J. Pharm. Pharmacol. 2000, 52: 1483–1490
Received March 24, 2000
Accepted July 26, 2000

Effect of Buthionine Sulphoximine, Glutathione and Methimazole on the Renal Disposition of Cisplatin and on Cisplatin-induced Nephrotoxicity in Rats: Pharmacokinetic–Toxicodynamic Analysis

KAZUHIKO HANADA, YUJI MUKASA, YUKO NOMIZO AND HIROYASU OGATA

Department of Biopharmaceutics, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose, Tokyo 204-8588, Japan

Abstract

The aim of this study was to classify the protective mechanisms of DL-buthionine-(*S*,-*R*)-sulphoximine, glutathione and methimazole on cisplatin-induced nephrotoxicity in rats. An E_{max} model was used to study the effect of these compounds on the pharmacokinetics of cisplatin, especially renal handling and intra-renal biotransformation.

Cisplatin (5 mg kg^{-1}) was administered as an intravenous bolus to rats treated with either 0·9% NaCl (control), buthionine sulphoximine, glutathione or methimazole. The blood urea nitrogen level was monitored to estimate cisplatin-induced nephrotoxicity. To estimate renal handling of cisplatin, cisplatin was infused intravenously to rats treated with 0·9% NaCl, buthionine sulphoximine, glutathione or methimazole. The concentrations of unchanged cisplatin in plasma, urine and kidney were determined by a post-column derivatization HPLC method. The relationship between the pharmacokinetics and toxicodynamics of cisplatin was analysed using a sigmoid E_{max} model.

All compounds studied ameliorated significantly the nephrotoxicity of cisplatin. The renal accumulation of cisplatin was reduced significantly by pretreatment with buthionine sulphoximine but not by either glutathione or methimazole. Although glutathione treatment did not affect the renal accumulation of cisplatin, it significantly decreased the binding of cisplatin to the intrarenal organelle and the decreased binding was well correlated to the decrease of the blood urea nitrogen level.

In summary, pharmacokinetic–toxicodynamic analysis will be useful for classifying the protective mechanism of cisplatin-induced nephrotoxicity.

Cisplatin (*cis*-diamminedichloro-platinum (II)) is one of the most potent agents for chemotherapy for various types of cancer. Cisplatin, however, causes several adverse effects, in particular severe nephrotoxicity is a dose-limiting factor (Deconti et al 1973).

Cisplatin undergoes ligand exchange reactions, which are virtually irreversible (Daley-Yates & McBrien 1984; Nagai et al 1996). In biological fluids, cisplatin is biotransformed through binding to low-molecular-mass substances (such as glutathione, methionine and cysteine) and to high-molecular-mass substances (such as albumin and nucleotides), and the resulting metabolites are called "mobile" and "fixed" metabolites, respectively (Farris et al 1985, 1988). Many investigators have studied the pharmacokinetics of cisplatin by measuring either total or filtered platinum (including cisplatin and its mobile metabolites) concentration. However, it is difficult to estimate exactly the pharmacokinetic–pharmacodynamics based on the mixed concentrations of these platinum species because the pharmacokinetics of these platinum species are different.

Nagai et al (1996) reported a relationship between nephrotoxicity and the disposition of cisplatin in cancer patients, suggesting that the nephrotoxicity induced by cisplatin was caused mainly by unchanged cisplatin. They also performed a quantitative pharmacokinetic–toxicodynamic analysis of cisplatin in rats (Nagai &

Correspondence: H. Ogata, Department of Biopharmaceutics, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose, Tokyo 204-8588, Japan.
E-Mail: hirogat@my-pharm.ac.jp

Ogata 1997) and, using a sigmoid E_{max} model, established a relationship between the nephrotoxicity induced by cisplatin (as estimated by blood urea nitrogen (BUN) level) and the area under the above-threshold cisplatin concentration of the plasma–time curve. They applied this model to the effect of sodium thiosulphate on the relationship between the pharmacokinetics and toxicodynamics of cisplatin (Nagai et al 1995). When cisplatin and sodium thiosulphate were administered concomitantly, the nephrotoxicity estimated by blood urea nitrogen was ameliorated. In this situation, the plasma filtered platinum (cisplatin plus mobile metabolites) concentration changed little, but the plasma cisplatin concentration decreased dramatically, because cisplatin bound strongly to the sodium thiosulphate. The result suggested that it was important to separate the platinum species into unchanged cisplatin and mobile metabolites, and the effect of sodium thiosulphate was associated to the alteration of the pharmacokinetics of cisplatin.

We recently extended this relationship to the area under the curve of cisplatin concentration in the kidney versus time (AUC_k) (Hanada et al 1999). This showed that the renal uptake of cisplatin was inhibited by the basic drugs disopyramide and verapamil unless there was a significant change in the pharmacokinetics of cisplatin in plasma, and this inhibition resulted in the amelioration of nephrotoxicity. In this way, pharmacokinetic–pharmacodynamic models can be used to identify whether the protective mechanism is related to pharmacokinetics and/or pharmacodynamics (or toxicodynamics).

