannitol) was dissolved in sterile water just before injection. Control animals were injected with 1 ml 0.14 M NaCl. Animals were kept under a 14–/10-h light-dark cycle, were fed standard laboratory chow (Hope Farms, Woerden, The Netherlands), and received water ad libitum. Rats were killed by exsanguination.

The liver, kidney, spleen, testis, uterus, and ovary were quickly removed, frozen on dry ice, and stored at $-80\,^{\circ}C$ for preparation of the cryostat sections and DNA isolation. The kidney and liver were also processed for K-plast embedding [19]: fresh tissues were fixed in Carnoy fixative ($CHCl_3$:methanol:HOAc; 6:3:1, by vol.) for 1 h at room temperature and were incubated for 30 min in 100% ethanol, twice for 30 min in acetone, overnight with K-plast (Medim GmbH, Giessen, Germany; monomer:softener, 8:2), and finally for 15 min with polymerization medium (monomer:softener, 8:2; 4% initiator). Polymerization took place overnight in a water bath at 37 °C in locked, O_2-free molds/containers. Liver and kidney sections that had been embedded in K-plast were deplasticized in acetone (2×30 min) and rehydrated via ethanol to water. Then they were processed as described below for the cryostat sections after fixation.

Immunocytochemical analysis of platinum-induced DNA modifications

The immunoperoxidase staining procedure was carried out as described elsewhere [26], with some modifications. The general outline of the present method was as follows: cryostat sections were fixed with methanol-H_2O_2 (methanol, 0.3% H_2O_2, v/v; 0.1% Na-azide, w/v), during which endogenous peroxidases were inactivated. Sections were rehydrated via ethanol to water, incubated for 3 min with proteinase K buffer [PK buffer: 20 mM TRIS/HCl, 5 mM ethylenediaminetetracetic acid (EDTA, pH 7.4)] at 37^0C and then incubated for 10 min with proteinase K (50 μg/ml) in PK buffer. The reaction was stopped by a 15-min period of incubation at room temperature in glycine [(0.2% glycine; 0.04% Triton X-100 in phosphate-buffered saline (PBS)]. Sections were transferred via water and 20% and 40% ethanol to ethanol-NaOH (40% ethanol in 0.05 N NaOH), incubated for 10 min at room temperature, and neutralized (5 s) in 1% acetic acid /40% ethanol (the incubations with NaOH and proteinase K were performed to denature the DNA and to increase the accessibility of the platinum-DNA adducts for the antibodies). Sections were rehydrated to water and rinsed with wash buffer (50 mM TRIS, 150 mM NaCl, 0.25% gelatin, 5 mM EDTA, 0.04% Triton X-100, pH 7.4) and PBS and were incubated for 1 h with 5% human serum (to reduce nonspecific antibody binding) and then overnight with rabbit antiserum NKI-A59 raised against cisplatin-modified calf-thymus DNA [29].

The antibodies bound to the platinated DNA were visualized by double PAP staining, i.e., sequential incubation of the sections with goat anti-rabbit immunoglobulin (GaR), peroxidase-(rabbit)anti-peroxidase complex (PAP), GaR, PAP, and, finally, 3,3′-diaminobenzidine-HCl/H_2O_2 as the peroxidase substrate. Each sample was stained in duplicate in two independent experiments. On each of the two independently stained slides the nuclear stain (defined as the sum of optical densities of the stained nuclear pixels [31]) of 20 randomly selected nuclei was expressed in arbitrary units (au). With regard to the heterogeneously stained areas in the kidney, representative nuclei throughout the kidney were selected and measured; the very darkly stained nuclei that were rather sparse in the sections are thus hardly represented in the mean nuclear stain values.

