Cancer Chemother Pharmacol (1991) 28: 427–433
034457049100129W

Cancer Chemotherapy and Pharmacology

Iron- and ascorbic acid-induced lipid peroxidation in renal microsomes isolated from rats treated with platinum compounds

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Received 16 May 1990/Accepted 20 March 1991

Summary. Renal microsomes isolated on day 3 from cisplatin (CDDP, single i.p. injection, 4 or 6 mg/kg)-treated rats were monitored for their susceptibility to lipid peroxidation as compared with microsomes from rats treated with carboplatin (CBDCA, 30 mg/kg), transplatin (TDDP, 6 mg/kg) or CDDP hydrolysis products (4 or 6 mg/kg) or from control animals. Cephaloridine (1 g/kg daily for 4 days, i.p. injection) was used as a positive control. The effect of CDDP on renal microsomal glucose-6-phosphatase activity was investigated in vivo and in vitro. Following treatment with CDDP and CDDP hydrolysis products vs CBDCA and TDDP treatment, microsomes revealed an enhanced susceptibility to lipid peroxidation in a Fe^{2+} and/or ascorbic acid stimulation system. Increased lipid peroxidation, expressed as an increase in malondialdehyde (MDA) generation, paralleled the alterations in body and kidney weight and the elevations of plasma creatinine and blood urea nitrogen concentrations. Injection of the antioxidant *N,N'*-diphenyl-*p*-phenylenediamine (DPPD, 0.5 g/kg, i.p.) at 24 h prior to CDDP treatment abolished the increased vulnerability of renal microsomes to lipid peroxidation. In vivo, only CDDP hydrolysis products exhibited a significant inhibitory effect on renal glucose-6-phosphatase activity. In vitro, rat renal and hepatic microsomal glucose-6-phosphatase activity was decreased by CDDP both time- and concentration-dependently. Nephrotoxicity induced by CDDP and CDDP hydrolysis products might be attributable to iron-dependent lipid peroxidation and microsomes might represent target organelles on a subcellular level.

Introduction

One of the most commonly used anticancer agents, cisplatin (CDDP), causes renal tubular toxicity as a major side effect, which can be documented in humans and rats as an increase in plasma creatinine and blood urea nitrogen concentrations and as proteinuria, enzymuria and electrolyte disturbances [16, 26]. The cellular mechanism of CDDP-induced nephrotoxicity has not been elucidated, but several studies have suggested that lipid peroxidation is involved [4, 19, 21, 23, 33, 39, 40]. A rise in levels of malondialdehyde (MDA), a lipid peroxidation product, has been observed in renal cortical slices following CDDP incubation in vitro [19, 40, 44] and in whole kidneys at 3 days after CDDP treatment in vivo [39]. Depletion of tissue glutathione, which may act as a radical scavenger, enhances CDDP-induced lipid peroxidation [31] and nephrotoxicity [28]. Antioxidants and radical scavengers diminish CDDP-induced lipid peroxidation and nephrotoxicity [10, 15, 19, 21, 38, 44]. Following a single dose of CDDP, specific platinum concentrations in the kidney have been found to be highest in nuclei and microsomes [7]. Microsomes contain polyunsaturated fatty acids, which are susceptible to peroxidation [32] and may therefore be target organelles for lipid peroxidation.

In the present study, the vulnerability to peroxidation of microsomes isolated from kidneys of rats that had been treated with CDDP and CDDP hydrolysis products was investigated and compared with that of microsomes isolated from carboplatin (CBDCA)- and transplatin (TDDP)-treated rats. CBDCA has been used instead of CDDP in clinical application because the former is less nephrotoxic and results in fewer gastro-intestinal disturbances [14]; TDDP is ineffective as an antitumor agent, showing different toxic properties in vitro and in vivo [21]. Cephaloridine, well known to be an inducer of lipid peroxidation in rat renal microsomes and rat renal cortical slices and thus known to be nephrotoxic [8, 17], was used as a positive control. Another goal of the present study was to examine the effect of the administration of an antioxidant, *N,N'*-diphenyl-*p*-phenylenediamine (DPPD), prior to CDDP treatment. Since radical-mediated lipid peroxidation depends on the availability of iron [29, 30], either Fe^{2+}, a combination of Fe^{2+} and the reducing agent ascorbic acid, or ascorbic acid alone were used as pro-oxidants.