Glutathione, a major non-protein thiol in mammalian cells, has many cellular functions, such as detoxification of exogenous substances, antioxidant properties and regulation of various enzyme activities (Meister 1994). The effect of glutathione inhibitors on cisplatin-induced nephrotoxicity is controversial. Mayer et al (1987) reported that after pretreatment of rats with DL-buthionine-(*S*,-*R*)-sulphoximine, a specific inhibitor of γ-glutamylcysteinylsynthetase, nephrotoxicity induced by cisplatin was reduced without reducing its antitumour activity. In contrast, significant increases of nephrotoxicity were observed after pretreatment with diethylmaleate, a non-specific glutathione inhibitor, or after pretreatment with diethylmaleate or buthionine sulphoximine in rats (Litterst et al 1985; Gemba et al 1992) and mice (Ishikawa et al 1990), respectively. The concomitant administration of glutathione reduced the nephrotoxicity of cisplatin, which was attributed to the antioxidative properties of glutathione (Zunino et al 1983, 1989). Similar results were obtained by administering methimazole, which also has antioxidant properties (Sausen et al 1992; Vail et al 1993).

Although many investigators have focused on the biochemical mechanisms by which these compounds reduce nephrotoxicity, it is possible that pharmacokinetic factors also contribute to this effect because these compounds have a sulphydryl group in their structure. Furthermore, the effects of these compounds on the pharmacokinetics of cisplatin cannot apparently be detected if only total platinum concentration is monitored, and little attention has been paid to this possibility.

Therefore, we have studied the effect of buthionine sulphoximine, glutathione and methimazole on the pharmacokinetic–toxicodynamic relationship with cisplatin, especially renal handling and intra-renal biotransformation, to clarify whether the protective mechanism results from pharmacokinetic or toxicodynamic interactions.

Materials and Methods

Materials

All reagents and chemicals used were of analytical grade except where stated otherwise. Cisplatin was a kind gift from Nippon Kayaku Co. (Tokyo, Japan). Buthionine sulphoximine, glutathione, methimazole and acivicin were obtained from Sigma Chemical Co. (St Louis, MO). The blood urea nitrogen assay kit was obtained from Wako Pure Chemical Industries (Tokyo, Japan). Cisplatin was dissolved at $1\,mg\,mL^{-1}$ in 0·9% (w/v) NaCl just before use. Buthionine sulphoximine was dissolved in 1 M HCl and subsequently readjusted to pH 7·0 with 1 M NaOH to obtain a 400 mM solution of buthionine sulphoximine (Mayer et al 1987).

Male Wistar rats (250–290 g) were maintained in a controlled environment on a standard laboratory pellet diet with water freely available. The animals were fasted for 18 h before the experiments, but were allowed water freely.

Effect of buthionine sulphoximine, glutathione and methimazole on cisplatin-induced nephrotoxicity

Rats were anaesthetized by intraperitoneal injection of sodium pentobarbital ($50\,mg\,kg^{-1}$). For the buthionine sulphoximine-treatment study, buthionine sulphoximine ($4\,mmol\,kg^{-1}$) was administered subcutaneously to rats 4 h before pentobarbital administration. Glutathione or methimazole were infused intravenously at a constant rate (18·3 or $2{\cdot}15\,mmol\,min^{-1}\,kg^{-1}$, respectively) for 1 h before and after cisplatin administration.

Cisplatin was administered as a bolus via the jugular vein at a dose of 2·5, 3·5, 4, 5 or 10 mg kg^{-1}. After the rats regained consciousness, they were maintained on a standard laboratory pellet diet with water freely available in a temperature-controlled environment.

Blood samples (0·3 mL) were taken from another jugular vein just before and on days 1, 3, 5 and 7 after administration of cisplatin, and were centrifuged at 1000 *g* for 5 min. The plasma was stored at −20°C until analysis of blood urea nitrogen.

Effect of buthionine sulphoximine, glutathione and methimazole on the renal accumulation of cisplatin

Rats were treated with buthionine sulphoximine, glutathione or methimazole as described above. At 3, 5, 15, 30 and 60 min after cisplatin administration, the rats were killed and ice-cold 0·9% (w/v) NaCl was injected into the heart to remove the blood from the other organs. The kidneys and liver were excised quickly, blotted on filter paper, weighed and homogenized with a fivefold volume of 0·9% (w/v) NaCl. The tissue homogenate was ultracentrifuged at 105 000 *g* for 65 min at 4°C (Beckman L8-60M). Blood samples were centrifuged at 1000 *g* for 5 min at 4°C, and the plasma and tissue supernatant were ultrafiltered at 4000 *g* for 30 min at 4°C using a membrane filter (Millipore filter UFC3GC, molecular weight cut-off 10 000). These samples were stored at −20°C until analysis. The cisplatin concentration was determined within three days.

