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"Two-route chemotherapy" using *cis*-diamminedichloroplatinum(II) and its antidote, sodium thiosulfate, combined with angiotensin II is effective against peritoneally disseminated cancer in rats*

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Summary. "Two-route chemotherapy" (TRC) using *cis*-diamminedichloroplatinum(II) (DDP) and its antidote, sodium thiosulfate (STS), combined with the angiotensin II (AT-II)-induced hypertension method was evaluated for its efficacy against peritoneally disseminated tumors in rats. A bolus i.p. injection of DDP (15 mg/kg) was given 1 min after the initiation of an AT-II (16.5 μg/kg) i.v. infusion lasting 11 min. Immediately after the termination of the AT-II infusion, 1,580 mg/kg STS was injected i.v. over a further 5 min. This modified TRC significantly improved the antitumor effect, evaluated by survival (increase in life span, 273%), compared with that achieved with other treatments, as follows: 15 mg/kg DDP i.p. and the concomitant i.v. infusion of 1,580 mg/kg STS (conventional TRC), 153% increase in life span; 5 mg/kg DDP i.p. with or without AT-II i.v. (167% and 107% increases in life span, respectively). As an index of nephrotoxicity, blood urea nitrogen (BUN) levels seen after modified TRC (21.1 mg/dl) were as low as those observed after conventional TRC (19.1 mg/dl), despite the postadministration of STS, and were much lower than those seen after DDP alone or DDP plus AT-II (35.6 and 35.7 mg/dl, respectively). Further evaluation of the effectiveness of modified TRC using various doses of DDP gave similar results. The feasibility of the administration of STS 10 min after DDP treatment was explained by the significant inhibition of DDP delivery to the kidney during the AT-II-induced hypertension. Thus, TRC combined with AT-II has a superior therapeutic effect against peritonitis carcinomatosa induced in rats.

Introduction

We previously designed a combination chemotherapy, termed two-route chemotherapy (TRC), in which a large amount of an anticancer drug was injected locally at the tumor site in combination with its antidote, which was given systemically [2]. This treatment was devised to increase the antitumor effect by giving high doses of drug at the tumor site while reducing the general toxicity of the anticancer drug with the antidote. Since *cis*-diamminedichloroplatinum(II) (DDP) is potent against a wide range of human tumors [42] and sodium thiosulfate (STS) effectively and safely detoxicates DDP in vivo [11, 14, 18, 40], we mainly used DDP as the anticancer agent and STS as its antidote. We have reported the remarkable effectiveness of TRC using DDP and STS against liver tumor [39], urinary bladder tumor [34], metastatic lung tumor [16], and limb tumor [15] in experimental animals. This combination chemotherapy has been used in clinical practice [12, 19, 29, 43].

Anticancer drugs are often injected i.p. for the treatment of peritoneally disseminated tumors, as i.p. administration produces greater and longer drug retention at the tumor site than does i.v. injection [4, 6, 30, 31]; however, the maximal dose to be given is limited by systemic toxicity, as seen in the case of i.v. administration. To overcome this limitation, we carried out TRC using DDP and STS for peritoneally disseminated tumors in mice and rats, where high doses of DDP were injected i.p., with significant increases in the therapeutic effects [17, 38]. However, there is room for improvement of the antitumor effect, because the therapeutic effect of i.p. DDP would be reduced by the concomitant i.v. infusion of STS, which diffuses rapidly from the systemic circulation into the peritoneal cavity; some investigators have pointed out that STS reduces not only DDP-induced nephrotoxicity but also the antitumor effect of the drug [1, 24, 27].

The delayed administration of STS in DDP chemotherapy without increasing nephrotoxicity was expected to improve the therapeutic effect; thus, we combined conventional TRC with the AT-II-induced hypertension method [22]. AT-II reduces renal blood flow [33], leading to a decrease in the delivery of DDP to the kidney during induced hypertension. This transient inhibition of DDP delivery to the kidney protected rats against DDP-induced nephrotoxicity, despite the delayed administration of STS [22]. On the other hand, in conventional TRC, STS must be given simultaneously with DDP to afford such protection [17]. This report describes the enhanced therapeutic effect obtained with modified TRC by giving STS i.v. 10 min after i.p. DDP compared with the efficacy of other treatments for peritoneally disseminated tumors in rats.

