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Inactivation of *cis*-diamminedichloroplatinum (II) in blood and protection of its toxicity by sodium thiosulfate in rabbits*

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Summary. The mode of inactivation of *cis*-diamminedichloroplatinum(II) (DDP) in the bloodstream and protection from its toxicity by sodium thiosulfate (STS) were investigated in rabbits.

Plasma ultrafiltrate in rabbits given 5 mg/kg DDP IV and various excess molar ratios of STS IV were assayed for the active platinum levels with a new microbiological assay system using an *E. coli* strain. The active platinum species in the plasma were inactivated completely by co-administration of a 400-fold excess of STS IV. The rabbits were almost completely protected against both BUN increase and body weight loss normally caused by DDP when 400-fold doses of STS were given. Diuretic effects were also observed.

Our data provide evidence for the basis of optimum use of STS to protect against DDP toxicity.

Introduction

Although local administration of anticancer drugs for regionally confined tumors is often superior to systemic administration [3, 13], the dose to be given is limited as there are side effects once the drug enters the systemic circulation.

We have devised a combination chemotherapy, termed 'two-route chemotherapy (TRC)', in which an anticancer drug and its antidote are given respectively, locally at the tumor site, and systemically. This therapy is capable of reducing the side effects of an anticancer drug, and has led on to investigations of the chemotherapeutic effect of two anticancer drugs in elevated doses. The first trial of TRC with a combination of mechlorethamine N oxide and its antidote cysteine, given via the hepatic artery and the tail vein, respectively, was effective for the treatment of rat liver tumors [1]. Recently, we obtained remarkable antitumor effects by giving a combination of DDP and STS for peritoneal dissemination, bladder tumors, and liver and lung metastasis in experimental animals [10, 11, 15–17], based on the evidence that STS inactivates the toxicity of DDP [6, 9]. This TRC with DDP and STS is now in clinical trial [7, 8], but the precise mode of protective action of STS against DDP toxicity has not been determined.

We now present evidence that this protective effect is due to inactivation of biologically active DDP in the bloodstream.

Materials and methods

Chemicals. DDP was provided by Nippon Kayaku Co. Ltd, Tokyo, Japan and STS was obtained from Wako Pure Chemical Industries, Ltd, Osaka, Japan. Each of these compounds was dissolved in 0.9% NaCl solution.

Assay of active platinum levels in plasma. Adult female rabbits (3.3–3.9 kg) were anesthetized by giving 30 mg/kg pentobarbital sodium IV. A heparinized polyethylene catheter (4 F) was inserted into the left common carotid artery to collect blood samples. DDP 5 mg/kg in a volume of 5.7 ml/kg body weight and STS at 50-, 100-, 200-, and 400-fold molar ratios to DDP (132, 264, 526, and 1054 mg/kg) or saline in a volume of 16 ml/kg body weight were given simultaneously, via the left and the right ear vein, respectively. DDP and STS solutions were infused at flow rates of 8.6 and 24 ml/min, respectively, with two infusion pumps (Harvard Apparatus, Model 975E, Boston, Mass, USA). DDP and STS were infused over the same period of time. Blood samples were collected through the catheter 5–180 min after the drug infusions, and ultrafiltrable plasma was separated by centrifugation at 4 °C for 20 min at 1000 *g* through Centriflo CF-25 filters (Amicon Corporation, Danvers, Mass, USA). The samples were frozen immediately in a dry ice-acetone bath and stored at −80 °C until analyzed for platinum content by a microbiological assay and by flameless atomic absorption spectrophotometry (Atomic Absorption Spectrophotometer, Type 180-70, HITACHI, Co., Ltd, Tokyo, Japan) [2]. The microbiological assay of DDP was developed recently in our laboratory to identify to active platinum species in the plasma fraction [12]. An *E. coli* strain, WP 100 (uvr^-, rec^-, trp^-) [18] was used. The bacterial cells grown at 37 °C in Davis minimum medium supplemented with 500 μg/ml tryptophan to a log phase were harvested by centrifugation, washed twice with PBS (0.067 *M* phosphate buffer, pH 7.4, plus 0.1 *M* NaCl), and suspended in PBS to give 2×10^9 cells/ml. The cell suspension was mixed 1:1