DNA isolation

The isolation of carboplatin-treated DNA has been described elsewhere [3]. Briefly: thawed tissues were homogenized to cell suspensions in 5 ml 10 mM TRIS-HCl/1 mM EDTA (pH 7.8; TE buffer), to which NH_4HCO_3 was added to a final concentration of 0.5 M just before use (TE-NH_3 buffer). After the addition of proteinase K (250 μg/ml), cells were lysed by the addition of sodium dodecyl sulfate (final concentration 1%, w/v) during a 10-min period of incubation at 37 °C, which was immediately followed by extraction with phenol for 10 min at 4 °C and precipitation of DNA from the water layer with ethanol. The DNA was washed, dissolved in 2 ml TE-NH_3 buffer supplemented with 250 μg proteinase K/ml, and dialyzed at 37 °C overnight against TE-NH_3 buffer and then twice for 1 h against TE buffer. Then, solutions were extracted with 2 ml chloroform/isoamylalcohol (24:1, v/v) and the DNA was precipitated and washed. After dissolution in 2 ml TE buffer, contaminating RNA was digested during a 2-h period of incubation at 37 °C with 75 μg RNase A/ml and 75 units RNase T1/ml. Subsequently, the DNA solutions were once more extracted with chloroform/isoamylalcohol, precipitated, and dissolved in digestion buffer (10 mM TRIS/HCl, 0.1 mM EDTA, 4 mM $MgCl_2$, pH 7.2).

After enzymatic digestion with nuclease P1 and DNase I to unmodified nucleotides and platinum-containing (di)nucleotides, the mixtures were incubated with proteinase K to destroy the remaining nuclease activity, after which the proteinase K was inactivated by heating. Then, the various adducts were separated by column chromatography and quantitated in an immunochemical assay [10, 11].

Immunochemical determination of the various platinum-DNA adducts

Quantitation of the platinum adducts present at identified positions in the column eluate of digested DNA was performed with specific antisera. In this method the dilutions of the fractions giving 50% inhibition of antibody binding in the competitive ELISA were determined and used to calculate the amounts of carboplatin-DNA digestion products [10, 11].

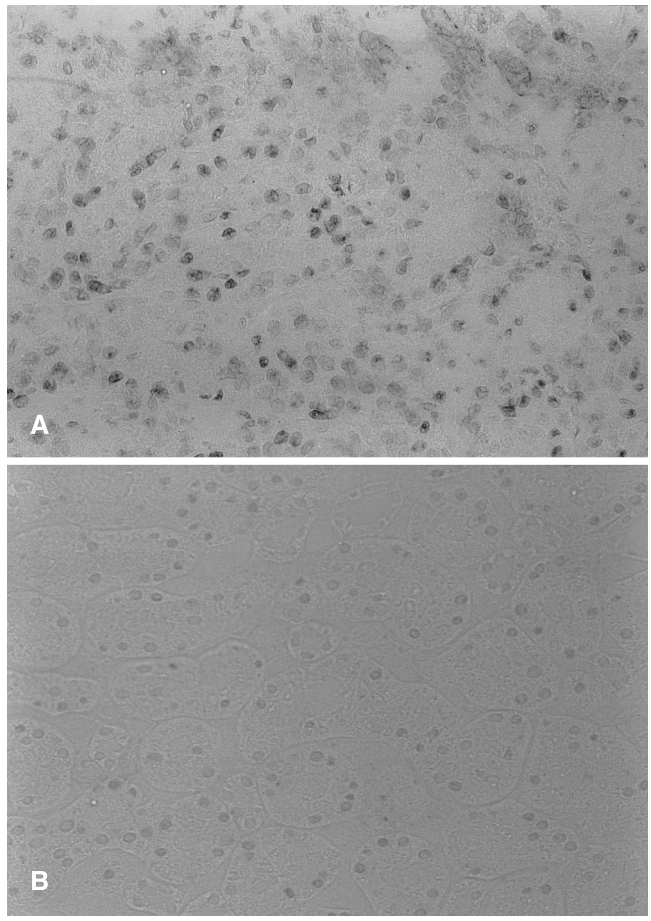

Fig. 1A,B Immunocytochemical staining of **A** a cryostat section (original magnification 200×) and **B** a K-plast-embedded section (original magnification 200×) of the cortex of a kidney at 48 h after i.p. administration of 80 mg/kg carboplatin to a male rat

