Cancer Chemother Pharmacol (1995) 36: 404–410

ORIGINAL ARTICLE

Naomi Nagai · Kazuhiro Hotta
Hidetoshi Yamamura · Hiroyasu Ogata

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

Received: 28 July 1994/Accepted: 30 December 1994

Abstract To investigate the mechanism underlying the protective effect against cisplatin (CDDP) nephrotoxicity of its antidote, sodium thiosulfate (STS), the effects of STS on the pharmacokinetics of unchanged CDDP and on the distribution of unchanged CDDP and high and low molecular mass metabolites (fixed and mobile metabolites) in the kidney 1 min after a bolus injection of CDDP (5 mg/kg) to rats were studied. A decrease in the plasma concentration of unchanged CDDP and an increase in the plasma concentration of mobile metabolites were observed in the rats after the bolus injection of CDDP in combination with STS infusion for 30 min (1200 mg/kg). Although STS accelerated platinum excretion during the first 10 min after CDDP injection, unchanged CDDP was not excreted in the urine in the STS-treated rats. Total kidney platinum 1 min after the bolus injection of CDDP was detected mainly as unchanged CDDP (86% of the total platinum) in the rats given CDDP alone. However, in the STS-treated rats, the total kidney platinum was decreased to 62% of the level in the rats given CDDP alone, and the platinum species detected in the kidney were mainly mobile metabolites. Only 24% of the total kidney platinum was detected as unchanged CDDP in the STS-treated rats. The loss of body weight and increases in BUN and serum creatinine levels usually observed after a bolus injection of CDDP were completely prevented by STS coadministration. The present study provides information about unchanged CDDP pharmacokinetics and the distribution of unchanged CDDP and some of its generic metabolites in the kidney when STS is coadministered as an antidote. These results show that the protective effect of STS against CDDP nephrotoxicity can be attributed to the formation of inactive mobile metabolites by a direct reaction between unchanged CDDP and STS in the systemic circulation, resulting in a reduction in the amount of unchanged CDDP in the kidney.

Key words Unchanged cisplatin · Pharmacokinetics · Sodium thiosulfate

N. Nagai · K. Hotta · H. Yamamura · H. Ogata (✉)
Department of Biopharmaceutics, Meiji College of Pharmacy, Yato-cho 1-22-1, Tanashi-shi, Tokyo 188, Japan

Introduction

Nephrotoxicity caused by cisplatin (CDDP) is a dose-limiting factor in CDDP chemotherapy [18, 20]. Sodium thiosulfate (STS) has been shown to exert a protective effect against CDDP toxicity in vitro and in vivo [1, 9, 12, 15, 24, 29, 31]. "Two-route chemotherapy" (TRC), in which a large amount of anticancer drug is injected locally at the tumor site in combination with its antidote given systemically, has been successfully applied to CDDP chemotherapy, using STS as the antidote, for treating liver [32], bladder [28], metastatic lung [13], limb [19] and peritoneally disseminated tumors [14, 17] in animals. It has also been used in clinical studies [10, 11] without evidence of severe nephrotoxicity. Single intravenous doses of CDDP are usually limited to 100–120 mg/m^2 because of its potential for causing severe nephrotoxicity, but STS allowed two- or threefold increases in the intraperitoneal or intravenous CDDP dose in these clinical trials [10, 11].

The mechanism by which STS protects against CDDP nephrotoxicity has been suggested to be direct inactivation of CDDP by a chemical interaction in the systemic circulation [6, 15, 33]. However, this theory is mainly based on studies of the pharmacokinetics of ultrafiltrable platinum, which includes inactive low molecular mass metabolites in addition to biologically active unchanged CDDP.

Recently, it has become possible to study the pharmacokinetics of unchanged CDDP in detail using a specific assay technique [25, 26]. The nephrotoxicity

of CDDP has now been shown to be more closely related to the pharmacokinetics of unchanged CDDP than to that of ultrafiltrable platinum. The purpose of the present study was to investigate the effects of STS on the pharmacokinetics of unchanged CDDP and the distribution of platinum species in the kidney. The protective mechanism of STS against CDDP nephrotoxicity is also discussed.

Materials and methods

Materials

Cisplatin (Randa) was a gift from Nippon Kayaku Co. (Tokyo, Japan). STS was purchased from Wako Pure Chemical Industries (Osaka, Japan). All other chemicals and reagents were of analytical grade.

Pharmacokinetic studies

CDDP and STS were dissolved in 0.9% w/v sodium chloride solution (1 mg/ml) just before use.

After an overnight fast, male Wistar rats (200–240 g) were anesthetized with ethyl carbamate (1 g/kg). A polyethylene catheter (PE-50) was inserted into the left jugular vein for STS or 0.9% w/v sodium chloride solution infusion, and into the left femoral artery for blood sampling. Two polyethylene catheters (PE-10) were inserted into the right and left ureters for urine sampling. Sodium chloride solution (0.9% w/v) containing inulin (5 mg/ml) was infused at a constant rate of 0.05 ml/min until a constant urine output was obtained. After 0.9% w/v sodium chloride solution containing both STS (200 mg/ml) and inulin (5 mg/ml) had been infused for 20 min, CDDP (5 mg/kg) or 0.9% w/v sodium chloride solution (5 ml/kg) was injected as a bolus via the right femoral vein. The STS infusion was continued for a further 10 min. The rats were sacrified 90 min after CDDP injection and the kidneys were immediately removed.