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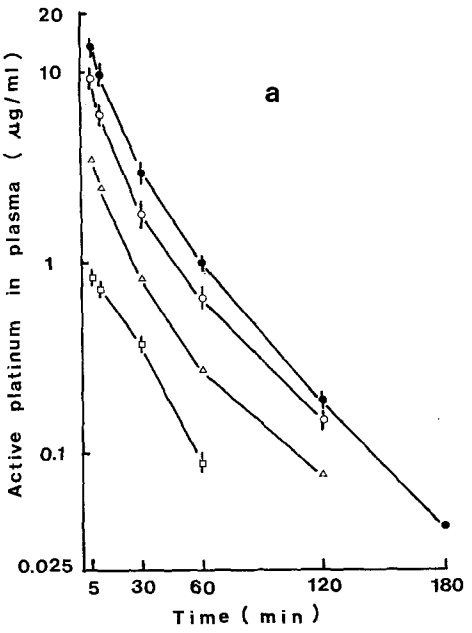

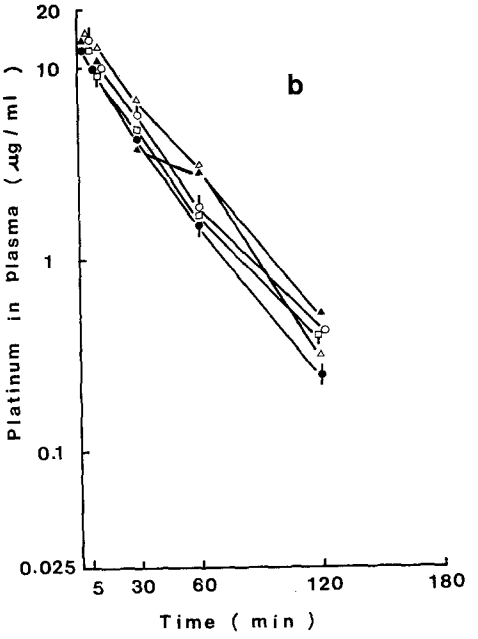

Fig. 1a, b. Platinum levels in plasma ultrafiltrate determined by microbiological assay **(a)** and atomic absorption spectrophotometry **(b)** after IV administration of DDP with or without STS IV to rabbits. ●, 5 mg/kg DDP plus saline; ○, 5 mg/kg DDP plus 50-fold molar ratios of STS to DDP; △, 5 mg/kg DDP plus 100-fold molar ratios of STS to DDP; □, 5 mg/kg DDP plus 200-fold molar ratios of STS to DDP; ▲, 5 mg/kg DDP plus 400-fold molar ratios of STS to DDP. *Bar* represents SEM ($n=2$). Active platinum levels determined by microbiological assay for 5 mg/kg DDP plus 400-fold STS at 0–180 min were less than detection limit (~0.025 μg/ml) **(a)**. Platinum levels determined by atomic absorption spectrophotometry at 180 min were less than detection limit (~0.1 μg/ml) in all cases **(b)**

with each of the ultrafiltrated samples that were approximately diluted with pooled plasma ultrafiltrate of untreated rabbits. The mixtures were incubated at 40 °C for 3 h with shaking and then diluted to 100-fold with PBS to halt the reaction; aliquots (0.1 ml) of serial dilutions were then plated with 2.5 ml soft agar overlay (10 μg/ml *L*-tryptophan, 0.6% NaCl, and 0.7% agar) onto plates containing Davis minimum agar medium supplemented with 0.4% glucose and 1.5% agar. The surviving colonies were scored after incubation of plates at 37 °C for 2 days. Active platinum levels of samples were quantitated by the standard dose-response curve of cell survival against known DDP concentrations in pooled plasma ultrafiltrate of untreated rabbits. The detection limit of DDP in this bioassay was 0.025 μg/ml. After the collection of blood samples the catheter was removed, the wound was sutured, and the rabbits were returned to their cages. Loss of body weight was monitored on various days after the treatments. BUN levels were measured by the urease method [4].

Urinary excretion of platinum. A polyethylene catheter (4 F) was inserted into each ureter of the anesthetized rabbits to collect urine samples. DDP 5 mg/kg and STS 1054 mg/kg (400-fold molar ratios to DDP) or saline were given in a similar manner as in the experiments for plasma platinum determination. Urine was accumulated and a sample (300 μl) was separated at 0.5, 1, 2, and 3 h after drug administration. Urine platinum concentrations were estimated by flameless atomic absorption spectrophotometry [14].