Effect of buthionine sulphoximine, glutathione and methimazole on the renal disposition and metabolism of cisplatin

Rats were anaesthetized by intraperitoneal injection of sodium pentobarbital (50 mg kg^{-1}). Polyethylene cannulae (0·28 mm i.d. × 0·61 mm o.d.) were implanted into the femoral vein and artery of one leg and into the ureters. For buthionine sulphoximine treatment, the rats were pretreated with buthionine sulphoximine as described above. NaCl (0·9%, w/v), glutathione or methimazole solutions containing 0·5% inulin were infused throughout the experimental periods via the jugular vein at a constant rate of 0·037 mL min^{-1}, 18·3 or 21·5 mmol min^{-1} kg^{-1}, respectively. At 60 min after starting the infusion, cisplatin was infused at a constant rate of 1·28 μmol min^{-1} kg^{-1} through the femoral venous cannula. Urine was collected at 90–120, 120–150, and 150–180 min after starting the cisplatin infusion, and blood samples were taken from the femoral artery at the midpoint of the urine sampling periods. Rats were killed 180 min after the start of the cisplatin infusion, and the excised kidneys were treated as described above.

In-vitro degradation of cisplatin in urine

The rats were treated as described above but cisplatin was not administered. Urine samples were collected 90–180 min after the start of infusion of 0·9% NaCl, glutathione or methimazole. A small volume (50 μL) of cisplatin (2 mg mL^{-1} in 0·9% NaCl) was added to 450 μL of the urine sample collected and the mixture was incubated at 37°C. The concentration of unchanged cisplatin was determined at 0, 1, 2, 4, 6 and 8 h.

Cisplatin uptake by rat renal cortical slices

Kidney cortical slices were obtained from male Wistar rats (200–250 g) using a Stadie-Riggs microtome as described by Harada et al (1998). The slices (thickness 0·3–0·4 mm, weight 20–40 mg) were incubated at 37°C and/or at 4°C for 60 min in an incubation medium consisting of (in mM): 120 NaCl, 16·2 KCl, 1 $CaCl_2$, 1·2 $MgSO_4$ and 0·1% inulin buffered with 10 mM phosphate buffer (pH 7·5).

Cisplatin uptake into the renal cortical slices was studied under three conditions. Renal cortical slices were obtained from rats pretreated with 0·9% (w/v) NaCl (5 mL kg^{-1}, control) or buthionine sulphoximine (4 mmol kg^{-1}) as described above. After preincubation of the cortical slices in an incubation medium with or without buthionine sulphoximine (8 mM), glutathione (0·5 mM) or methimazole (0·5 mM) for 5 min, cisplatin was added to the incubation medium (final cisplatin concentration was 50 μM) and the incubation was continued at 37 or 4°C. After 60-min incubation, the slices were placed on ice, blotted on filter paper, weighed and homogenized with a 20-fold volume of 0·05% Triton X-100 solution. Atomic platinum and inulin (as a marker of extra-cellular fluid) in the incubation medium and slices were determined.

Assay procedures

Non-protein sulphydryl in tissues was determined by the method of Levi et al (1980). Inulin concentrations were determined by the method of White & Samson (1954). The concentrations of unchanged cisplatin in filtrates were determined by post-column derivatization HPLC method (Hanada et al 1995). Atomic platinum concentrations in tissue homogenates and supernatants (cytosol

fraction) were determined by atomic absorption spectrophotometry (Hitachi Z-9000, Japan).

Data analysis

All data are presented as mean ± s.d. Platinum in the tissues was expressed as micrograms of platinum/gram wet weight of tissue (μg Pt (g tissue)$^{-1}$). The cisplatin concentration in the kidney was corrected by a factor of 0·7 because 30% of unchanged cisplatin is lost during ultracentrifugation (Hanada et al 1995). The area under the curve of concentration of unchanged cisplatin in the plasma or kidney versus time ($AUC_{p\infty}$ or $AUC_{k\infty}$, respectively), total systemic clearance (CL_t) and renal clearance (CL_r) values were calculated as follows:

$$AUC_{p\infty} = AUC_{p_{0-tl}} + C_{pt_l}/k_{ep}$$

$$AUC_{k\infty} = AUC_{k_{0-tl}} + C_{kt_l}/k_{ek}$$

$$CL_t = \text{infusion rate}/Cp_{ss}$$

$$CL_r = \text{urinary excretion rate}/Cp_{ss}$$

where $AUC_{p_{0-tl}}$ and $AUC_{k_{0-tl}}$ are the areas under the curves of concentrations in plasma and kidney versus time from time zero to the final sampling time (t_l), respectively, as calculated by the trapezoidal rule; C_{pt_l} and C_{ktl} are the concentrations in plasma and kidney, respectively at t_l; k_{ep} and k_{ek} are the terminal elimination rate constants from plasma and kidney, respectively; and Cp_{ss} is the plasma cisplatin concentration at steady state. The concentrations of platinum metabolites in renal cytosol and organelles were calculated as follows:

platinum metabolites concentration in cytosol = total platinum concentration in cytosol − concentration of unchanged cisplatin

platinum metabolites concentration in organelles = total platinum concentration in homogenate − total platinum concentration in cytosol

The area under the curve of concentration of platinum metabolites in renal organelles versus time from time zero to the final sampling time (AUC_o) was calculated by the trapezoidal rule.