Blood samples were taken 5, 15 and 30 min before CDDP injection and 1, 5, 15, 30, 50 and 75 min after. Urine samples were collected during the following eight periods: 40–20 min, 20–10 min and 10–0 min before CDDP injection, and 0–10 min, 10–20 min, 20–40 min, 40–60 min and 60–90 min after. The blood samples were immediately centrifuged (1,000 *g*) for 3 min at room temperature. The resulting plasma was immediately subjected to ultrafiltration (4,000 *g*) at 4°C for 30 min using a membrane with a pore size of 10,000 Da molecular mass cut-off (UFC 3GC; Japan Millipore, Tokyo, Japan). Each sample (plasma, plasma ultrafiltrate and urine) was stored below − 20°C until analysis.

Platinum distribution in the kidney

After an overnight fast, five male Wistar rats (220–270 g) were anesthetized with ethyl carbamate (1 g/kg). Infusion of STS and administration of CDDP were carried out according to the procedure described above. Blood was taken 1 min after the CDDP injection (5 mg/kg), and the rat was sacrificed immediately. The kidneys were removed and immersed in ice-cold 0.9% w/v sodium chloride solution. After the capsules had been removed, the kidneys were blotted on filter paper, weighed and homogenized in ice-cold 0.9% w/v sodium chloride solution (1 : 4 w/v). The cytosol fraction and cytosolic ultrafiltrate were prepared according to previously reported procedures [7]. Briefly, the homogenate was ultracentrifuged (100, 000 *g*) at 4°C for 60 min. A 450-µl sample of the supernatant was subjected to ultrafiltration (4,000 *g*) at 4°C for 30 min using the same membrane described above. Each sample (kidney homogenate, cytosol and cytosolic ultrafiltrate) was frozen and stored below − 20°C until analysis.

Determination of CDDP nephrotoxicity

The following four treatments were given to separate groups each of five male Wistar rats (250–300 g). (1) 0.9% w/v sodium chloride solution (5 ml/kg) bolus injection; (2) CDDP (5 mg/kg) bolus injection; (3) STS (1200 mg/kg) infusion for 20 min before and 10 min after 0.9% w/v sodium chloride solution (5 ml/kg) bolus injection; (4) STS (1200 mg/kg) infusion for 20 min before and 10 min after CDDP (5 mg/kg) bolus injection. Blood samples (0.4 ml) were collected before and 1, 2, 3 and 5 days after treatment for monitoring blood urea nitrogen (BUN) and serum creatinine (SCr). At 5 days after treatment, the rats were sacrificed and the kidneys were homogenized with 0.25% Triton X-100 (1: 8 w/v) for platinum analysis.

Analysis

The concentration of unchanged CDDP in plasma ultrafiltrate, urine, and kidney cytosolic ultrafiltrate was determined by high-performance liquid chromatography (HPLC) using post-column derivatization as reported previously [7, 16]. The system consisted of a Shimadzu HPLC system (Kyoto, Japan) in combination with a Hitachi NO. 3013-N analytical column, two HPLC pumps (Shimadzu LC-9A, Kyoto, Japan) and two different sizes of PTFE tube for post-column derivatization. Samples (50 µl) of plasma ultrafiltrate, diluted (1 : 10 v/v) urine or kidney cytosolic ultrafiltrate were injected directly into the analytical column. The column was eluted with acetonitrile/10m*M* sodium chloride (15 : 85 v/v) at a constant flow rate of 0.9 ml/min.

The platinum concentration in the kidney homogenate, kidney cytosol and plasma and kidney cytosolic ultrafiltrates was determined using a Hitachi Model Z-9000 atomic absorption spectrometer (Hitachi, Tokyo, Japan). Analytical conditions were as follows: a 30-s dry stage at a programmed temperature between 80°C and 120°C, a 17-s ash stage at 1300°C, a 15-s atomization stage at 2800°C and a 5-s clean stage at 3000°C. A 20-µl sample was applied to the tube cuvette and the 265.9-nm line was monitored. In the plasma and urine, the concentration of CDDP was evaluated as unchanged CDDP (Upt), ultrafiltrable platinum (Free Pt) and low molecular mass metabolites (mobile metabolites, MM) calculated as follows:

Upt = unchanged CDDP in plasma ultrafiltrate or in urine, determined by the HPLC method (platinum equivalent)
Free Pt = platinum in plasma ultrafiltrate or in urine, determined by atomic absorption spectrometry
MM = Free Pt − Upt

In the kidney, the concentrations of Upt, MM and high molecular mass metabolites (fixed metabolites, FM) were calculated as reported previously [7]:

Upt = unchanged CDDP in cytosolic ultrafiltrate, determined by the HPLC method (platinum equivalent)/0.7
Upt_{loss} = Upt − unchanged CDDP in cytosolic ultrafiltrate, determined by the HPLC method (platinum equivalent)
MM = platinum in cytosolic ultrafiltrate − unchanged CDDP in cytosolic ultrafiltrate, determined by the HPLC method (platinum equivalent)
FM = (platinum in kidney homogenate − platinum in cytosolic ultrafiltrate) − Upt_{loss}

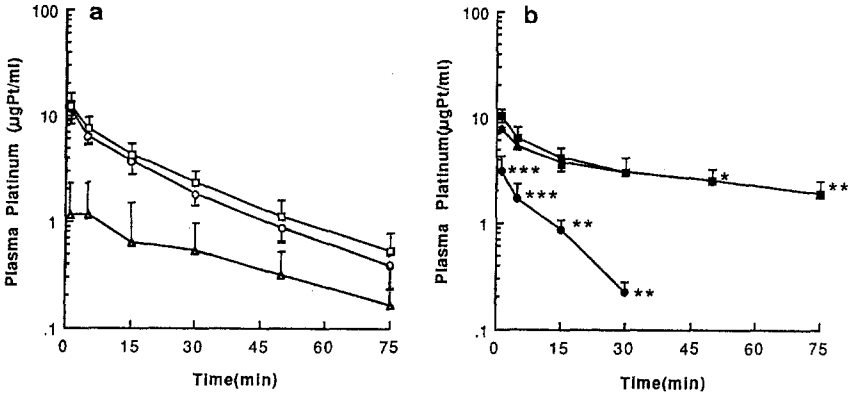

Fig. 1a, b Plasma concentrations of unchanged CDDP, ultrafiltrable platinum and mobile metabolites in rats following a bolus injection of CDDP alone (5 mg/kg) (**a**) or with STS infusion (1200 mg/kg) (**b**). ○, ● unchanged CDDP; □, ■ ultrafiltrable platinum; △, ▲ mobile metabolites. Values are expressed as mean ($n = 5$) ± SD. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. The mobile metabolite concentrations in the STS-treated rats were significantly different from those in the control rats during the study ($P < 0.001$)

where 0.7 is a correction factor representing the recovery of unchanged CDDP from the kidney homogenate, and Upt_{loss} is the concentration of decomposed unchanged CDDP which is transformed to FM [7].

The concentration of STS in plasma and urine was measured by the iodine titration method, as described previously [4, 14]. The inulin concentration was measured spectrophotometrically by the method of Davidson and Sackner [5]. BUN and SCr were measured using a diagnosis kit (Urease Test Wako; Wako Co., Tokyo, Japan) and the Folin-Wu method [3], respectively.

Pharmacokinetic analysis

Pharmacokinetic parameters after the bolus injection of CDDP (5 mg/kg), the area under the plasma concentration-time curve from time zero to infinity (AUC_{inf}), the excretion of unchanged CDDP in the urine from time zero to infinity (amount or percentage of dose, Ae_{inf} or Ae_{inf} (%), total clearance (Cl_t) and renal clearance (Cl_r) were calculated according to a non-compartmental method as follows:

$$AUC_{inf} = AUC_{(0-t_1)} + C_{pt_1}/\beta$$

$$Ae_{inf} = Ae_{(0-t_2)} + (dAe/dt)_{t_2}/\beta_r$$

$$Ae_{inf}\ (\%) = Ae_{inf}/D \cdot 100$$

$$Cl_t = D/AUC_{inf}$$

$$Cl_r = Ae_{inf}/AUC_{inf}$$

where $AUC_{(0-t_1)}$ and $Ae_{(0-t_2)}$ are the AUC and Ae from time zero to the final sampling time (t_1) and to the midpoint of the final sampling interval (t_2): C_{pt_1} and $(dAe/dt)_{t_2}$ are the plasma concentration and the urinary excretion rate of unchanged CDDP at t_1 and t_2, β and β_r are the terminal elimination rate constants of unchanged CDDP in the plasma and urine, and D is the dose of CDDP.

Statistics

All data are expressed as means ± SD. The significance of the differences in the pharmacokinetic parameters and the kidney platinum levels between treatment with CDDP alone and that with CDDP and STS combined were determined using Student's *t*-test. Nephrotoxicity was analysed by one-way ANOVA or the Kruskal-Wallis *H*-test [30]. The multiple range test between treatments was carried out if a significant difference between means was indicated [30]. Differences were considered to be significant for *P*-values < 0.05.

Results

Effect of STS on the pharmacokinetics of unchanged CDDP

The effects of STS on the plasma concentrations of unchanged CDDP, ultrafiltrable platinum and mobile metabolites are shown in Fig. 1. The plasma concentration of STS increased rapidly during STS infusion, the mean concentration being 2.69 ± 0.3 mg/ml at 1 min following CDDP injection (Table 1). Both unchanged CDDP and ultrafiltrable platinum were eliminated bi-exponentially in the rats given CDDP by bolus (control rats). When CDDP was given during STS infusion, the plasma concentration of unchanged CDDP at 1 min was significantly lower (mean 3.09 μgPt/ml) than in the control rats (mean 11.67 μgPt/ml), and could not be detected in plasma at 50 min after CDDP injection (< 26 ngPt/ml, the assay limit with a 50-μl injection). In the rats given CDDP with STS, although the ultrafiltrable platinum level in plasma was almost the same as in the control rats during the first 15 min after CDDP injection, the elimination of ultrafiltrable platinum in the terminal phase was slower than in the control rats.