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Table 1. Effect of treatment with platinum compounds on the body and kidney weight of rats

	Day	Control	CDDP (6 mg/kg)	CDDP hydrolysis products (6 mg/kg)	CBDCA (30 mg/kg)	TDDP (6 mg/kg)
% of initial body weight	0	100 ±1.97	100 ±1.75	100 ±1.86	100 ±1.89	100 ±0.67
	3	105.38 ±1.6	88.46*, ** ±2.09	83.72*, ** ±2.1	99.64* ±1.71	100.82 ±2.42
% of control kidney weight	3	100 ±1.58	115.85* ±1.92	110.28* ±4.05	96.95 ±7.54	102.71 ±6.29
Kidney weight body weight ratio ($x10^{-3}$)	3	3.18	4.24*	4.41*	3.25	3.42

The table contains data ($\bar{x} \pm SEM$) from at least 20 rats/group used for several microsomal preparations. Kidney weight was calculated as the weight of both kidneys divided by 2

* $P < 0.05$ as compared with the corresponding control value on day 3,
** $P < 0.05$ as compared with the corresponding control value on day 0

Results of previous studies have shown that the CDDP-induced decrease in gluconeogenesis in renal cortical slices in vitro could not be altered by antioxidants [19, 21]. The decrease in gluconeogenesis might be caused by the direct inhibition of glucose-6-phosphatase [18] or via lipid peroxidation [12, 18]. The in vitro effect of CDDP on renal microsomal glucose-6-phosphatase activity was therefore investigated time- and concentration-dependently and was compared with the in vivo effect. Furthermore, the in vitro effect of CDDP, which only occasionally induced transient and mild increases in liver function tests [42], on hepatic microsomal glucose-6-phosphatase activity was investigated.

Materials and methods

Drugs and chemicals. CDDP, cephaloridine and corn oil were purchased from Sigma (St. Louis, Mo., USA); CBDCA was obtained from Medac (Hamburg, FRG); and TDDP was supplied by Aldrich (Steinheim, FRG). Ascorbic acid and iron(II) chloride were obtained from Merck (Darmstadt, FRG), and DPPD was purchased from Fluka (Buchs, Switzerland).

Experimental protocols and assay procedures. Male Wistar rats (240–280 g) obtained from Winkelmann (Borchen, FRG) were housed in ventilated rooms equipped with regular light cycles and controlled temperature and humidity. Rats were provided with a standard diet (Altromin) and water ad libitum. Animals were given a single i.p. injection of CDDP (4 or 6 mg/kg), TDDP (6 mg/kg) or CBDCA (30 mg/kg) dissolved in sterile isotonic saline solution or of CDDP hydrolysis products (4 or 6 mg/kg) prepared as previously described [20]. Cephaloridine (1 g/kg i.p.) was given once daily for 4 consecutive days. For antioxidant studies, rats were treated with DPPD (0.5 g/kg i.p.) dissolved in corn oil at 24 h prior to CDDP injection. Control rats were treated likewise with corn oil.

After 3 days, the body weight of rats that had been treated with platinum compounds was determined and blood samples were taken to quantitate plasma creatinine and blood urea nitrogen concentrations (Test-Combinations, Boehringer Mannheim, FRG). After 72 h, animals were killed by cervical dislocation and the kidneys were removed immediately, decapsulated and weighed for determination of kidney/body weight ratios. Microsomal fractions of kidneys from pretreated rats (lipid peroxidation experiments) were prepared using a rapid isolation method [1]. For in vitro experiments (glucose-6-phosphatase activity), renal or hepatic microsomes from untreated rats were prepared as previously described [21]. Both isolation methods [1, 36] revealed the same specific glucose-6-phosphatase activity for renal microsomes. The microsomal pellets were washed, frozen in liquid nitrogen and stored at –80°C. All microsomal suspensions were assayed for protein content [35] and glucose-6-phosphatase activity [2]. Microsomes from cephaloridine-treated rats were prepared after 5 days.