Results

Effect of STS on active platinum levels in plasma

Figure 1 shows the active platinum level, determined by microbiological assay (Fig. 1a), and platinum content, determined by atomic absorption spectrophotometry (Fig. 1b), in filtrable fractions of plasma after IV administration of DDP, with or without varying doses of STS. The

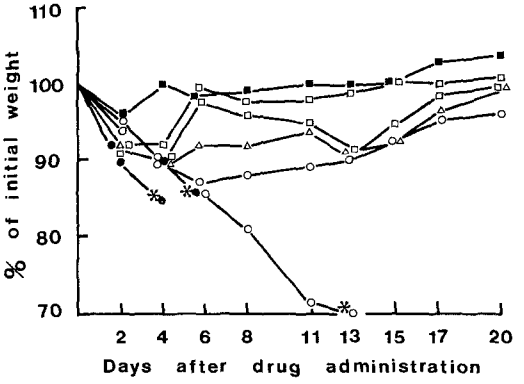

Fig. 2. Changes in body weight after DDP administration with or without STS. ●, 5 mg/kg DDP plus saline; ○, 5 mg/kg DDP plus 50-fold molar ratios of STS to DDP; △, 5 mg/kg DDP plus 100-fold molar ratios of STS to DDP; □, 5 mg/kg DDP plus 200-fold molar ratios of STS to DDP; ■, 5 mg/kg DDP plus 400-fold molar ratios of STS to DDP. Asterisks indicate rabbits that died of DDP toxicity.

Table 1. BUN levels on day 4 after DDP administration with various doses of STS

Treatment	No. of rabbits	BUN (mg/dl)
Untreated control	4	15.9 ± 1.9 (mean ± SE)
DDP 5 mg/kg IV + saline IV	2	74.2[a]
DDP 5 mg/kg IV + 50-fold molar ratios of STS to DDP IV	2	61.2, 44.5
DDP 5 mg/kg IV + 100-fold molar ratios of STS to DDP IV	1	16.4
DDP 5 mg/kg IV + 200-fold molar ratios of STS to DDP IV	2	14.3, 15.8
DDP 5 mg/kg IV + 400-fold molar ratios of STS to DDP IV	1	14.7

[a] One rabbit died before BUN was measured

amount of filtrable platinum represents the active platinum levels when DDP only was given [5, 12]. Co-administration of varying excess doses of STS with DDP had no effect on the elimination curve of the filtrable platinum levels in plasma (Fig. 1 b). However, the active platinum levels were reduced in proportion to increasing dose of STS (Fig. 1 a); approximately 30%, 70%, and 90% of the active platinum detected 5 min after administration of DDP alone was inactivated by 50-, 100- and 200-fold molar ratios of STS to DDP, respectively. Moreover, a complete inactivation of DDP was observed by 400-fold molar ratios of STS. Elimination curves of active platinum both in single DDP infusion and in combination with various doses of STS (50- to 200-fold molar ratios to DDP) showed a monophasic pattern, with similar slopes. These results indicated that STS inactivated active platinum in blood, and that the inactivated platinum did not subsequently return to an active form.

Effect of STS on the side effects of DDP

Figure 2 shows the loss of body weight after administration of DDP, with or without STS. Rabbits given DDP alone showed the most severe loss of body weight and died of DDP toxicity within 6 days after the treatment. Protection from body weight loss tended to depend on the increasing doses of STS, and the nadir of weight loss was within 5% of the initial weight in rabbits given DDP in combination with 400-fold molar ratios of STS.

BUN levels on day 4 after the treatments are summarized in Table 1. In rabbits given 5 mg/kg DDP alone and in combination with 50-fold molar ratios of STS, striking increases in BUN levels were observed. In rabbits given 5 mg/kg DDP with 100-fold molar ratios of STS and over, increases in BUN levels were prevented.

Effect of STS on urinary excretion of platinum

Figure 3 shows the urine output during 3 h after administration of IV DDP (5 mg/kg), with or without STS. Rabbits given DDP with STS excreted much larger volumes of urine than did the control rabbits given DDP alone. Figure 4 shows the urinary excretion of platinum in rabbits given IV DDP, with or without IV STS. Platinum tended to be excreted more rapidly in rabbits given DDP in com-

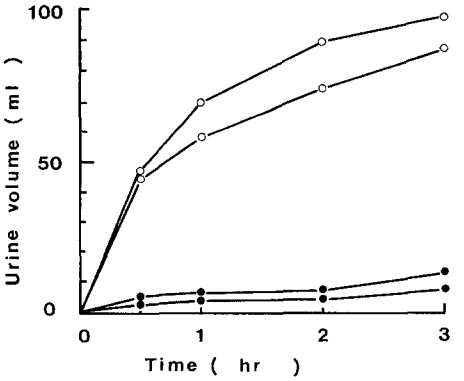

Fig. 3. Urine output after administration of DDP IV with or without STS IV: ○, 5 mg/kg DDP plus 400-fold molar ratios of STS; ●, 5 mg/kg DDP plus saline