Figure 2 shows the time-courses of cumulative excretion of platinum in urine (as a percentage of the dose). In the control rats, about 35% of the dose was excreted in the urine over 90 min after the CDDP injection, and this was detected mainly as unchanged CDDP (about 30% of the dose). The excretion of mobile metabolites was one-sixth (about 5% of the dose) of the unchanged CDDP excretion. In contrast, in the STS-treated rats, platinum was excreted more rapidly during the first 10 min, and only mobile metabolites were detected in the urine.

Table 1 shows the pharmacokinetic parameters for unchanged CDDP, ultrafiltrable platinum and mobile metabolites following the bolus injection of CDDP.

Table 1 Pharmacokinetic parameters of platinum species after bolus injection of CDDP (5 mg/kg) to rats with or without STS treatment. Plasma concentration data were analyzed according to the non-compartmental method (C_p plasma concentration, Cl_t total clearance, Cl_r real clearance, AUC_{inf} area under plasma concentration-time curve from zero to infinity, Ae_{inf}(%) cumulative amount percent excreted in urine from zero to infinity). Values are expressed as mean ($n = 5$) ± SD. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

	Without STS	With STS
Unchanged CDDP		
C_p 1 min (μgPt/ml)	11.67 ± 2.3	3.09 ± 1.2***
Cl_t (ml/min/kg)	17.55 ± 4.2	90.08 ± 29.0*
Cl_r (ml/min/kg)	5.83 ± 1.3	0.0
AUC_{inf} (μgPt/ml·min)	195.0 ± 44.5	39.08 ± 12.4**
Ae_{inf}(%)	34.4 ± 9.1	0.0
Ultrafiltrable platinum		
C_p, 1 min(μgPt/ml)	12.53 ± 4.0	10.47 ± 1.4
AUC_{inf}(μgPt/ml·min)	231.1 ± 72.4	448.0 ± 182.3*
Ae_{inf}(%)	40.7 ± 7.6	28.6 ± 5.4*
Mobile metabolites		
C_p, 1 min(μgPt/ml)	1.13 ± 1.4	7.75 ± 0.2***
AUC_{inf}(μgPt/ml·min)	49.6 ± 53.1	433.7 ± 180.7**
Ae_{inf}(%)	7.8 ± 7.7	28.6 ± 5.4**
STS		
C_p, 1 min(mg/ml)		2.69 ± 0.3

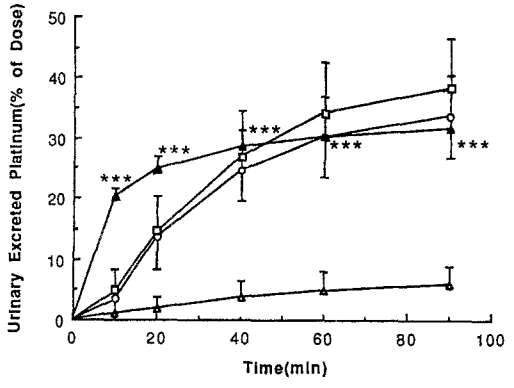

Fig. 2 Cumulative urinary excretion of unchanged CDDP, ultrafiltrable platinum and mobile metabolites in rats following a bolus injection of CDDP alone (5 mg/kg) or with STS infusion (1200 mg/kg). ○ unchanged CDDP; □ ultrafiltrable platinum; △, ▲ mobile metabolites. Values are expressed as mean ($n = 5$) ± SD. $^{***}P < 0.001$

Although the ultrafiltrable platinum plasma concentrations at 1 min in the STS-treated rats were not significantly different from those in the control rats, the platinum species found in the plasma differed between the two groups. More than 90% of the ultrafiltrable platinum was found to be unchanged CDDP in the plasma of the control rats. On the other hand, more than 70% was present as mobile metabolites in the plasma of the STS-treated rats. The total clearance (Cl_t) of unchanged CDDP was 17.55 ± 4.2 ml/min per kg and renal clearance (Cl_r) was 5.83 ± 1.3 ml/min per kg (33.2% of Cl_t) in the control rats. The cumulative excretion of unchanged CDDP in the urine was 34.4 ± 9.1% of the dose in the control rats. In the STS-treated rats, the urinary excretion of platinum was reduced (28.6% of the dose) compared with that in the control rats (40.7% of the dose). Unchanged CDDP was not excreted in urine and Cl_r of unchanged CDDP was zero. All excreted platinum was detected as mobile metabolites in the STS-treated rats.