Materials and methods

Chemicals. DDP (Nippon Kayaku Co., Ltd., Tokyo, Japan), AT-II (Sigma Chemical Co., St. Louis, Mo), and STS (Wako Pure Chemical Industries, Ltd.; Osaka, Japan)

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were dissolved in 0.9% NaCl solution (saline) at the desired concentrations. Each solution was prepared just before use.

Animals and tumors. Female Wistar-King-Aptekman (WKA) rats weighing 170–210 g at 10–12 weeks of age were used for all experirments. They were obtained from the Animal Center of Kyushu University and were given free access to food pellets and tap water.

A transitional cell carcinoma of the bladder (RBT-1) induced in WKA rats with *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine [8] was maintained in serial transplantations by i.m. injection into the hind limb of the rats. The tumor was excised, minced, suspended in Hanks' balanced salt solution (Nissui Seiyaku Co., Tokyo, Japan), and passed through a metal sieve to obtain a single-cell suspension. After a trypan blue exclusion test (viability, >90%), $1-1.5 \times 10^6$ viable cells per rat were inoculated i.p.

Chemotherapy experiments. Chemotherapy experiments were carried out on either day 4 or day 7 after the i.p. inoculation of 1×10^6 viable RBT-1 cells. All rats were anesthetized i.v. with sodium pentobarbital (15 mg/kg).

The top of Fig. 1 shows a protocol of the modified TRC. AT-II (1 μg/ml) was infused in each rat at 3.3 ml/200 g body weight for 11 min via the right femoral vein using an infusion pump (model STC-521; Terumo Co., Ltd., Tokyo, Japan). At 1 min after the start of the AT-II infusion, 15 mg/kg DDP was given i.p. as a bolus injection at 3 ml/200 g body weight. Immediately after the termination of the AT-II infusion, 1,580 mg/kg STS (200-fold molar ratio to 15 mg/kg DDP) was given at 4 ml/200 g body weight via the left femoral vein for a further 5 min. Hence, the STS infusion was started 10 min after i.p. DDP in the modified TRC group. On the other hand, in the conventional TRC group, STS was infused for 5 min without AT-II starting 1 min after the i.p. administration of 15 mg/kg DDP because this protocol has shown the best therapeutic effect [17]. In treatment with DDP alone or DDP plus AT-II, 5 mg/kg DDP was injected i.p. without STS. To equalize the hydration effect, sterile saline was given to each rat.

At several doses of DDP, the therapeutic effects of each treatment were repeatedly evaluated in the same manner as above. In this trial, treatments were carried out on day 4 after the i.p. inoculation of 1.5×10^6 viable RBT-1 cells. DDP doses were set at 10, 15 and 20 mg/kg in the TRC groups and 3, 5, and 7 mg/kg in the groups given DDP alone. In the TRC groups, doses of STS were fixed at a 200-fold molar ratio to each DDP dose.

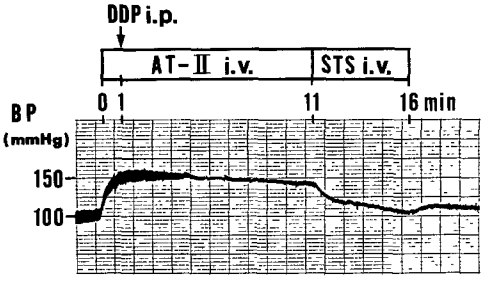

Fig. 1. Protocol of two-route chemotherapy combined with angiotensin II with blood pressure monitoring during treatment. *BP*, arterial blood pressure; *DDP*, 15 mg/kg i.p.; *AT-II*, 16.5 μg/kg i.v.; *STS*, 1,580 mg/kg i.v. (200-fold molar ratio to DDP)

Evaluation of antitumor and side effects. The survival in each group was monitored to evaluate the antitumor effect. Side effects were evaluated by blood urea nitrogen (BUN) levels, loss of body weight, and decreases in leukocytes. As an index of nephrotoxicity, a major dose-limiting factor of DDP [21, 41], BUN levels in blood samples taken from the tail vein of tumor-bearing rats were assessed by the urease technique [7] on day 4, a time at which BUN levels in DDP-treated rats usually reach their maximum [34]. The rats bearing i.p.-disseminated tumors were weighed on days 1, 4, 7, and 10 after each treatment. To determine the possible hematological disorders, leukocytes in blood samples drawn from the tail vein of non-tumor-bearing rats were counted on days 4, 8, and 12 after each treatment.