The degradation of cisplatin in urine was analysed as an apparent first-order process and the rate constant was calculated by a linear regression technique using WinNonlin (Pharmacyt, Scientific Consulting, NC).

Cisplatin uptake by rat renal cortical slices was expressed as concentration ratio of slice to medium (S/M) for platinum, corrected for the extravesicular space.

The relationships between AUC and maximum blood urea nitrogen level were analysed with a sigmoid E_{max} model. A non-linear least-squares regression technique was applied using the NLS program (Sugiyama et al 1987):

$$\text{BUN level (mg dL}^{-1}) = BUN_{base} + (BUN_{max}AUC^{\gamma}/(AUC50^{\gamma} + AUC^{\gamma}))$$

where BUN_{base} and BUN_{max} are the baseline and maximum blood urea nitrogen levels, respectively, and AUC50 and γ are 50% of the AUC for the maximum blood urea nitrogen level and the slope factor, respectively.

Statistical analysis

The statistical analysis was performed using one-way analysis of variance. Dunnett's test was applied if a significant difference among means was detected, and differences were considered to be statistically significant when $P < 0{\cdot}05$.

Results

Effect of buthionine sulphoximine, glutathione and methimazole on cisplatin-induced nephrotoxicity in rats

Four hours after buthionine sulphoximine administration glutathione concentration, estimated as non-protein thiol, in the kidney and liver was decreased by 55 and 53% of the control value, respectively (kidney: 0·56 ± 0·08 vs 0·31 ± 0·05 mg (g tissue)$^{-1}$; liver: 1·14 ± 0·17 vs 0·61 ± 0·06 mg (g tissue)$^{-1}$).

Figure 1 shows the effect of buthionine sulphoximine, glutathione and methimazole on cisplatin-induced nephrotoxicity as estimated by blood urea nitrogen. Blood urea nitrogen levels were changed little by treatment with buthionine sulphoximine, glutathione or methimazole alone (data not shown), indicating that these compounds themselves did not influence renal function at the doses studied. Blood urea nitrogen was increased significantly at 3 and 5 days after cisplatin administration. When the animals were pretreated with buthionine sulphoximine, blood urea nitrogen levels at 3 and 5 days after cisplatin administration were suppressed significantly compared with cisplatin (5 mg kg^{-1}) alone. These results suggested that buthionine sulphoximine treatment could

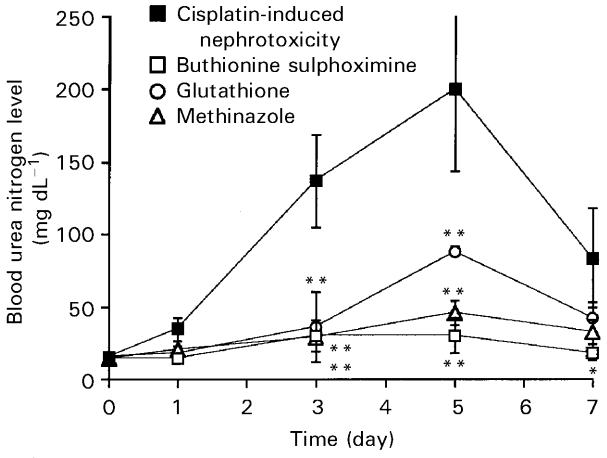

Figure 1. Effects of buthionine sulphoximine, glutathione and methimazole on cisplatin-induced nephrotoxicity in rats. Each point represents the mean ± s.d. (n = 4). $^{**}P < 0.01$ compared with cisplatin.

ameliorate cisplatin-induced nephrotoxicity, as estimated by blood urea nitrogen level, in rats. Plasma creatinine levels were also decreased in the same manner as those of blood urea nitrogen (data not shown). Similarly, the increase of blood urea nitrogen levels was also suppressed by co-administration of either glutathione or methimazole when compared with cisplatin alone. These results are consistent with those reported previously (Zunino et al 1983, 1989; Mayer et al 1987; Sausen et al 1992; Yoon et al 1993).

Effects of buthionine sulphoximine, glutathione and methimazole on the pharmacokinetics of cisplatin

None of the compounds studied influenced the CL_t of cisplatin (Table 1), but CL_r was decreased significantly by pretreatment with buthionine sulphoximine and infusion of glutathione without any significant changes in glomerular filtration rate (GFR). CL_r/GFR (clearance ratio) was significantly decreased to unity by either buthionine sulphoximine or glutathione. The apparent kidney-to-plasma cisplatin concentration ratio (K_{papp}) was significantly decreased by pretreatment with buthionine sulphoximine only.