Figure 3 shows the time courses of the urine flow rate and the glomerular filtration rate (GFR) between 40 min before and 90 min after the CDDP injection. The urine flow rate increased markedly after the start of STS infusion (the second urine collection period), resulting in about 10- to 25-fold higher levels during STS infusion than in the pretreatment period (the first urine collection period). The GFR decreased gradually in the STS-treated rats. No statistically significant differences in urine flow rate and GFR were observed in the STS-treated rats with and without CDDP injection.

Effect of STS on the distribution of platinum species in the kidney

The concentrations of platinum species in the plasma and in the kidney 1 min after the bolus injection of CDDP are shown in Table 2. In the control rats, unchanged CDDP was the main platinum species in the plasma and kidney. On the other hand, the concentrations of unchanged CDDP in the plasma and kidney of the STS-treated rats were approximately one-fifth of those in the control rats. Total kidney platinum in the STS-treated rats (24.90 μgPt/g tissue) decreased to about 60% of the level in the control rats (39.96 μgPt/g tissue). In the control rats, kidney platinum was present mainly (86%) as unchanged CDDP, whereas mobile metabolites were the main kidney platinum species (about 52%) found in the STS-treated rats. The tissue to plasma ratio (T: P ratio) of unchanged CDDP was not significantly different between control and STS-treated rats.

Effect of STS on CDDP nephrotoxicity

Changes in the body weight, BUN and SCr levels of the rats over the 5 days following the bolus injection of CDDP with or without STS treatment are shown in Fig. 4. After the bolus injection of CDDP without STS infusion, rapid increases in BUN and SCr and loss of body weight were observed, representing acute nephrotoxicity induced by CDDP. However, these toxicity markers did not significantly vary from the normal range in the rats given CDDP and STS. The kidney platinum level at 5 days was significantly lower in the rats given CDDP with STS than in those given CDDP alone (Table 2).

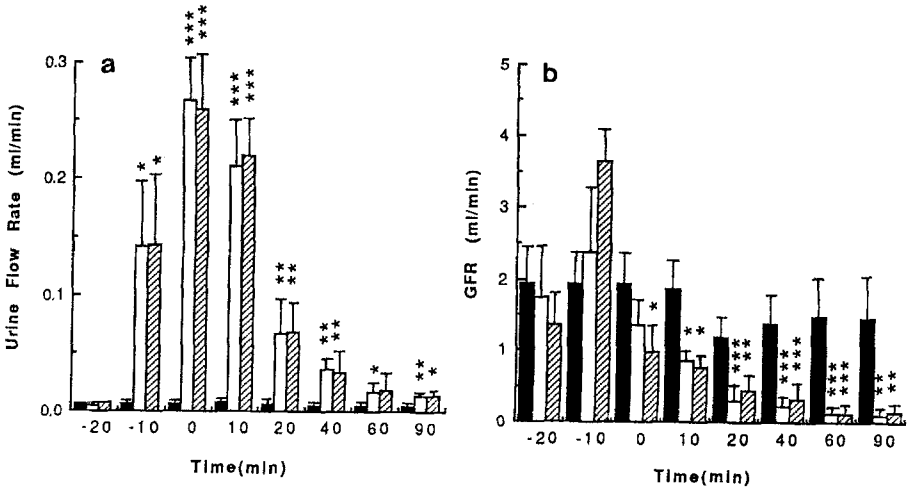

Fig. 3a, b Time courses for urine flow rate (**a**) and glomerular filtration rate (**b**) in rats given CDDP alone (5 mg/kg) or with STS infusion (1200 mg/kg). ■ CDDP bolus injection, $n = 5$; □ CDDP bolus injection with STS infusion, $n = 5$; □ STS infusion alone, $n = 3$. CDDP injection was given at time zero. Values were significantly ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$) different from those in the rats given CDDP alone

Table 2 Plasma and kidney concentrations of platinum species after bolus injection of CDDP (5 mg/kg) to rats with or without STS treatment (*Upt* unchanged CDDP, *Total Pt* kidney platinum, *Fpt* ultrafiltrable platinum, *MM* mobile metabolites, *FM* fixed metabolites, $T:P_{CDDP}$ tissue to plasma ratio of unchanged CDDP 1 min after CDDP administration, $Total\ Pt_{5day}$ kidney platinum 5 days after CDDP administration). Values are expressed as mean ($n = 5$) ± SD. $**P < 0.01$, $***P < 0.001$

	Without STS	With STS
Plasma		
Fpt (μgPt/ml)	13.60 ± 2.3	11.71 ± 1.0
Upt (μgPt/ml)	13.49 ± 1.5	2.71 ± 1.7***
MM (μgPt/ml)	0.22 ± 0.5	9.00 ± 2.0***
Kidney		
Total Pt (μgPt/g tissue)	39.96 ± 5.5	24.90 ± 5.9**
Upt (μgPt/g tissue)	34.41 ± 2.7	5.90 ± 1.5***
MM (μgPt/g tissue)	2.39 ± 0.9	13.07 ± 5.4**
FM (μgPt/g tissue)	3.56 ± 4.5	5.92 ± 1.1
$T:P_{CDDP}$(ml/g tissue)	2.51 ± 0.4	2.25 ± 1.0
Total $Pt_{5\ day}$(μgPt/g tissue)	12.25 ± 3.2	6.90 ± 1.9**

Discussion

STS is known to be a strong nucleophile, and reacts irreversibly with CDDP to form $Pt(S_2O_3)_4$ in plasma [2, 12, 14, 27]. This has been proposed as one of the mechanisms by which STS reduces CDDP nephrotoxicity [6, 15, 33]. The diuretic action of STS [15, 22] has also been suggested as another protective mechanism.