Blood pressure monitoring during TRC combined with AT-II. Arterial blood pressure during modified TRC was recorded using a transducer (P23 ID; Gould, Inc., Oxnard, Calif) connected to a blood pressure meter (AP-611G; Nihon Kohden Kogyo Co., Ltd., Tokyo, Japan) by a polyethylene catheter placed in the femoral artery of the representative tumor-bearing rats.

Platinum concentrations in the kidney. To assess the time course of platinum (Pt) concentrations in the kidney, rats were given 15 mg/kg DDP i.p. according to the protocols of modified TRC, conventional TRC, and treatment with DDP alone. All rats were killed and bilateral kidneys were excised from 5 min to 24 h after DDP administration. The Pt concentrations were estimated by flameless atomic absorption spectrophotometry (Atomic Absorption Spectrophotometer type 180-70; Hitachi Co., Ltd., Tokyo, Japan) [3].

Statistical analysis. The probability of significant differences in survival was calculated by the generalized Wilcoxon test [23]. Student's *t*-test was used to determine significant differences in body weight, BUN levels, the number of leukocytes, and Pt concentrations.

Results

Antitumor effect of TRC combined with AT-II

Table 1 shows the survival of rats given each treatment on day 4 or day 7 after the i.p. inoculation of tumor cells. Rats given modified TRC combined with AT-II (group A) survived significantly longer than those receiving any other treatment; the two rats in this group survived for over 150 days after the inoculation, with no tumor recurrence. Conventional TRC without AT-II (group B) and treatment with i.p. DDP plus AT-II (group C) resulted in a slight increase in the antitumor effect compared with treatment using i.p. DDP alone (group D). The rats usually died of cachexia due to the i.p.-disseminated tumors. A massive, bloody ascites and infiltrations of tumor nodules into the abdominal organs were observed.

Side effects of TRC combined with AT-II

Table 2 shows BUN levels on day 4 after each treatment as an index of nephrotoxicity. Although a high dose of DDP (15 mg/kg) was given to each rat in the conventional TRC

Table 1. Effects of various treatments on survival of rats bearing i.p.-disseminated tumors

Treament[a]	Survival[b] (days)					
	Treatment on day 4			Treatment on day 7		
	Range	Median	%ILS[c]	Range	Median	%ILS
A. DDP, 15 mg/kg i.p. + AT-II, 16.5 μg/kg i.v. + STS, 1,580 mg/kg i.v.	34 – > 150	56.0 (9)[d]	273	28 – 60	49.0 (9)	227
B. DDP, 15 mg/kg i.p. + saline i.v. + STS, 1,580 mg/kg i.v.	33 – 54	38.0 (9)	153	23 – 45	29.0 (8)	93
C. DDP, 5 mg/kg i.p. + AT-II, 16.5 μg/kg i.v. + saline i.v.	29 – 48	40.0 (9)	167	25 – 43	30.5 (8)	103
D. DDP, 5 mg/kg i.p. + saline i.v.	29 – 42	31.0 (8)	107	21 – 34	26.5 (8)	77
E. Untreated control	13 – 16	15.0 (10)		13 – 16	15.0 (10)	

[a] Treatments were given on day 4 or 7 after the i.p. inoculation of 10^6 viable RBT-I cells. Group A: STS was injected i.v. 10 min after i.p. DDP. Group B: STS was given 1 min after DDP

[b] Probability was calculated by the generalized Wilcoxon test. Day 4 treatment: A, B, C, D vs E, $P < 0.001$; A vs C, D, $P < 0.01$; A vs B and B, C vs D, $P < 0.05$; B vs C, not significant. Day 7 treatment: A, B, C, D vs E, $P < 0.001$; A vs D, $P < 0.01$; A vs B, C, $P < 0.05$; B vs C and B, C vs D, not significant

[c] %ILS, percentage of increase in life span = $(T/C - 1) \times 100$, where T is the median survival of the treatment group and C is the median survival of the control group

[d] Numbers in parentheses represent the number of rats; the results are the combinated data from two experiments

group (group B), BUN levels were almost equal to those in the untreated control group ($P > 0.05$). In the modified TRC group (group A), BUN levels were as low as those in the conventional TRC group, in spite of the (10-min) delayed administration of STS ($P > 0.05$). On the other hand, in the groups given DDP alone (group D) or DDP plus AT-II (group C), BUN levels were significantly higher than those in the TRC groups ($P < 0.01$). When STS was injected i.v. 15 min after i.p. DDP in the modified TRC group, nephrotoxicity was obvious: BUN levels were > 40 mg/dl (data not shown).