Results

Visualization of DNA damage in tissues from rats treated with carboplatin or cisplatin

Platinum-induced DNA damage in rats treated with platinum compounds was visualized at the cellular level by staining cryostat and K-plast-embedded tissue sections with NKI-A59 antiserum. Staining according to the original protocol [26] gave a relatively high cytoplasmatic background signal, but the method could be improved by an additional incubation step with proteinase K, which resulted in a lower background signal (in the control samples) and a higher specific nuclear stain in both carboplatin- and cisplatin-treated animal tissues (data not shown). With this adapted protocol platinum-induced DNA modifications were determined in liver, kidney, spleen, testis, and combined ovary and uterus samples after i.p. administration of different doses of carboplatin to rats. The tissues embedded in K-plast showed a better morphology than did the cryostat sections, but the intensity of the staining was lower (cf. Fig. 1A and Fig. 1B). Therefore, quantitation of the nuclear stain levels was performed only on the cryostat sections. With this method, no specific nuclear stain was detected in the spleen, testis, ovary, or uterus.

Dose-response relationships found for the liver and kidney samples at 20 h after carboplatin treatment are shown in Fig. 2A; at this time point significant levels of bifunctional adducts were expected to be present. No significant difference in adduct levels was observed between male and female tissues. For purposes of comparison, platinum-DNA adduct levels were studied in liver and kidney samples obtained from female rats at 4 h after cisplatin treatment (see Fig. 2B), the time point at which maximal bifunctional cisplatin-DNA adduct levels would be expected. After both carboplatin and cisplatin treatment, adduct levels were higher in the kidney than in the liver. A carboplatin dose about 15–20 times higher than that of cisplatin was needed to obtain equal levels of nuclear stain. The nuclear stain seen in the liver was homogeneous in both

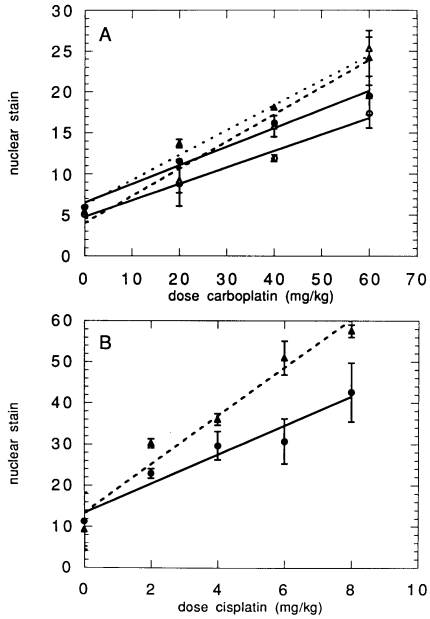

Fig. 2A,B Relationship between the dose of **A** carboplatin or **B** cisplatin given and the platinum-induced DNA damage measured immunocytochemically (antiserum NKI-A59) in the liver (*circles*) and kidney (*triangles*; *dotted lines*), of male (*white symbols*) and female (*black symbols*) rats. Carboplatin- and cisplatin-treated rats were killed at 20 and 4 h, respectively, after drug administration. Platinum-induced DNA damage is expressed in arbitrary units (au) of nuclear stain. Each point represents the mean value ± range for at least 2 independent stainings