The loss of body weight and increases in BUN and SCr observed in the rats following the bolus injection of CDDP were almost completely prevented by STS coadministration. The mean plasma concentration of unchanged CDDP 1 min following the CDDP injection with STS was reduced to 26.5% of that in the control rats. Although the ultrafiltrable platinum concentration was not significantly different between the two groups, the active platinum level (unchanged CDDP) was markedly reduced by STS coadministration. This, however, was not detected by monitoring only ultrafiltrable platinum determined by atomic absorption spectrometry. Iwamoto et al. reported similar results after measuring plasma con-centrations of active platinum species following administration of CDDP (5 mg/kg) to rabbits using a microbiological assay [15]. Therefore, it is suggested that monitoring of unchanged CDDP is very important when STS is co-administered as an antidote.

The kidney platinum concentration was significantly reduced and the distribution pattern of platinum species 1 min following the CDDP injection was changed drastically in the STS-treated rats. In the control rats, unchanged CDDP was the main platinum species (86%) at 1 min following the CDDP injection. However, unchanged CDDP was only 24% of the total platinum in the STS-treated rats. The tissue to plasma ratio of unchanged CDDP was 2.51 in the control rats and 2.25 in the STS-treated rats. Therefore, the transport system into the kidney for unchanged CDDP may remain the same even when STS is coadministered. The significant reduction in kidney platinum and the change in the platinum distribution pattern have mainly been ascribed to the reaction of unchanged CDDP with STS and increased formation of mobile metabolites in plasma.

Uozumi and Litterst reported that platinum concentrations in kidney homogenate or in each subcellular fraction are not significantly different between control and STS-treated rats in spite of the pronounced protective effect of STS against CDDP nephrotoxicity [31]. In their study, platinum was determined as total platinum using radioactive labelling. They suggested that the distribution of active CDDP could be an important protective mechanism. Our results showed that the level of unchanged CDDP in the kidney was drastically reduced while mobile metabolite levels increased in the STS-treated rats. Therefore, although total platinum levels in the STS-treated rats were not significantly

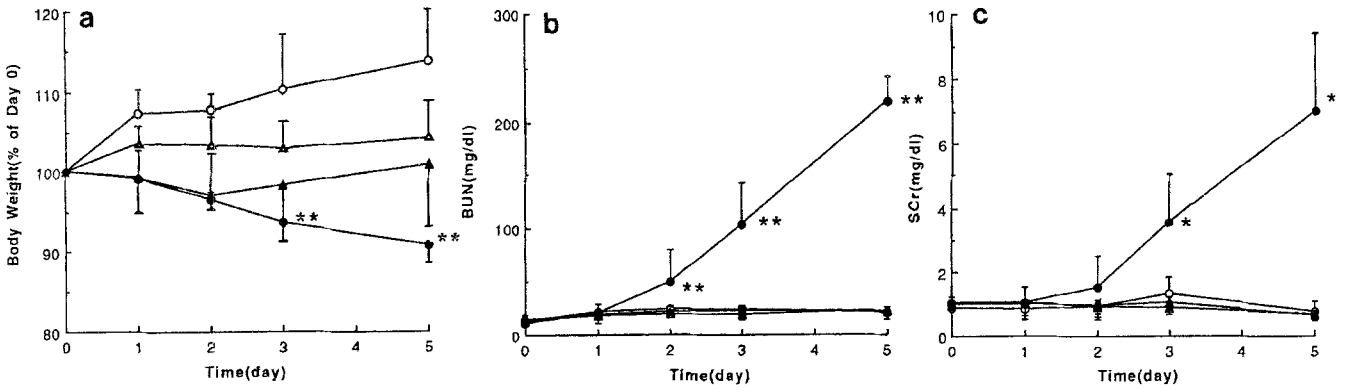

Fig. 4a-c Changes in body weight (**a**), BUN (**b**) and SCr (**c**) levels in rats. ○ 0.9% w/v sodium chloride solution 5 ml/kg i.v. ● CDDP 5 mg/kg i.v., △ 0.9% w/v sodium chloride solution 5 ml/kg i.v. with STS infusion (1200 mg/kg). ▲ CDDP 5 mg/kg i.v. with STS infusion (1200 mg/kg). Values are expressed as mean ($n = 5$) ± SD. Significant differences for body weight were detected between rats given CDDP alone and rats given 0.9% w/v sodium chloride solution alone or with STS infusion. Significant differences for BUN and SCr were detected between rats given CDDP alone and other groups. $^*P < 0.05$, $^{**}P < 0.01$

different from those in the control rats in the previous study [31], active CDDP might be reduced. Changing the distribution pattern of platinum species might therefore be the main protective mechanism of STS against CDDP nephrotoxicity, rather than reducing kidney platinum levels.