For determination of microsomal lipid peroxidation, incubations of rat renal microsomes (final concentration, 0.1–0.15 mg protein/ml) were performed in 1 ml TRIS-HCl buffer (30 mM TRIS-HCl, 100 mM KCl; pH 7.4) at 37°C under a pure oxygen atmosphere. A metabolic shaker (Braun, Melsungen, FRG) was used. To stimulate lipid peroxidation, media contained either Fe^{2+} (0.1 mM), ascorbic acid (1 mM), or both pro-oxidants (0.01 or 0.1 mM Fe^{2+} and 0.1 or 1 mM ascorbic acid). Incubations were carried out for different periods (0–240 min). Levels of the lipid peroxidation breakdown product MDA were determined using the thiobarbituric acid assay [6].

Incubations of renal or hepatic microsomes (final concentration, 0.35 mg protein/ml) for the determination of glucose-6-phosphatase activity in vitro were performed in a TRIS-HCl buffer (6 mM TRIS-HCl, 200 mM KCl; pH 7.4) at 37°C in 100% O_2 using the metabolic shaker. Incubations were carried out for different periods (0–6 h); except for control samples, different concentrations of CDDP were added (0.167–1.67 mM). For some experiments the antioxidant DPPD (2 μM) was added to control or CDDP-containing media. Activity of glucose-6-phosphatase was determined spectrophotometrically [2] as recently described [21].

Statistical analysis. Mean values (±SD or SEM) were calculated. Student's *t*-test was used for data analysis, whereby a value of $P < 0.05$ was considered to be significant.

Results

Effect of treatment with platinum compounds on body and kidney weight

On day 3, body weight was significantly decreased in rats that had been treated with CDDP (6 mg/kg) and CDDP hydrolysis products (6 mg/kg) as compared with control animals. CBDCA (30 mg/kg) showed only a slight effect, whereas TDDP (6 mg/kg) had no significant influence on body weight (Table 1). Kidney weight was significantly increased in animals that had been treated with CDDP

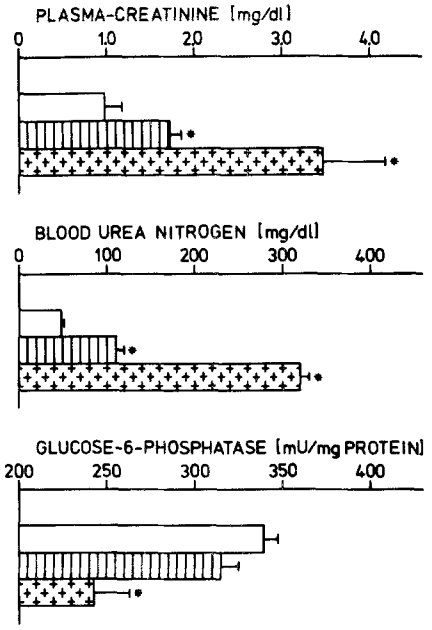

Fig. 1. Effect of treatment with platinum compounds on concentrations of plasma creatinine and blood urea nitrogen and on renal microsomal glucose-6-phosphatase activity at 3 days post-treatment in rats. Symbols represent $\bar{x} \pm$ SEM of at least 20 rats or of at least 4 different microsomal preparations. * $P < 0.05$ as compared with the corresponding control value. ▭, control; ▥, CDDP (6 mg/kg i.p.); ⊞, CDDP hydrolysis products (6 mg/kg i.p.)