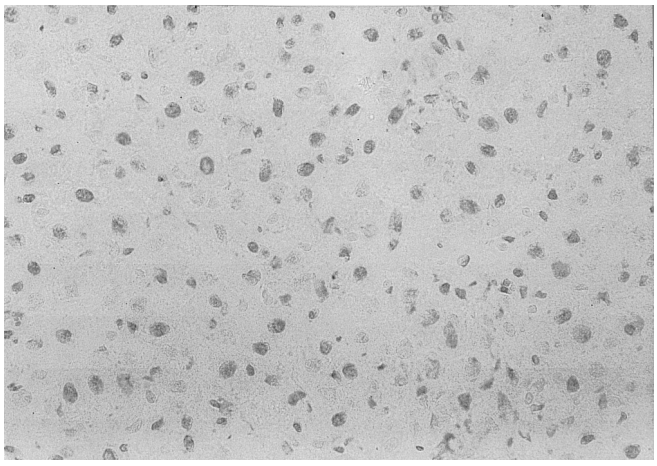

Fig. 3 Immunocytochemical staining of a cryostat section of the liver at 48 h after i.p. administration of 80 mg/kg carboplatin to a female rat (original magnification 200×)

carboplatin- and cisplatin-treated rats (Fig. 3). The nuclear stain observed in the cortex of the kidneys after carboplatin treatment was stronger than that seen in the medulla, and the proximal tubules in the outer cortex occasionally (< 1% of the cells in the cortex)

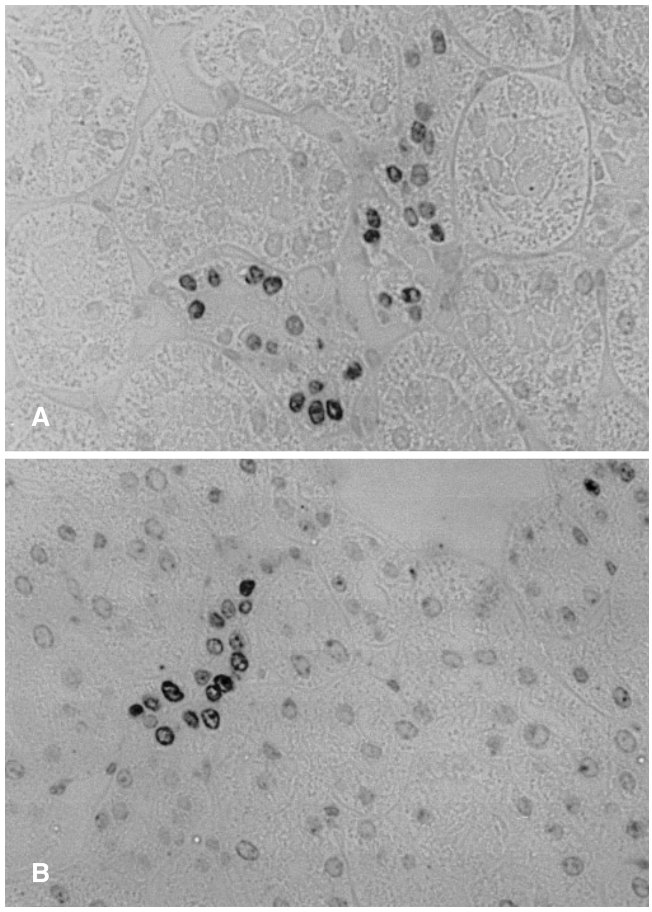

Fig. 4A Immunocytochemical staining of a K-plast-embedded section of the outer cortex of the kidney at 48 h after i.p. administration of 80 mg/kg carboplatin to a male rat (original magnification 400×). **B** Immunocytochemical staining of a K-plast-embedded section of the inner cortex at 48 h after i.p. administration of 8 mg/kg cisplatin to a female rat (original magnification 400×)

showed a darker nuclear stain than did the other cells of the cortex (Fig. 4A). The nuclear stain observed in the cortex of cisplatin-treated kidneys was darker and more heterogeneous. The heterogeneity was particularly prominent in the inner cortex (1–5% of the total cells; Fig. 4B), where groups of very darkly stained nuclei (probably in the distal tubules or the proximal convoluted tubules) were visible.

Formation and persistence of carboplatin-induced DNA damage in the liver and kidney

After a single dose of 80 mg/kg carboplatin, DNA-damage formation and persistence were followed in the liver and kidney. As shown in Fig. 5, within the first 8 h after the injection the initially weak nuclear stain (background) rapidly increased to a more or less stable level, which was followed by a slow decrease after 48 h.