The diuretic action of STS [15, 22] has also been suggested as another protective mechanism. It has been suggested that increased urine output might accelerate platinum excretion and might dilute the platinum concentration in urine [15, 17, 19]. In the present study, urine output was increased markedly and excretion of platinum during the first 10 min following CDDP injection was accelerated in the STS-treated rats. Accelerated platinum excretion in urine has been reported both in humans and in rabbits [8, 15]. However, in these studies, platinum species were not examined separately. In the present study, unchanged CDDP was not detected in the urine and the excreted platinum species comprised only mobile metabolites in the STS-treated rats. Finally, the Cl_r of unchanged CDDP was calculated to be zero after STS coadministration. STS is eliminated quickly from the plasma by glomerular filtration with a biological half-life of about 10 min [14]. The urinary STS concentration in the present study was between 20 and 40 mg/ml (data not shown). Unchanged CDDP has been reported to be inactivated completely by a 400-fold molar ratio of STS [15]. Therefore, the reason why unchanged CDDP was not detected in urine after STS infusion could be that STS reacts with unchanged CDDP to form mobile metabolites in urine as well as in plasma. However, in the present study, the excretion of mobile metabolites in the STS-treated rats was estimated to be smaller than in the control rats (Table 1). The GFR-dependent excretion of mobile metabolites after STS infusion may explain this. Although excretion of mobile metabolites was accelerated during the first 10 min after CDDP injection, a delay in mobile metabolite elimination was observed during the terminal phase only in the STS-treated rats (Figs. 1 and 2). This might be related to the decrease in GFR after STS infusion. Although data are not shown in the figure, the slope of the regression line between GFR and Cl_r for mobile metabolites was greater for the STS-treated rats (about 3.1) than for the diuretic-treated rats (about 1.7). This may support the theory of the formation of mobile metabolites in the renal tubule after the process of glomerular filtration, when it can be assumed that the Cl_r of mobile metabolites is not changed by STS. Another possible reason why unchanged CDDP was not detected in the urine is that transport of unchanged CDDP into the renal tubule might be inhibited by STS. However, we have no specific data to clarify the Cl_r of unchanged CDDP after STS infusion. When furosemide was administered in the other studies, urine output was accelerated markedly, and CDDP nephrotoxicity was partly reduced but could not be eliminated [21, 23, 34]. Therefore, the diuretic effect induced by STS cannot be considered as a direct protective mechanism against CDDP nephrotoxicity.

In conclusion, the mechanism underlying the protective effect of STS against CDDP nephrotoxicity appears to be an increase in unchanged CDDP total clearance due to a direct reaction of CDDP with STS in the systemic circulation. As a result, unchanged CDDP levels in the kidney can be kept below the critical level for CDDP nephrotoxicity.

References

1. Abe R, Akiyoshi T, Tsuji H, Baba T (1986) Protection of antiproliferative effect of cis-diamminedichloroplatinum(II) by sodium thiosulfate. Cancer Chemother Pharmacol 18: 98
2. Basolo F, Pearson RG (1958) A study of metal complexes; mechanisms of inorganic reactions, 2nd edn. Wiley, New York