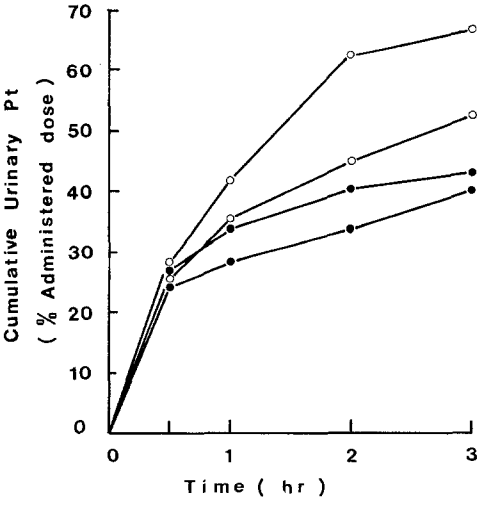

Fig. 4. Urinary excretion of platinum after administration of DDP IV with or without STS IV. ○, 5 mg/kg DDP plus 400-fold molar ratios of STS to DDP; ●, 5 mg/kg DDP plus saline

bination with STS than in the control rabbits given DDP alone. Urine platinum concentrations 3 h after drug administrations were 0.558 and 1451 mg/ml (mean 1.0 mg/ml) in two rabbits given a single DDP treatment, and 0.096 and 0.120 mg/ml (mean 0.108 mg/ml) in two rabbits given DDP with STS. Thus, platinum concentrations in the urine of rabbits given STS was approximately one-tenth that found in the control rabbits.

Discussion

Although it has been well established that STS affords protection against side effects of DDP [7, 8, 10, 11, 15–17], the mechanism of this protection is poorly understood. We speculated that STS might bind directly to DDP in the bloodstream to form an inactive complex, because (1) high doses of STS plus DDP completely protect bacteria against DDP cytotoxicity [9]; (2) the concomitant IP administration of extremely high doses of STS with a lethal dose of DDP produced no toxicity in mice [6]; and (3) STS protected mice against both renal and hematologic toxicities [10].

The present study demonstrated that DDP given IV to rabbits is inactivated in blood by STS given IV and that the inactivated form of platinum does not subsequently revert to the active form (Fig. 1). Therefore, it is suggested that in TRC, DDP entering the systemic circulation from the tumor area will be inactivated by excess STS administered systemically; the protection by STS against renal and hematologic toxicities of DDP may be due to a reduction in the delivery of active platinum to the kidney and bone marrow.

Our present results are inconsistent with those of Howell et al., who reported that active platinum in the bloodstream after DDP administration was not reduced by STS administration [7, 8]. This discrepancy may relate to procedures, as Howell et al. used DDTC to separate platinum from plasma ultrafiltrate by chelation to estimate the active platinum species; however, the inactive complex of STS and DDP might also be separated by DDTC and be estimated as the active form. In contrast, the microbiological assay of DDP, which allows for detection of only its active form, seems to be an optimal method for the special case of combination treatment with DDP and STS. As DDP inactivation is dependent on amounts of STS in the bloodstream, it is important to determine what molar ratios of STS to DDP are required to protect against various side effects. In previous studies, renal toxicity of DDP was completely avoided by 200-fold molar ratios of STS to DDP [11, 15, 17]. In the present study, BUN increase was avoided by 100-fold molar ratios of STS (Table 1); however, complete inactivation of DDP in blood was observed with STS at 400-fold molar ratios (Fig. 1); and nearly complete protection against body weight loss was achieved at 400-fold molar ratios of STS (Fig. 2). Since STS has a diuretic effect (Figs. 3 and 4), the prevention of renal toxicity at the 100-fold molar ratios of STS to DDP may be due partly to dilution of the remaining active platinum species in the renal tubules. A combination of 400-fold molar ratios of STS to DDP and over may be required to protect against other side effects of DDP in addition to renal toxicity.

Our present data provide a basis for the optimal use of STS in the protection of DDP toxicity in the clinical application of the combination treatment with DDP and STS. Not only side effects but also antitumor effects may be diminished when both DDP and STS are given IV, since STS inactivates DDP in blood. In addition, when administered after DDP had already been taken up into normal cells, STS did not protect cells against DDP toxicity [9]. Therefore, DDP should be given locally into the tumor area and STS systemically before leaking of DDP into the systemic circulation by which normal cells are attacked.

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