Figure 2 shows changes in the average body weight of rats after each treatment. The groups given modified TRC (group A) or treatment with i.p. DDP alone (group D) showed a similar pattern of change: their body weight decreased until days 1–4 but gradually reverted to the initial level before day 10. The maximal loss of body weight in these groups was within 10% of the initial weight. The

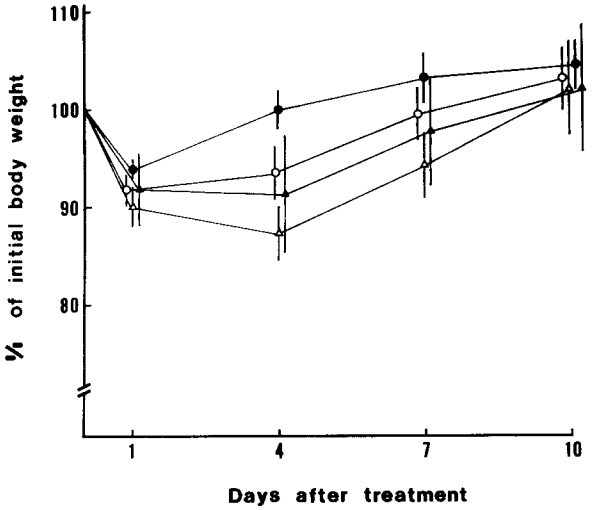

Fig. 2. Changes in the average body weight of rats after each treatment. ○, DDP 15 mg/kg i.p. + AT-II 16.5 μg/kg i.v. + STS 1,580 mg/kg i.v.; ●, DDP 15 mg/kg i.p. + STS 1,580 mg/kg i.v.; △, DDP 5 mg/kg i.p. + AT-II 16.5 μg/kg i.v.; ▲, DDP 5 mg/kg i.p. Each point is the mean level of 8 or 9 rats. *Bars*, SD

Table 2. BUN levels after various treatments in rats

Treatment[a]	Number of rats	BUN (mg/dl)[b] (mean ± SE)	*P*-value[c]
A. DDP, 15 mg/kg i.p. + AT-II, 16.5 μg/kg i.v. + STS, 1,580 mg/kg i.v.	9	21.1 ± 0.8	NS
B. DDP, 15 mg/kg i.p. + saline i.v. + STS, 1,580 mg/kg i.v.	9	19.1 ± 1.1	NS
C. DDP, 5 mg/kg i.p. + AT-II, 16.5 μg/kg i.v. + saline i.v.	9	35.6 ± 2.7	< 0.01
D. DDP, 5 mg/kg i.p. + saline i.v.	8	35.7 ± 2.0	< 0.01
E. Untreated control	10	19.4 ± 1.0	

[a] Treatments were given on day 4 after the i.p. inoculation of 10^6 viable RBT-I cells. Group A: STS was injected i.v. 10 min after i.p. DDP. Group B: STS was given 1 min after DDP

[b] BUN levels in blood samples were measured 4 days after each treatment

[c] Probability was calculated by Student's *t*-test: NS, not significant; A vs C, D and B vs C, D, $P < 0.01$; A vs B and C vs D, NS

Table 3. Changes in the number of leukocytes after various treatments in rats

Treatment[a]	Days after treatment	Number of WBC[b] (mean ± SE)	*P*-value[c]
A. DDP, 15 mg/kg i.p.	4	5,324 ± 653[d1]	< 0.05
+ AT-II, 16.5 μg/kg i.v.	8	6,030 ± 719	NS
+ STS, 1,580 mg/kg i.v.	12	7,538 ± 623	NS
B. DDP, 15 mg/kg i.p.	4	6,062 ± 486[d2]	< 0.05
+ saline i.v.	8	6,446 ± 438	NS
+ STS, 1,580 mg/kg i.v.	12	8,150 ± 535	NS
C. DDP, 5 mg/kg i.p.	4	5,138 ± 569[d3]	< 0.01
+ AT-II, 16.5 μg/kg i.v.	8	5,682 ± 591	< 0.05
+ saline i.v.	12	8.069 ± 613	NS
D. DDP, 5 mg/kg i.p.	4	5,226 ± 668[d4]	< 0.05
+ saline i.v.	8	6,078 ± 567	NS
	12	7,552 ± 515	NS
E. Untreated control		7,514 ± 407	