3. Bonsnes RW, Taussky HH (1945) On the colorimetric determination of creatinine by the jaffe reaction. J Biol Chem 158: 581
4. Brun C (1950) Thiosulfate determination in kidney function test. J Lab Med 35: 152
5. Davidson WD, Sackner MA (1963) Simplification of the anthrone method for the determination of inulin in clearance studies. J Lab Clin Med 62: 351
6. Elferink F, Vijgh JF van der, Klein I, Pinedo HM (1986) Interaction of cisplatin and carboplatin with sodium thiosulfate: reaction rates and protein binding. Clin Chem 32: 641
7. Hanada K, Nagai N, Ogata H (1995), Quantitative determination of unchanged cisplatin in rat kidney and liver by high-performance liquid chromatography. J Chromatogr 663: 181
8. Hirosawa A, Sakai S, Iizuka K, Niitani H, Hayashibara K, Tsuboi E (1987) Combination effects of cis-dichlorodiammineplatinum(II) and sodium thiosulfate on renal dysfunction. Jpn J Cancer Chemother 14: 2870
9. Howell SB, Taetle R (1980) The effect of sodium thiosulfate on cis-dichlorodiammineplatinum(II) nephrotoxicity and antitumor activity in the L1210 leukemia. Cancer Treat Rep 64: 611
10. Howell SB, Pfeifle CL, Wung WE, Olshen RA, Lucas WE, Yon JL, Green M (1982) Intraperitoneal cisplatin with systemic thiosulfate protection. Ann Intern Med 97: 845
11. Howell SB, Pfeifle CE, Wung WE, Olshen RA (1983) Intraperitoneal cis-diamminedichloroplatinum with systemic thiosulfate protection. Cancer Res 43: 1426
12. Ishizawa M, Taniguchi S, Baba T (1981) Protection by sodium thiosulfate and thiourea against lethal toxicity of cis-diamminedichloroplatinum(II) in bacteria and mice. Jpn J Pharmacol 31: 883
13. Iwamoto Y, Aoki K, Kawano T, Baba T (1984) Upper hemibody infusion of cis-diamminedichloroplatinum(II) followed by systemic antidote, sodium thiosulfate, for lung metastasis in rats. Clin Exp Metastasis 2: 261
14. Iwamoto Y, Kawano T, Uozumi J, Aoki K, Baba T (1984) "Two-route chemotherapy" using high-dose ip cisplatin and iv sodium thiosulfate, its antidote, for peritoneally disseminated cancer in mice. Cancer Treat Rep 68: 1367
15. Iwamoto Y, Kawano T, Ishizawa M, Aoki K, Kuroiwa T, Baba T (1985) Inactivation of cis-diamminedichloroplatinum(II) in blood and protection of its toxicity by sodium thiosulfate in rabbits. Cancer Chemother Pharmacol 15: 228
16. Kinoshita M, Yoshimura N, Ogata H, Tsujino D, Takahashi T, Takahashi S, Wada Y, Someya K, Ohno T, Masuhara K, Tanaka Y (1990) High-performance liquid chromatographic analysis of unchanged cis-diamminedichloroplatinum (cisplatin) in plasma and urine with post-column derivatization. J Chromatogr 529: 462
17. Kobayashi H, Hasuda K, Aoki K, Kuroiwa T, Taniguchi S, Baba T (1989) "Two-route chemotherapy" using cis-diamminedichloroplatinum(II) and its antidote, sodium thiosulfate, combined with angiotensin II is effective against peritoneally disseminated cancer in rats. Cancer Chemother Pharmacol 24: 141
18. Krakoff IH (1979) Nephrotoxicity of cis-dichlorodiammineplatinum(II). Cancer Treat Rep 63: 1523
19. Kuroiwa T, Aoki K, Taniguchi S, Hasuda K, Baba T (1987) Efficacy of two-route chemotherapy using cis-diamminedichloroplatinum(II) and its antidote, sodium thiosulfate, in combination with angiotensin II in a rat limb tumor. Cancer Res 47: 3618
20. Madias NE, Harrington JT (1978) Platinum nephrotoxicity. Am J Med 65: 307
21. Reference deleted
22. Newman EV, Gilman A, Philips FS (1946) The renal clearance of thiosulfate in man. Bull Johns Hopkins Hosp 79: 229
23. Pera MF, Harder HC (1979) Effects of mannitol or furosemide diuresis on cis-dichlorodiammineplatinum-(II) antitumor activity and toxicity to host-renewing cell populations in rats. Cancer Res 39: 1279
24. Pfeifle GE, Howell SB, Felthouse RD (1985) High-dose cisplatin with sodium thiosulfate protection. J Clin Oncol 3: 237
25. Reece PA, Stafford I, Davy M, Freeman S (1987) Disposition of unchanged cisplatin in patients with ovarian cancer. Clin Pharmacol Ther 42: 320
26. Reece PA. Stafford I, Davy M, Morris R, Freeman S (1989) Influence of infusion time on unchanged cisplatin disposition in patients with ovarian cancer. Cancer Chemother Pharmacol 24: 256
27. Riabchikov DI (1941) The reactions with thiosulfate as a method for determining the nature of isomerism of platinum complex compounds. Comp Rend Acad Sci URSS 32: 344
28. Sagiyama K. Uozumi J, Aoki K, Baba T (1983) Efficacy of "two-route chemotherapy" using intra-arterial cisplatin and iv sodium thiosulfate, its antidote, in rat bladder tumor. Cancer Treat Rep 67: 567
29. Saito D, Fujii T, Yoshida S, Ohkura H, Oguro Y (1990) Effect of sodium thiosulfate on the anti-tumor effect and nephrotoxicity of CDDP in human gastric cancer. Jpn J Cancer Chemother 17: 959
30. Sakuma A (1977) Analysis of variance; nonparametric procedures. In: Sakuma A (ed) Statistical methods in pharmacometrics, vols I, II. University of Tokyo Press, Tokyo, I: p220, II: p1
31. Uozumi J, Litterst CL (1986) The effect of sodium thiosulfate on subcellular localization of platinum in rat kidney after treatment with cisplatin. Cancer Lett 32: 279
32. Uozumi J, Sagiyama K, Aoki K, Iwamoto Y, Baba T (1983) Effectiveness of "two-route chemotherapy" using cisplatin and its antidote, sodium thiosulfate, on lifespan of rats bearing metastatic liver tumors. Cancer Treat Rep 67: 1067
33. Uozumi J, Ishizawa M, Iwamoto Y, Baba T (1984) Sodium thiosulfate inhibits cis-diamminedichloroplatinum(II) activity. Cancer Chemother Pharmacol 13: 82
34. Ward JM, Grabin AF, LeRoy AF, Young DM (1977) Modification of the renal toxicity of cis-dichlorodiammineplatinum(II) with furosemide in male F344 rats. Cancer Treat Rep 61: 375