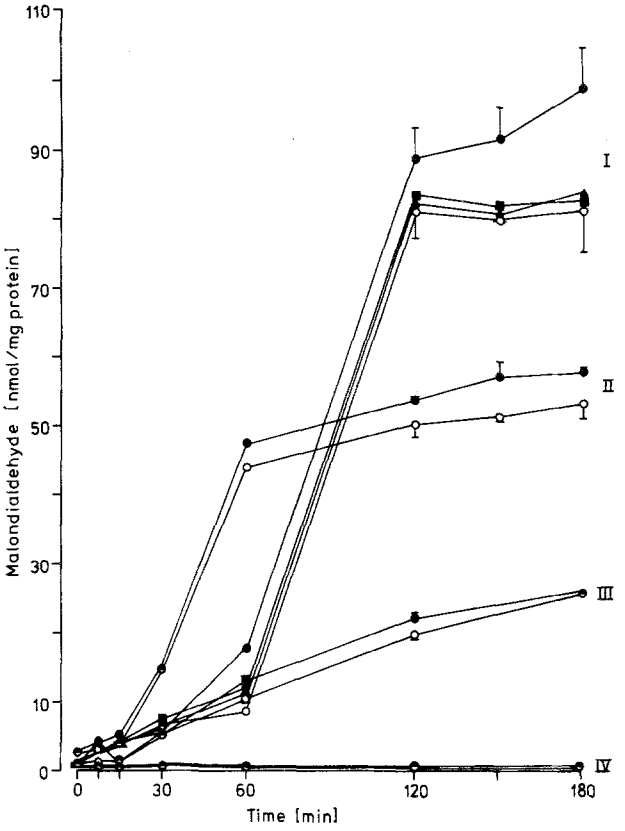

Fig. 2. Effect of treatment with platinum compounds on renal microsomal MDA generation at 3 days post-treatment in rats. Microsomes were incubated in TRIS-HCl buffer supplemented with various pro-oxidants. Symbols represent $\bar{x}$ or $\bar{x} \pm$ SD of at least 4 experiments using microsomes from at least 2 different preparations. Post-treatment MDA generation was significantly different from the corresponding control values ($P < 0.05$) where SD were indicated. *I*, Fe^{2+} (0.1 mM) and ascorbic acid (1 mM); *II*, Fe^{2+} (0.1 mM); *III*, ascorbic acid (1 mM); *IV*, without stimulation; ○, control; ●, CDDP (6 mg/kg i.p.); ▲, CBDCA (30 mg/kg i.p.); ■, TDDP (6 mg/kg i.p.)

(6 mg/kg) and CDDP hydrolysis products (6 mg/kg) as compared with controls (Table 1). The body weight of rats that had been given cephaloridine (1 g/kg daily for 4 days) was significantly decreased, and kidney weight was significantly increased on day 5 (data not shown).

Effect of treatment with platinum compounds on concentrations of plasma creatinine or blood urea nitrogen and on renal microsomal glucose-6-phosphatase activity

Levels of plasma creatinine and blood urea nitrogen were significantly elevated at 3 days after treatment with CDDP (6 mg/kg) and CDDP hydrolysis products (6 mg/kg; Fig. 1). Plasma creatinine and blood urea nitrogen levels in rats that had received CDDP hydrolysis products were >2-fold those in CDDP-treated animals. The activity of glucose-6-phosphatase in renal microsomes as measured immediately after isolation was not decreased in CBDCA (30 mg/kg)-, TDDP (6 mg/kg)- or cephaloridine (1 g/kg daily for 4 days)-treated rats (data not shown), tended to be diminished in CDDP (6 mg/kg)-treated animals, and was significantly reduced in those treated with CDDP hydrolysis products (6 mg/kg; Fig. 1). In contrast, additional in vivo experiments revealed no change in the glucose-6-phosphatase activity in hepatic microsomes from rats treated with CDDP (6 mg/kg) or CDDP hydrolysis products (6 mg/kg; data not shown).

Effect of treatment with platinum compounds on renal microsomal MDA generation

In the absence of stimulation with pro-oxidants, microsomes isolated from CDDP (6 mg/kg)-treated rats did not exhibit significantly more vulnerability to lipid peroxidation than those from control animals (Fig. 2). After 120 min, stimulation with Fe^{2+} (0.1 mM) or ascorbic acid (1 mM) led to the generation of higher levels of MDA in microsomes isolated from CDDP-treated rats than in those isolated from control animals. The total amount of MDA generated after Fe^{2+} stimulation was up to 2-fold that following ascorbic acid stimulation (Fig. 2). The combination of Fe^{2+} and ascorbic acid (0.1/1 mM) was the most effective promoter of MDA generation (Fig. 2; Table 2, 3), and microsomes from CDDP-treated rats showed a significantly increased vulnerability to lipid peroxidation as compared with control microsomes. In contrast, microsomes from TDDP (6 mg/kg)- and CBDCA (30 mg/kg)-treated rats showed no significant increase in MDA generation as compared with control microsomes in the Fe^{2+} ascorbic acid stimulation system (Fig. 2).