[a] Non-tumor-bearing rats were treated. Group A: STS was injected i.v. 10 min after i.p. DDP. Group B: STS was given 1 min after DDP

[b] Leukocytes (WBC) were counted from rats' blood samples on days 4, 8, 12 after each treatment. Each group consisted of 5 rats

[c] Probability was calculated by Student's *t*-test: NS, not significant; d1 vs d2, d3, d4, NS

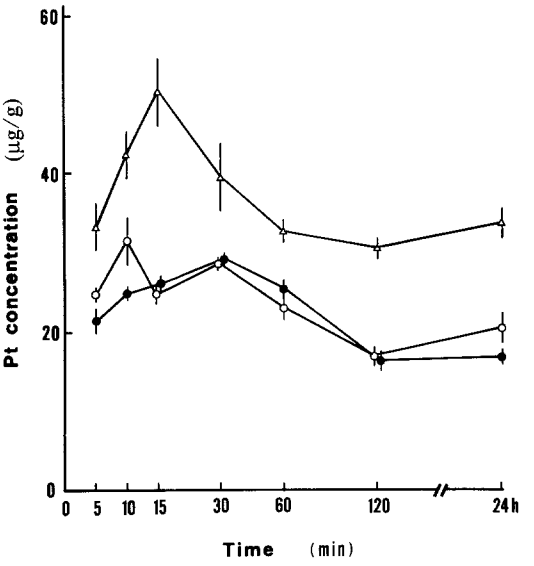

Fig. 3. Platinum (*Pt*) concentrations in the kidney after i.p. DDP administration. Pt concentrations were measured by rats given DDP as follows: ○, DDP 15 mg/kg i.p. + AT-II 16.5 μg/kg i.v. + STS 1,580 mg/kg i.v., STS being given 10 min after i.p. DDP; ●, DDP 15 mg/kg i.p. + STS 1,580 mg/kg i.v., STS being given 1 min after DDP; △, DDP 15 mg/kg i.p. Each point is the mean level of 6 rats. *Bars*, SE

weight loss in the group given DDP plus AT-II (group C) exceeded 10% on day 4 but was not significantly greater than that in groups A and D ($P > 0.05$). Body weight loss in the conventional TRC group (group B) was slight compared with that in the other groups.

Table 3 shows changes in the number of leukocytes after each treatment as an index of hematological disorders. The number of leukocytes did not significantly differ among the four treatments. Although a slight decrease was observed on day 4 after each treatment, it was transient and recovery occurred by day 12.

Blood pressure monitoring during TRC combined with AT-II

During TRC combined with AT-II, the systolic arterial blood pressure rapidly elevated from 100 to 150 mmHg within about 1 min after the start of the AT-II infusion and remained at about 150 mmHg for 10 min during the infusion (bottom of Fig. 1). This elevated level was not affected by i.p. DDP. The blood pressure gradually decreased when STS was injected i.v. for a further 5 min following AT-II.

Platinum concentrations in the kidney

Figure 3 shows the time course of Pt concentrations in the kidney from 5 min to 24 h after i.p. DDP. In the modified TRC group, AT-II was given i.v. for 10 min after the i.p. injection of 15 mg/kg DDP. The Pt concentration at 5 min (24.7 ± 1.2 μg/g) and 10 min (31.6 ± 3.1 μg/g) in this group was much lower than that at 5 min (33.3 ± 3.1 μg/g) and 10 min (42.6 ± 2.9 μg/g) in the group given 15 mg/kg DDP i.p. alone ($P < 0.05$). The Pt concentration between 15 min and 24 h in the group given i.p. DDP alone was much higher than that in the modified TRC group ($P < 0.01$).

The time course of Pt levels in the conventional TRC group was similar to that in the modified TRC group, although concomitant i.v. STS with i.p. DDP reduced Pt concentrations at 5 and 10 min after the initial i.p. injection of DDP slightly more effectively than did the transient AT-II-induced hypertension treatment ($P < 0.05$).