These drugs have a chemical structure containing a sulphur atom, and if cisplatin binds chemically to these drugs in biological fluids, especially in urine, it is possible that CL_r may be underestimated. Therefore, we determined the degradation rate constants of cisplatin in urine obtained from rats treated with 0·9% NaCl, buthionine sulphoximine, glutathione or methimazole. The apparent degradation rate constants (K_{deg}) of cisplatin in urine obtained from rats treated with 0·9% NaCl or buthionine sulphoximine were $0{\cdot}052 \pm 0{\cdot}009$ and $0{\cdot}060 \pm 0{\cdot}006\ h^{-1}$, respectively. The K_{deg} values of cisplatin in urine obtained from rats treated with glutathione or methimazole ($0{\cdot}249 \pm 0{\cdot}018$ and $0{\cdot}187 \pm 0{\cdot}011\ h^{-1}$) were significantly larger ($P < 0{\cdot}005$) compared with the 0·9% NaCl-treated group.

Effects of buthionine sulphoximine, glutathione and methimazole on cisplatin uptake into rat renal cortical slices

We studied the effect of buthionine sulphoximine on cisplatin uptake into the kidney using renal cortical slices to elucidate whether the protective mechanism of buthionine sulphoximine pretreatment resulted from inhibition of cisplatin uptake into the kidney or from other mechanisms.

Cisplatin uptake (S/M ratio) at 37°C by renal cortical slices from a buthionine sulphoximine-pretreated rat was decreased significantly ($1{\cdot}25 \pm 0{\cdot}04$) compared with slices from 0·9% NaCl-pretreated rats ($1{\cdot}79 \pm 0{\cdot}10$). Uptake of cisplatin into slices from untreated rats was not inhibited significantly when the slices were incubated with buthionine sulphoximine, glutathione or methimazole (Figure 2).

Analysis of the protective mechanisms by the sigmoid E_{max} model

Blood urea nitrogen levels at 5 days after cisplatin administration were plotted against $AUC_{p\infty}$ and

Table 1. Effect of buthionine sulphoximine, glutathione and methimazole on the pharmacokinetics and renal accumulation of cisplatin.

	CL_t (mL min^{-1} kg^{-1})	CL_r (mL min^{-1} kg^{-1})	CL_r/GFR	K_{papp}[a]
Control	21·0 ± 1·5	7·87 ± 2·24	1·32 ± 0·04	3·52 ± 0·85
Buthionine sulphoximine	21·7 ± 1·9	4·50 ± 1·09*	1·03 ± 0·16*	2·35 ± 0·42*
Glutathione	21·1 ± 3·4	4·42 ± 1·33*	0·61 ± 0·20*	3·90 ± 1·31
Methimazole	20·3 ± 4·6	5·44 ± 1·33	0·91 ± 0·45	2·98 ± 0·35

[a]K_{papp}, kidney-to-plasma cisplatin concentration ratio. Each value represents the mean ± s.d. (n = 4). *Significantly different from cisplatin ($P < 0{\cdot}05$).

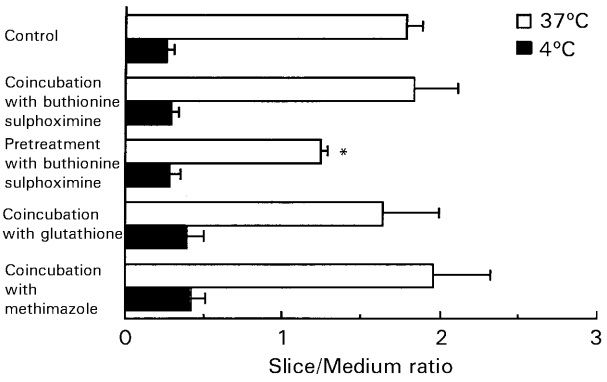

Figure 2. Effects of pretreatment with buthionine sulphoximine or incubation with buthionine sulphoximine on the cisplatin uptake into rat renal cortical slices. The slices were prepared from rats that had been pretreated with subcutaneous buthionine sulphoximine (4 mmol kg^{-1}) or 0·9% (w/v) NaCl. The slices were pre-incubated with or without 8 mM buthionine sulphoximine for 15 min, 75 μL 3·33 mM cisplatin was added (initial cisplatin concentration was 50 μM) and the reaction was incubated for 60 min at 37 and 4°C. Each column represents mean ± s.d. (n = 4). *$P < 0.01$ compared with cisplatin.

$AUC_{k\infty}$ (Figure 3) to clarify the protective mechanisms of buthionine sulphoximine, glutathione and methimazole. The resulting data after treatment with buthionine sulphoximine, glutathione or methimazole were shifted from a curve obtained from total plasma cisplatin alone, i.e. AUC_p. When AUC values from the curve of intact cisplatin concentration in the kidney versus time (AUC_k) were related to blood urea nitrogen levels, the data obtained after pretreatment with buthionine sulphoximine were almost on the curve. Furthermore, when AUC values determined from the curve of organelle platinum concentration in the kidney vs time (AUC_o) were plotted against blood urea nitrogen levels at 5 days after cisplatin administration, the data sets obtained from buthionine sulphoximine pretreatment and glutathione co-administration were on the curve.