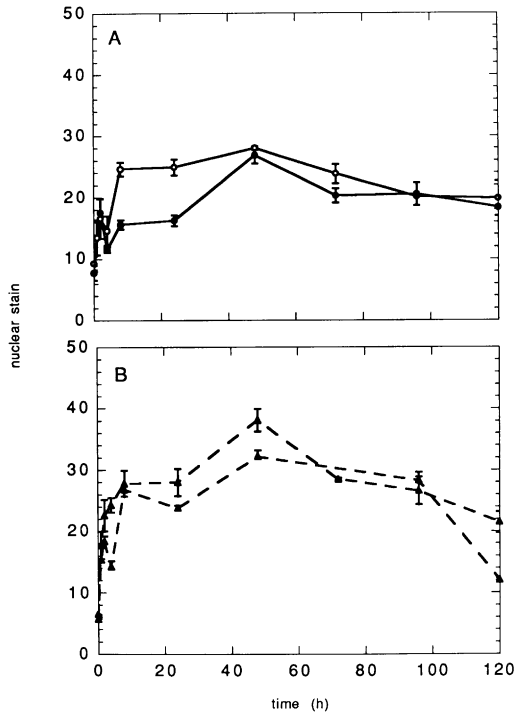

Fig. 5A,B Formation and persistence of DNA damage in **A** liver and **B** kidney sections from carboplatin-treated (80 mg/kg) rats as measured by immunocytochemistry (*white symbols* Tissues from male rats, *black symbols* tissues from female rats). Each point represents the mean value ± range for at least 2 independent stainings

Determination of the various carboplatin-DNA adducts

The levels of the monoadduct Pt-G and the bifunctional adducts Pt-AG, Pt-GG, and G-Pt-G, were measured immunochemically in digested DNA from the kidney, liver, spleen, testis, and combined uterus and ovary samples collected at 8 and 48 h after carboplatin administration. The adducts were detectable in all tissues studied, with levels ranking as follows: kidney > liver, combined uterus/ovary > spleen > testis (see Table 1 for the individual samples and Fig. 6 for the averaged data for the male and female samples). No major difference in adduct levels was observed between male and female rats. At 8 h after administration of carboplatin the relative occurrence of the Pt-GG (29–38%), G-Pt-G (28–36%), and Pt-AG (22–33%), adducts was comparable for the kidney, liver, spleen, and combined uterus/ovary samples (the testis data were omitted because of the very low and, therefore, not very accurate adduct levels), whereas the percentage of the monofunctional Pt-G adducts (3–14%) was much lower. At 48 h the percentages of Pt-GG adducts had significantly increased to 40–58% of the total adducts (Table 1). As can be seen from Table 2, the decrease observed in total adduct levels between 8 and 48 h amounted to 32–42% in the kidney, 29–32% in the liver, and 3–32% in the spleen, whereas the data for the testis (Table 1) suggest that hardly any adducts were removed during that interval.

Discussion

We studied the formation and persistence of carboplatin-induced DNA damage in tissues from rats with an immunocytochemical method and in chromatographed, digested DNA samples with a competitive ELISA. Linear correlations (correlation coefficients of 0.99) between the delivered dose of carboplatin and the resulting platinum-induced DNA damage were observed for the liver and kidney samples as measured immunocytochemically with NKI-A59 antiserum.