Table 2. Effect of treatment with platinum compounds and cephaloridine on renal microsomal MDA generation in rats

Incubation condition		Incubation period	
Pro-oxidants	Substance tested	180 min	240 min
0.1 mM Fe^{2+} and 1 mM ascorbic acid	Control	79.67 ± 3.72	79.09 ± 4.14
	CDDP	93.3 ± 5.11*	94.08 ± 5.4*
	CDDP hydrolysis products	104.83 ± 4.19*	111.14 ± 4.64*
	Cephaloridine	108.04 ± 4.2*	113.12 ± 1.44*
0.01 mM Fe^{2+} and 0.1 mM ascorbic acid	Control	78.59 ± 6.61	79.33 ± 3.5
	CDDP	88.42 ± 4.31	86.82 ± 4.6*
	CDDP hydrolysis products	97.72 ± 3.43*	98.32 ± 4.6*
	Cephaloridine	100.67 ± 3.04*	102.65 ± 6.69*

Renal microsomes prepared at 72 h after the administration of platinum compounds (4 mg/kg i. p.) or 120 h after cephaloridine treatment (1 g/kg daily, i. p.) were incubated in TRIS-HCl buffer containing the pro-oxidants Fe^{2+} (0.1 or 0.01 mM) and ascorbic acid (1 or 0.1 mM). Data represent $\bar{x} \pm SD$ expressed in nmol/mg protein

* $P < 0.05$ as compared with the corresponding control value

Table 3. Effect of the injection of the antioxidant DPPD on renal microsomal MDA generation in CDDP-treated rats

Incubation time (min)	Control corn oil-treated rats	CDDP- and corn oil-treated rats	Control DPPD- and corn oil-treated rats	CDDP-, DPPD- and corn oil-treated rats
120	80.97 ± 3.68	88.9* ± 3.99	3.73 ± 1.01	4.68 ± 0.95
180	80.95 ± 5.78	98.55* ± 5.77	4.31 ± 0.69	4.78 ± 0.55

Renal microsomes prepared at 72 h after CDDP injection (6 mg/kg i. p.) were incubated in a TRIS-HCl buffer medium containing the pro-oxidants Fe^{2+} (0.1 mM) and ascorbic acid (1 mM). DPPD (0.5 mg/kg i. p., dissolved in corn oil) was given at 24 h prior to CDDP injection. Data represent $\bar{x} \pm SD$ expressed in nmol/ng protein

* $P < 0.05$ as compared with the corresponding control value

A dose of 4 mg/kg was used for lipid peroxidation experiments in rats that had been treated with CDDP hydrolysis products because animals receiving a dose of 6 mg/kg were in extremely poor condition and the protein content, glucose-6-phosphatase activity and MDA generation varied considerably in the microsomal preparations from these animals, whereby the specific activity of glucose-6-phosphatase was reduced by about 30% as compared with that of preparations from control and CDDP-treated rats. Microsomes from rats that had been given CDDP hydrolysis products (4 mg/kg) were more vulnerable to lipid peroxidation than were those from CDDP (4 mg/kg)-treated animals. The former exhibited MDA generation similar to that displayed by microsomes prepared from cephaloridine (1 g/kg daily for 4 days)-treated rats (Table 2). Overall, 10-fold lower concentrations of Fe^{2+}/ascorbic acid induced comparable MDA generation (Table 2).

Effect of the antioxidant DPPD on renal microsomal MDA generation after CDDP treatment

A single dose of the antioxidant DPPD (0.5 g/kg) given 24 h prior to CDDP treatment or control treatment resulted in a strong depression of Fe^{2+}/ascorbic acid (0.1/1 mM)-stimulated MDA generation (Table 3). After DPPD pretreatment, no significant difference in MDA generation was observed between microsomes isolated from CDDP-treated rats and those isolated from control rats (Table 3). Pretreatment of rats with the corn oil vehicle had no significant effect on MDA generation in microsomes isolated from control or CDDP-treated rats (Table 3) as compared with animals that had not been treated with corn oil (Fig. 2).