Discussion

Glutathione and methimazole were administered by constant infusion because the drug-drug interactions may be more readily detectable. All compounds studied significantly suppressed the increase of the blood urea nitrogen level induced by cisplatin administration.

A pharmacokinetic and toxicodynamic model reported previously was applied to clarify whether the protective mechanisms of these compounds were related to pharmacokinetics and/or toxicodynamics in rats. The blood urea nitrogen level 5 days after cisplatin administration was not related to the $AUC_{p\infty}$ of intact cisplatin in plasma after co-administration of any of the compounds studied. An in-vivo renal clearance study also showed that systemic clearance of intact cisplatin was not affected by the compounds. In animals pretreated with buthionine sulphoximine, the blood urea nitrogen level fitted well to the $AUC_{k\infty}$ of intact cisplatin in the kidney using a sigmoid E_{max} model. The $AUC_{k\infty}$ in rats treated with buthionine sulphoximine was decreased by 41% of control (cisplatin 5 mg kg^{-1} alone), and the blood urea nitrogen level 5 days after cisplatin administration

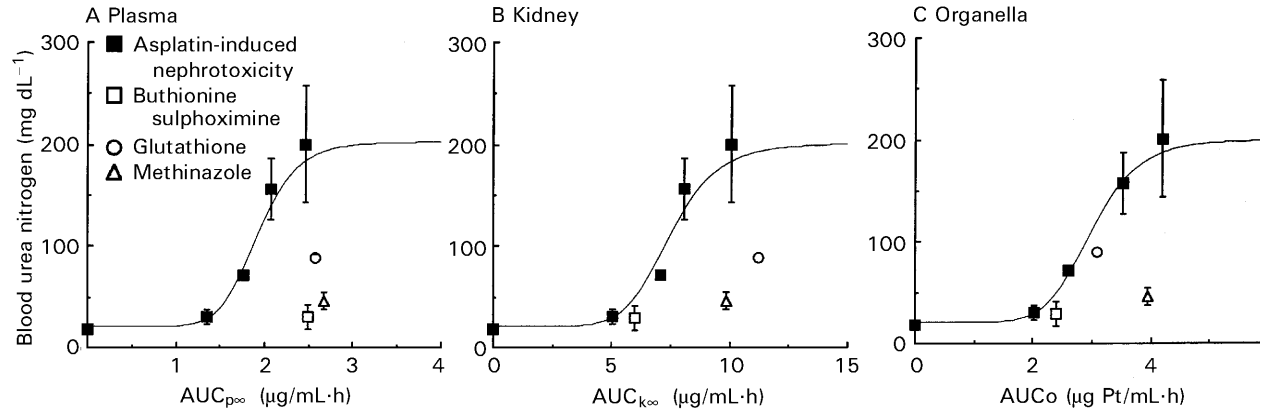

Figure 3. Effects of buthionine sulphoximine, glutathione and methimazole on the relationships between blood urea nitrogen level and area-under the curve of cisplatin concentration in plasma and kidney vs time in rats. The lines show computer simulations following the equation:

$$\text{Plasma, BUN (mg dL}^{-1}) = 20 \cdot 0 + ((202\ AUC_p^{8 \cdot 85})/(1 \cdot 93^{8 \cdot 85} + AUC_p^{8 \cdot 85}))$$

$$\text{Kidney, BUN (mg dL}^{-1}) = 20 \cdot 4 + ((197\ AUC_k^{7 \cdot 73})/(7 \cdot 55^{7 \cdot 73} + AUC_k^{7 \cdot 73}))$$

$$\text{Organella, BUN (mg dL}^{-1}) = 19 \cdot 6 + ((201\ AUC_o^{7 \cdot 22})/(3 \cdot 12^{7 \cdot 22} + AUC_o^{7 \cdot 22}))$$

was decreased by 60% of control. These results suggested that the protective mechanism of buthionine sulphoximine against cisplatin-induced nephrotoxicity might be due to a decrease in the renal accumulation of cisplatin. When cisplatin was co-administered with glutathione, the blood urea nitrogen level 5 days after cisplatin administration was related to AUC_o, but not to $AUC_{p\infty}$ or $AUC_{k\infty}$. This suggested that the platinum bound to the organelle fraction was the more direct function of cisplatin-induced nephrotoxicity. If the relationship between free and bound cisplatin was not changed by co-administered compounds, $AUC_{k\infty}$ could be reasonably substituted by AUC_o. This seemed to be the case for buthionine sulphoximine pretreatment. However, for glutathione co-administration it could be speculated that the binding of intact cisplatin to cellular organelles might be decreased by glutathione. This hypothesis was supported by the fact that the concentration of non-protein sulphydryl groups in the kidney was significantly increased after administration of glutathione (Zunino et al 1989).