No specific nuclear staining was observed in the spleen, ovary, uterus, or testis. With regard to the testis, very low levels of platinum-DNA adducts were measured with the ELISA and, therefore, no major nuclear staining could be expected. The adduct levels found in the spleen and the ovary/uterus, however, were much higher and the lack of nuclear staining therefore has to reflect a lower susceptibility of these tissues to the nuclear staining procedure. Possibly, the treatment of the sections with proteinase K and NaOH had not sufficiently increased the accessibility of the platinum-DNA adducts for the antibodies in these tissues, resulting in a lower sensitivity of the assay. Also, the relatively high degree of background staining seen in these tissues diminished the sensitivity of the immunocytochemical assay. NKI-A59, raised against cisplatin-treated DNA, is believed to recognize bifunctional intrastrand platinum-DNA cross-links or changes in the DNA structure such as the kink(s) [22] induced by these adducts ([29; unpublished results). With this assay, positive correlations have been found between nuclear stain levels and cell survival for a number of cell lines [27] and between nuclear stain levels in buccal cells and the tumor response in patients [2]. The formation of carboplatin-DNA adducts in rat tissues is rather slow (Fig. 5) as compared with the formation of cisplatin-DNA adducts [26] but is in agreement with the slow adduct formation observed in cultured cells [3] and in buccal and peripheral blood cells from patients after carboplatin treatment [8].

The relatively large difference in nuclear stain observed between the livers of male and female rats at 8 and 24 h (Fig. 5A) is in conflict with the results obtained in dose-response experiments (Fig. 2A; at 24 h after administration of carboplatin) and with the ELISA data (Table 1), in which no significant difference between the male and female livers was observed. This discrepancy seems to be the result of the variation of the assay when platination levels are determined at the detection limit. With the more sensitive ELISA, however, the various platinum-DNA adducts were detected

Table 1 Carboplatin-DNA adducts in rat tissues

Tissue	Time[a]	Sex	Adducts (fmol/ μg DNA)[b] Pt-G	(%)	Pt-AG	(%)	Pt-GG	(%)	G-Pt-G	(%)	Σ adducts
Kidney	8	M	3.4 ± 0.3	(7)	14.4 ± 0.3	(32)	12.8 ± 2.0	(29)	14.4 ± 1.3	(32)	44.9
	8	F	2.8 ± 0.7	(7)	10.2 ± 4.5	(24)	15.4 ± 0.8	(36)	14.5 ± 0.2	(34)	43.0
	48	M	1.6 ± 0.5	(5)	5.1 ± 1.3	(17)	17.7 ± 1.0	(58)	6.1 ± 0.6	(20)	30.5
	48	F	0.7	(3)	6.2 ± 1.4	(25)	13.7 ± 0.5	(55)	4.3 ± 0.8	(17)	24.9
Liver	8	M	1.9 ± 0.2	(7)	7.4 ± 1.6	(28)	8.6 ± 1.6	(33)	8.4 ± 0.6	(32)	26.2
	8	F	0.9 ± 0.3	(3)	5.9 ± 2.1	(24)	9.3 ± 0.4	(37)	8.9 ± 0.1	(36)	24.9
	48	M	1.0 ± 0.6	(6)	4.6 ± 0.2	(26)	8.0 ± 0.2	(45)	4.1 ± 0.0	(23)	17.7
	48	F	0.5 ± 0.1	(3)	5.3 ± 0.1	(30)	7.4 ± 0.8	(42)	4.4 ± 0.1	(25)	17.6
Spleen	8	M	0.8 ± 0.2	(8)	3.3 ± 0.2	(33)	3.1 ± 0.2	(30)	2.9 ± 0.1	(29)	10.1
	8	F	1.4 ± 0.6	(14)	2.3 ± 0.8	(23)	3.6 ± 1.1	(35)	2.8 ± 0.1	(28)	10.1
	48	M	1.2 ± 0.8	(12)	2.6 ± 0.3	(26)	4.0 ± 0.7	(40)	2.1 ± 0.3	(22)	9.8
	48	F	0.4 ± 0.2	(6)	2.2 ± 0.1	(31)	2.8 ± 0.1	(40)	1.6 ± 0.0	(23)	6.9
Testis	8		0.6	(17)	1.3 ± 0.5	(42)	0.7 ± 0.1	(22)	0.6 ± 0.0	(19)	3.2
	48		1.0 ± 0.8	(35)	0.7 ± 0.3	(22)	0.7 ± 0.1	(25)	0.5 ± 0.1	(18)	2.9
O + U[c]	8		2.7 ± 0.5	(10)	5.7 ± 1.5	(22)	9.7 ± 0.8	(38)	7.6 ± 0.6	(30)	25.2
	48		1.2 ± 0.4	(8)	3.1 ± 1.1	(20)	6.9 ± 0.4	(45)	4.1 ± 0.1	(27)	15.4