Effect of CDDP on renal and hepatic microsomal glucose-6-phosphatase activity in vitro

A time-dependent decrease in glucose-6-phosphatase activity was observed in renal microsomes from control rats during the 6-h incubation period (Fig. 3) but not in hepatic microsomes from controls (Fig. 4). The addition of CDDP to the incubation medium resulted in a time- and concentration-dependent diminution of glucose-6-phosphatase activity in renal and hepatic microsomes as compared with the corresponding control values (Figs. 3, 4). DPPD (2 μM) did not exhibit a protective effect against the CDDP-induced decrease in glucose-6-phosphatase activity in renal or hepatic microsomes (data not shown).

Discussion

The present study showed that renal microsomes isolated from rats that had been treated with CDDP and CDDP

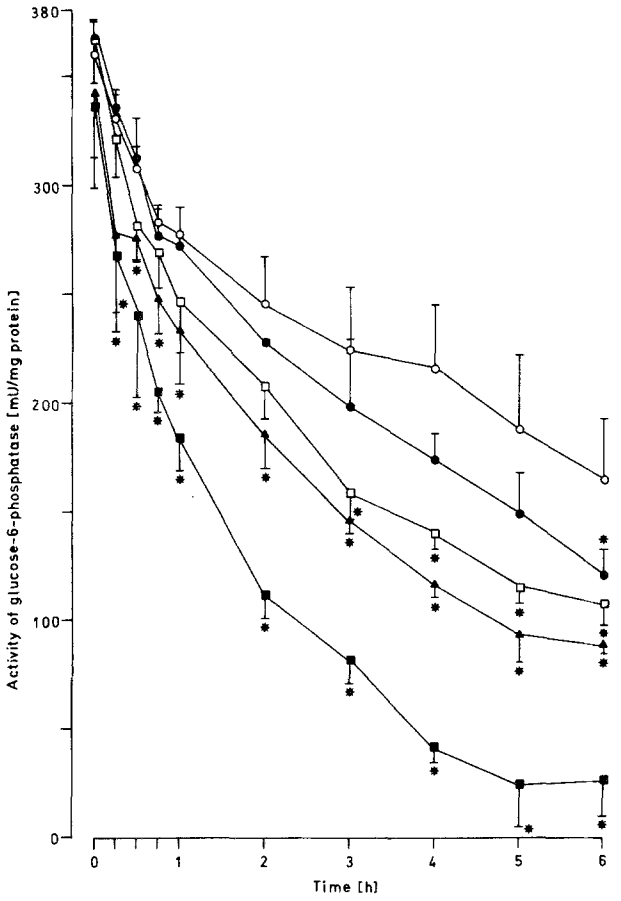

Fig. 3. Effect of CDDP on rat renal microsomal glucose-6-phosphatase activity in vitro. Microsomes were incubated in TRIS-HCl buffer containing CDDP. Symbols represent $\bar{x} \pm SD$ of at least 4 experiments. * $P < 0.05$ vs the corresponding control value. ○, Control; ●, 0.167 mM CDDP; □, 0.251 mM CDDP; ▲, 0.417 mM CDDP; ■, 1.67 mM CDDP