The nephrotoxicity of cisplatin was reduced also when methimazole was administered concomitantly. However, for methimazole the AUC_o of platinum was not related to the blood urea nitrogen level 5 days after cisplatin administration, suggesting that methimazole reduced the nephrotoxicity of cisplatin via toxicodynamic rather than pharmacokinetic mechanisms. Administering methimazole up to 4 h after cisplatin administration ameliorated the nephrotoxicity of cisplatin (Sausen et al 1992). Again, this suggested that methimazole affected the toxicodynamics rather than pharmacokinetics of cisplatin because the half-life of cisplatin is very short (approximately 14 min). These results suggested that the effects of the tested compounds on the pharmacokinetics and toxicodynamics of cisplatin could be analysed by the modified sigmoid E_{max} model based on the target concentration in the kidney.

The clearance ratio and K_{papp} of cisplatin were significantly decreased by pretreatment with buthionine sulphoximine, suggesting that uptake of cisplatin into the kidney via the basolateral membrane may be inhibited by buthionine sulphoximine. It is known that cisplatin uptake into rat renal cortical slices is temperature-dependent and the mechanism of cisplatin uptake by renal cortical slices is thought to involve partly the organic cation transport systems (Safirstein et al 1984; Harada et al 1998). However, cisplatin was not transported by the proton/cation exchange transport system in brush-border membranes (our unpublished data). The uptake of cisplatin into kidney slices was inhibited by pretreatment of the animals with buthionine sulphoximine but not by co-incubation of the slices with buthionine sulphoximine. Buthionine sulphoximine treatment may have decreased the transport activity of the organic cation transport system because buthionine sulphoximine reduced the cellular concentration of the sulphydryl groups, which are essential for this transport system in the kidney (Sokol et al 1986).

Although the CL_r of cisplatin was significantly decreased when co-administered with either glutathione or methimazole, these decreases may be artifactual. Cisplatin was significantly degraded in urine obtained from rats after infusion of either glutathione or methimazole. Consequently, the cisplatin that had undergone glomerular filtration and/or active secretion was degraded in the urine, resulting in the apparently low CL_r. Intact glutathione undergoes glomerular filtration but is hydrolysed rapidly by the tubular luminal transpeptidase and aminopeptidase, and the catabolized cysteine is absorbed into the tubular cell from the luminal side (Meister 1994).

The stability of cisplatin in plasma and kidney must also be considered. In plasma, the concentration of cisplatin was changed little in the presence of glutathione or methimazole (data not shown). On the other hand, the uptake of cisplatin into the renal slices was not significantly inhibited by either glutathione or methimazole. The K_{papp} value after co-administration of either glutathione or methimazole was also unchanged. This value is a function of both the rate of uptake into, and the rate of elimination from the kidney. The absence of any effect of glutathione on K_{papp} may be due to the fact that the rate of elimination from the kidney was apparently unchanged, although the rate of formation of mobile cisplatin metabolites was increased.

In summary, cisplatin-induced nephrotoxicity in rats was ameliorated by buthionine sulphoximine, glutathione and methimazole. In renal tissue buthionine sulphoximine may decrease the uptake of cisplatin, whilst glutathione may decrease the binding of cisplatin to organelles. We suggest that these are the principal mechanisms of amelioration of cisplatin toxicity by these compounds and pharmacokinetic–toxicodynamic analysis will be useful for classifying the protective mechanism of cisplatin-induced nephrotoxicity.

References

Daley-Yates, P. T., McBrien, D. C. H. (1984) Cisplatin Metabolites in plasma, a study of their pharmacokinetics

and importance in the nephrotoxic and antitumour activity of cisplatin. Biochem. Pharmacol. 33: 3063–3070

Deconti, R. C., Toftness, B. R., Lange, R. C., Creasey, W. A. (1973) Clinical and pharmacological studies with *cis*-diamminedichloroplatinum (II). Cancer Res. 33: 1310–1315

Farris, F. F., Dedrick, R. L., King, F. G. (1988) Cisplatin pharmacokinetics: applications of a physiological model. Toxicology Letters 43: 117–137

Farris, F. F., King, F. G., Dedrick, R. L., Litterst, C. L. (1985) Physiological model for the pharmacokinetics of cis-dichlorodiammineplatinum (II) (DDP) in the tumored rat J. Pharmacokinet Biopharm. 13: 13–40

Gemba, M., Yamaguchi, T., Kambara, K., Suzuki, A., Kawai, Y. (1992) Increases in urinary enzyme excretion in rats depleted of glutathione inhibited by scavenger of oxygen free radicals. J. Pharmacobio-Dyn. 15: 513–518

Hanada, K., Nagai, N., Ogata, H. (1995) Quantitative determination of unchanged cisplatin in rat kidney and liver by high-performance liquid chromatography. J. Chromatogr. B 663: 181–186