[a]Time (h) after injection of carboplatin (i.p., 80 mg/kg)
[b]Mean values ± range for two independently performed ELISAs, each in four dilutions; between brackets the relative occurrence is given as % of total adducts
[c]Combined samples of the ovary and uterus

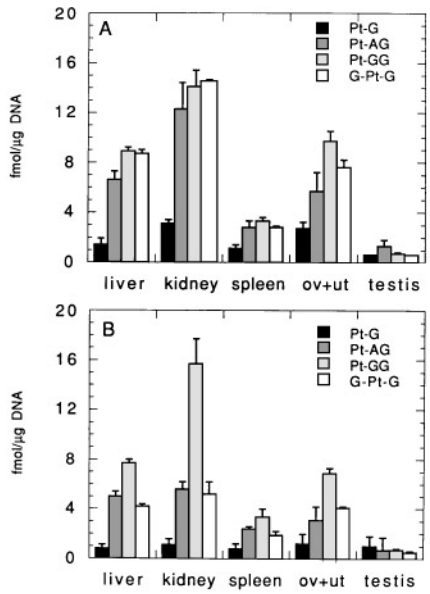

Fig. 6A, B DNA-adduct levels determined in various tissues from male and female rats at **A** 8 and **B** 48 h after carboplatin administration (80 mg/kg). The data on the liver, kidney and spleen are averaged values (± range) for the male and female samples. The testis and the combined ovary and uterus (*ov + ut*) data are mean values (± range) for single samples analyzed in two independently performed ELISA experiments

in all tissues tested, with their (total) levels ranking in the follwing order: kidney > liver, combined ovary/uterus > spleen > testis. The same rank order has been found for the total platinum concentration detected in

Table 2 Repair of DNA adducts occurring between 8 and 48 h after carboplatin treatment of rats.

Tissue		Decrease in adduct level (%) Pt-G	Pt-AG	Pt-GG	G-Pt-G	Σ adducts
Kidney	(M)	53	65	0	58	32
	(F)	75	39	11	70	42
Liver	(M)	47	38	7	51	32
	(F)	44	10	20	51	29
Spleen	(M)	0	21	0	28	3
	(F)	71	4	22	43	32
O + U[a]		55	46	29	46	39

[a]Combined samples of the ovary and uterus

these tissues during 24 h after the treatment of mice with carboplatin or cisplatin [24] and after the treatment of rats with cisplatin [12, 21, 26].

The relative occurrence of the various adducts in the tissues of the rats at 8 h after administration of carboplatin [Pt-GG (34%); Pt-AG (27%); G-Pt-G (32%); Pt-G (7%)] was different from that reported in rats at 1 and 24 h after administration of cisplatin [Pt-GG (61%); Pt-AG (15%); G-Pt-G (17%); Pt-G (6%)] [12] but was in agreement with data obtained in carboplatin-treated cultured cells [3] and in white blood cells of patients (unpublished data).

Clear differences in repair were observed between the various adducts and between the tissue types (Tables 1, 2). When the total DNA-platination levels (calculated from the sum of the adducts) detected at 8 and 48 h

after carboplatin treatment were compared, a substantial decrease in DNA platination was observed in the kidney (32–42%), liver (29–32%), and combined ovary/uterus samples (39%), whereas the repair levels seen in the testis (9%) and, possibly, in the spleen (2–32%) were lower. That the repair of platinum-induced DNA damage in the testis is low, if not completely absent, is in agreement with the observation that germ-cell tumor cells, which are very sensitive to cisplatin, are deficient in the repair of cisplatin-induced bifunctional adducts [14].