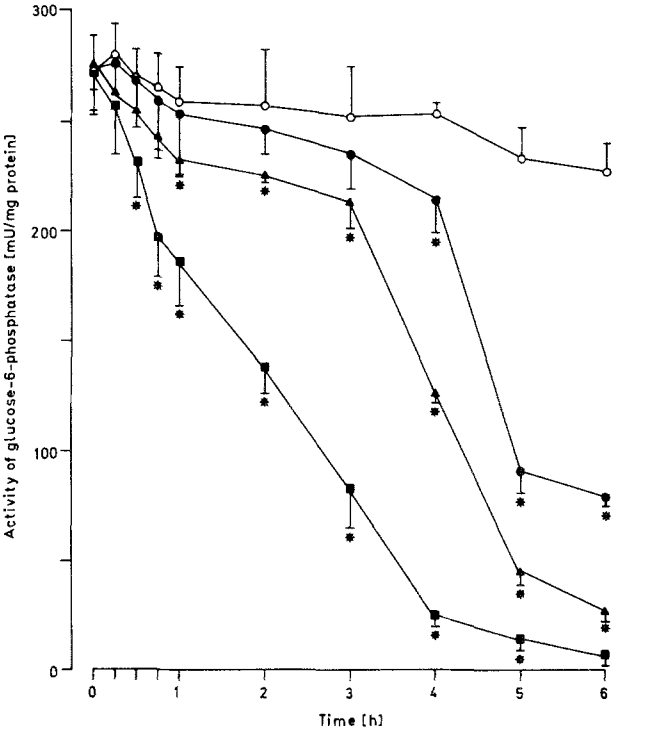

Fig. 4. Effect of CDDP on rat hepatic microsomal glucose-6-phosphatase activity in vitro. Microsomes were incubated in TRIS-HCl buffer containing CDDP. Symbols represent $\bar{x} \pm SD$ of at least 4 experiments. * $P < 0.05$ vs the corresponding control value. ○, Control; ●, 0.167 mM CDDP; ▲, 0.417 mM CDDP; ■, 1.67 mM CDDP

hydrolysis products demonstrated an increased vulnerability to lipid peroxidation in a Fe^{2+} and/or ascorbic acid stimulation system. In contrast, rat renal microsomes isolated from CBDCA- or TDDP-treated rats did not show enhanced susceptibility to lipid peroxidation. It has previously been shown that CDDP promotes lipid peroxidation in renal cortical slices following CDDP incubation in vitro, whereas CBDCA exerts only a slight effect on MDA generation [21]. The nephrotoxicity of CDDP and CDDP hydrolysis products might be attributable to lipid peroxidation, since the observed increase in renal microsomal MDA generation paralleled the changes in body and kidney weight in animals that had received CDDP and CDDP hydrolysis products. As further assessed as an increase in plasma creatinine and blood urea nitrogen concentrations, such nephrotoxicity was observed in rats that had been given CDDP- and CDDP hydrolysis products. In contrast, TDDP- or CBDCA-treated animals did not develop pronounced symptoms of nephrotoxicity-like alterations in these parameters [26, 27], and in the present study these rats exhibited only slight changes in body or kidney weight.

Hydrolysis products of CDDP might be generated intracellularly after uptake of the drug in the kidney due to the low intracellular chloride concentration, which attenuates the stability of CDDP [34]. The hydrolysis products are more nephrotoxic than is CDDP itself [9]. Microsomes isolated from rats that had been treated with CDDP hydrolysis products demonstrated MDA generation higher than that exhibited by microsomes prepared from rats that had been given an equivalent dose of CDDP; the MDA generation observed in the former reached the same levels found in microsomes from cephaloridine-treated rats. However, the results do not answer the question as to whether or not the effect of cephaloridine and that of CDDP hydrolysis products on microsomal MDA generation are caused by the same mechanism.

Ferrous ions and/or ascorbic acid are important initiators of tissue peroxidation [11, 13]. Fe^{2+} [13], and optimal Fe^{3+}:Fe^{2+} ratio [3, 5] or an Fe^{2+}-dioxygen-Fe^{3+} complex [29] have been proposed as initiators of lipid peroxidation. In the present study, Fe^{2+} ions were more effective than ascorbic acid in stimulating lipid peroxidation in the TRIS-HCl buffer system. In contrast, a previous study has shown that in a phosphate buffer system, Fe^{2+} ions had only a marginal stimulatory effect on lipid peroxidation as compared with ascorbic acid [23]. Phosphate could act as an iron chelator [24]. In both buffer systems, a combination of Fe^{2+} and ascorbic acid (molar ratio, 1:10) was the most effective promoter of lipid peroxidation. The lag period at the beginning of Fe^{2+}/ascorbic acid-stimulated lipid peroxidation might be explained by the reducing property of ascorbic acid, which keeps Fe^{2+} in the reduced state until a sufficient Fe^{2+}:Fe^{3+} ratio has been attained.

Differences in MDA production between microsomes isolated from CDDP-treated rats and those from control rats were highest in the Fe^{2+}/ascorbic acid stimulation system. CDDP could show the ability to alter microsomal iron stores and may thus increase the vulnerability of microsomal membranes to lipid peroxidation. Elevated iron levels have been observed in kidneys of mice [37] and rats [33] following CDDP treatment, and iron overload may enhance the rate of lipid peroxidation in animal organs [43]. Much of the endogenous iron in microsomes occurs as ferritin, and anticancer drugs such as Adriamycin display the ability to release iron from ferritin in a microsomal system [41]. Results from our laboratory (unpublished data) show that CDDP may also exhibit this ability.