Hanada, K., Odaka, K., Kudo, A., Ogata, H. (1999) Effects of disopyramide and verapamil on renal disposition and nephrotoxicity of cisplatin in rats. Pharm. Res. 16: 1589–1595

Harada, Y., Kumagai, E., Nagai, N., Hanada, K., Ogata, H. (1998) Uptake of cisplatin and its metabolites into rat and rabbit renal cortical slices. Pharm. Pharmacol. Commun. 4: 455–458

Ishikawa, M., Takayanagi, Y., Sasaki, K. (1990) Enhancement of cisplatin toxicity by buthionine sulfoximine, a glutathione-depleting agent, in mice. Res. Commun. Chem. Pathol. Pharmacol. 67: 131–141

Levi, J., Jacobs, C., Kalman, S. M., Mctigue, M., Weiner, M. W. (1980) Mechanism of cis-platinum nephrotoxicity (Effects of sulfhydryl groups in rat kidneys). J. Pharmacol. Exp. Ther. 213: 545–549

Litterst, C. L., Bertolero, F., Uozumi, J. (1985) The role of glutathione and metallothionein in the toxicity and subcellular binding of cisplatin. In: McBrien, D. C. H., Slater, T. F. (eds) Biochemical Mechanisms of Platinum Antitumour drugs. IRL Press, Oxford, pp 227–254

Mayer, R. D., Lee, K., Cockett, T. K. (1987) Inhibition of cisplatin-induced nephrotoxicity in rats by buthionine sulfoximine, a glutathione synthesis inhibitor. Cancer Chemother. Pharmacol. 20: 207–210

Meister, A. (1994) Glutathione, ascorbate, and cellular protection. Cancer Res. 54: 1969s–1975s

Nagai, N., Ogata, H. (1996) The renal clearance of unchanged cisplatin during furosemide and mannitol diuresis is dependent on glomerular filtration rate in rats. J. Pharm. Sci. 85: 720–724

Nagai, N., Ogata, H. (1997) Quantitative relationship between pharmacokinetics of unchanged cisplatin and nephrotoxicity in rats – importance of area under the concentration-time curve (AUC) as the major toxicodynamic determination in vivo. Cancer Chemother. Pharmacol. 40: 11–18

Nagai, N., Hotta, K., Yamamura, H., Ogata, H. (1995) Effects of sodium thiosulfate on the pharmacokinetics of unchanged cisplatin and on the distribution of platinum species in rat kidney: protective mechanism against cisplatin nephrotoxicity. Cancer Chemother. Pharmacol. 36: 404–412

Nagai, N., Kinoshita, M., Ogata, H., Tsujino, O., Wada, Y., Someya, K., Ohno, T., Masuhara, K., Tanaka, Y., Kato, K., Nagai, H., Yokoyama, A., Kurita, Y. (1996) Relationship between pharmacokinetics of unchanged cisplatin and nephrotoxicity after intravenous infusions of cisplatin to cancer patients. Cancer Chemother. Pharmacol. 39: 131–137

Safirstein, R., Miller, P., Guttenplan, J. B. (1984) Uptake and metabolism of cisplatin by rat kidney. Kidney Int. 25: 753–758

Sausen, P. J., Elefarra, A. A., Cooley, A. J. (1992) Methimazole protection of rats against chemically induced kidney damage in vivo. J. Pharmacol. Exp. Ther. 260: 393–401

Sokol, P. P., Holohan, P. D., Ross, C. R. (1986) Essential disulfide and sulfhydryl groups for organic cation transport in renal brush-border membranes. J. Biol. Chem. 261: 3282–3287

Sugiyama, Y., Hanano, M., Sawada, Y. (1987) Program NLS (in Japanese).In: Hanano, M. (ed.) Pharmacokinetics. Nanzando Press, Tokyo, pp 113–127

Vail, D. M., Elfarra, A. A., Cooley, A. J., Panciera, D. L., MacEwen, E. G., Soergel, S. A. (1993) Methimazole as a protectant against cisplatin-induced nephrotoxicity using the dog as a model. Cancer Chemother. Pharmacol. 33: 25–30

White, R. P., Samson, F. E. (1954) Determination of inulin in plasma and urine by use of anthrone. J. Lab. Clin. Med. 43: 475–478

Yoon, E. J., Kim, S. H., Lee, M. G. (1993) Effects of sodium thiosulfate on the pharmacokinetics of total platinum after intravenous administration of cisplatin to rabbits. Int. J. Pharm. 99: 343–349

Zunino, F., Tofanetti, O., Besati, A., Cavalletti, E., Savi, G. (1983) Protective effect of reduced glutathione against cis-dichlorodiammine platinum (II) – induced nephrotoxicity and lethal toxicity. Tumori 69: 105–111

Zunino, F., Pratesi, G., Micheloni, A., Cavalletti, E., Sala, F., Tofanetti, O. (1989) Protective effect of reduced glutathione against cisplatin-induced renal and systemic toxicity and its influence on the therapeutic activity of the antitumor drug. Chem. Biol. Interactions 70: 89–101