The decreases seen in the platination levels of the kidney, liver, spleen, and ovary/uterus samples were mainly due to repair (in decreasing order) of the G-Pt-G, Pt-G, and Pt-AG adducts, whereas the Pt-GG adducts seemed to be more persistent. Although less obvious, during the first 24 h after treatment of rats with cisplatin, a less efficient repair of Pt-GG as compared with the other bifunctional adducts has been observed too [12]. Our data are also in agreement with in vitro experiments showing that repair by ABC excinuclease of the DNA adducts with diamminocyclohexane (DACH) or ethylenediamine (EN) as nitrogen ligands occurs in the following order: G-Pt-G > Pt-G > Pt-AG > Pt-GG [20]. Also, Huang et al. [16] have reported a relatively poor repair activity by the human incision nuclease of Pt-GG adducts as compared with G-Pt-G adducts, together with the observation that high-mobility-group (HMG)-domain proteins are capable of inhibiting the repair of Pt-GG but not G-Pt-G adducts due to the binding of these proteins to the former adducts. However, Visse et al. [32] have reported that the incision of DNA by the *Escherichia coli* Uvr ABC endonuclease in an in vitro system is much more efficient in DNA containing a cisplatin Pt-GG adduct than in DNA harboring a G-Pt-G adduct.

In the present study, the ratio of the total adduct levels detected in the kidney and the liver at between 8 and 48 h after administration of carboplatin was about 1.6 (Table 1), which is lower than the ratios of 2.5–3.9 previously obtained at between 1 and 24 h after treatment of rats with cisplatin [12].

Another, remarkable difference between the effects of carboplatin and those of cisplatin was observed in the nuclear staining patterns of kidney sections. The highest nuclear staining in the carboplatin-treated kidney was mainly located in the outer cortex and was more homogeneous than that seen after cisplatin treatment, whereas cisplatin caused very intense staining in a limited area, probably the proximal (convoluted) tubules in the inner cortex (Fig. 4). The cells in this area are known to be involved in active secretory processes that are supposed to remove cisplatin and cisplatin-protein complexes, whereas carboplatin, which is less reactive with proteins, is removed by glomerular filtration. The presence of high levels of nuclear staining in the inner cortex also corresponds to the damage observed histologically and biochemically after treatment of these cells with cisplatin in cell culture [7] as well as in vivo [1, 25], whereas no damage was observed after carboplatin treatment. The differences observed in the DNA damage to the various cell types in the kidney may be the reason for the difference in the nephrotoxicity of carboplatin versus cisplatin.

When the formation of carboplatin and cisplatin adducts in the various tissues are compared, several differences become noticeable. In addition to the slower formation of the carboplatin-DNA adducts, it becomes apparent that doses of carboplatin much higher than those of cisplatin are required to obtain equal platination levels and degrees of cytotoxicity. Carboplatin doses about 15–20 times higher than those of cisplatin were needed to get comparable nuclear stain levels in the kidney and liver (after 20 and 4 h, respectively; Fig 2). To achieve comparable total platinum-DNA levels (measured by AAS) in the liver, an approximately 30-fold higher dose of carboplatin was required (unpublished data). Similar ratios (5–20) between carboplatin and cisplatin doses have been found in other animal models when equitoxicity and similar platinum content or platinum-DNA levels have been pursued [24]. The same holds for cells (20 to 40-fold) [18, 28] and for the dose given to patients (5 to 20-fold) [2]. However, doses of carboplatin much higher (> 100-fold) than those of cisplatin are required to obtain equal levels of DNA platination in vitro [3, 17, 18]. No simple explanation for these observations can be given, but it has been suggested that in cells, carboplatin is activated by enzymatic cleavage [6] or by the presence of free oxygen radicals [30].

In conclusion, not only our in vitro study [3] but also the present in vivo study indicates that carboplatin is not simply a slowly reacting substitute for cisplatin. Carboplatin does not show a preference for binding on GG sequences, and the level and location of DNA damage can differ from that seen after cisplatin treatment, as was found for certain cell types in the kidney, which may explain the different toxicity patterns of these drugs.

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