In accordance with the observation of elevated serum iron levels following CDDP administration, CDDP may compete with iron at available protein-binding sites [25]. Since other trace elements such as copper are significantly altered in kidneys after treatment with CDDP [37], this may contribute to enhanced susceptibility to lipid peroxidation. For example, copper could exert a potentiating effect on ferrous ion-induced lipid peroxidation [3], although the possibility that copper deficiency might enhance the nephrotoxic effect of CDDP has been raised [33]. One should assume that there would be an increase in renal microsomal lipid peroxidation in CDDP-treated rats without stimulation by exogenous Fe^{2+} or/and ascorbic acid.

Although we did not observe these alterations in the present study, it has been shown that in the absence of further stimulation, microsomes isolated from CDDP-treated rats contain more MDA than do those isolated from control rats [4]. The discrepancy between these results might be due to differences in the preparations and in the treatment protocol (in the previous study, CDDP was given i.p. three times at intervals of 21 days), which might have led to the detection of small amounts of lipid peroxidation products in the absence of further incubation and stimulation. Therefore, CDDP may induce alterations in trace element stores as well as in antioxidative capacity (e.g. endogenous vitamin E or glutathione), thus favouring conditions (e.g. optimal Fe^{2+}:Fe^{3+} ratio) for increased microsomal lipid peroxidation. However, the reason for the difference in the extent of iron mobilisation by CBDCA and TDDP vs CDDP remains unclear.

Pretreatment of rats with the antioxidant DPPD resulted in a highly marked decrease in Fe^{2+}/ascorbic acid-stimulated lipid peroxidation. The increased vulnerability of renal microsomes isolated from CDDP-treated rats as compared with those from control rats could be no longer detected. Pretreatment with DPPD prior to the injection of CDDP prevented the weight loss and attenuated the increase in blood urea nitrogen concentration caused by CDDP [15]. DPPD does not alter the uptake of CDDP in rat renal cortical slices in vitro [21]. The clinical use of antioxidants might help to reduce CDDP-induced renal toxicity and, hence, to enhance the tolerability of CDDP.

Previous in vitro results indicated a CDDP-induced decrease in gluconeogenesis in renal cortical slices, which could not be altered by DPPD [19]. The results of the present study suggest that direct inhibition of glucose-6-phosphatase might mediate this CDDP-induced decrease in gluconeogenesis. Similarly, the CDDP-induced time- and concentration-dependent decrease in glucose-6-phosphatase activity could not be influenced by the antioxidant DPPD [21]. The question as to whether or not the effect of CDDP on glucose-6-phosphatase and gluconeogenesis plays a major role in CDDP-induced nephrotoxicity in vivo remains open. As observed in the present study, CDDP hydrolysis products but not CDDP itself significantly reduced microsome-specific glucose-6-phosphatase activity in vivo. In vitro, both hepatic and renal microsomal glucose-6-phosphatase levels were affected by CDDP, and DPPD exerted no protective effect on this activity. CDDP is known to be nephrotoxic but not hepatotoxic, except for occasional, transient and mild elevations in liver function tests [42]. This might be caused by differences in the accumulation of CDDP in organs and in subcellular fractions in vivo [7]. After CDDP treatment, kidneys retained a higher concentration of CDDP than did livers; furthermore, the affinity of CDDP was higher for renal microsomes than for hepatic microsomes [7].

In conclusion, renal microsomes may be primary target organelles for lipid peroxidation induced by CDDP and CDDP hydrolysis products on a subcellular level. The role of iron in CDDP-induced lipid peroxidation and nephrotoxicity should be addressed in further investigations.

Acknowledgements. The authors thank Ms. G. Hackländer, Ms. M. Kunkel, Ms. T. Kock and Ms. S. Steinberg for their excellent technical assistance and Ms. A. Hasse for her help in preparing the manuscript. This work was presented in part during the Fourth International Nephrotoxicity Symposium at the University of Surrey in Guildford, England [22].

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