Chemotherapy experiment using various doses of DDP

To confirm the superiority of modified TRC, antitumor and side effects were compared at various doses of DDP among modified TRC, conventional TRC, and treatment with i.p. DDP alone, using the same protocols mentioned above (Table 4).

In the modified TRC (A) groups, survival was prolonged by increasing the dose of DDP, although the group given 20 mg/kg DDP showed a slight elevation in BUN levels (27.8 ± 2.2 mg/dl). On the other hand, in the conventional TRC (B) groups, the antitumor effect was not improved by increasing the dose of DDP. The modified TRC group given 15 mg/kg DDP showed much longer survival than the conventional TRC group given 20 mg/kg DDP (38 vs 27 days; $P < 0.05$), although these two groups showed much the same BUN levels.

Side effects observed in the group given 7 mg/kg i.p. DDP alone were severe: BUN levels were 50.4 ± 3.4 mg/dl, and the maximal loss of body weight on day 7 was 19.4% of the initial weight. Despite this severe toxicity, survival in this group was almost equal to that in the modified TRC group given 20 mg/kg DDP, which did not induce severe toxicity (44.5 vs 41.0 days; not significant). In the group treated with 3 mg/kg DDP alone, neither nephrotoxicity (BUN levels, 22.1 ± 0.9 mg/dl) nor an antitumor effect (68% ILS) was evident. Consequently, the group treated with 5 mg/kg i.p. DDP alone showed the best ther-

Table 4. Survival and BUN levels in rats given various doses of DDP in each treatment

Treatment[e]	DDP dose (mg/kg)	Number of rats	Survival[f] (days)			BUN (mg/dl)[h] (mean ± SE)
			Range	Median	%ILS[g]	
A. DDP i.p.	10	8	21–54	30.5[a1]	177	22.4 ± 0.8
+ STS i.v.	15	8	28–56	38.0[a2]	245	22.1 ± 1.4
+ AT-II i.v. (16.5 μg/kg)	20	8	25–62	41.0[a3]	273	27.8 ± 2.2
B. DDP i.p.	10	7	22–40	29.0[b1]	164	20.1 ± 0.5
+ saline i.v.	15	7	25–45	29.0[b2]	164	19.6 ± 0.7
+ STS i.v.	20	8	24–48	27.0[b3]	145	19.4 ± 0.6
C. DDP i.p.	3	8	13–23	18.5[c1]	68	22.1 ± 0.9
+ saline i.v.	5	7	17–36	24.0[c2]	118	32.4 ± 1.4
	7	8	30–76	44.5[c3]	305	50.4 ± 3.4
D. Untreated control		10	10–12	11.0[d]		20.0 ± 1.1

[e] Treatments were given on day 4 after the i.p. inoculation of 1.5×10^6 viable RBT-I cells. Group A: STS was injected i.v. 10 min after i.p. DDP. Group B: STS was given 1 min after DDP. STS was given at a 200-fold molar ratio to DDP in groups A and B. The results are the combinated data from two experiments

[f] Probability was calculated by the generalized Wilcoxon test: all a, b, c vs d and a1 vs c1, $P < 0.01$; a2 vs b1, b2, b3, c2 and a3 vs b3, $P < 0.05$; a1 vs b1 and a3 vs c3, NS

[g] %ILS, percentage of increase in life span = $(T/C - 1) \times 100$, where T is the median survival of treatment group and C is the median survival of the control group

[h] BUN levels in blood samples were measured 4 days after each treatment

apeutic effects of the groups treated with DDP alone (C), but survival in the former group was much shorter than that in the modified TRC group given 15 mg/kg i.p. DDP (C, 24 days, vs A, 38 days; $P < 0.05$), in spite of higher BUN levels in the former (C, 32.4 mg/dl, vs A, 22.1 mg/dl; $P < 0.05$).

Discussion

We obtained evidence that modified TRC had therapeutic effects superior to those of conventional TRC in peritoneally disseminated tumors in rats. STS protects normal tissues (particularly the kidney) against DDP-induced toxicity [11, 14, 18, 40], presumably by a direct reaction of STS with DDP in the extracellular fluid [18, 40]. This reaction forms an inactive complex, $Pt[(S_2O_3)_4]^{6-}$ [32], which is rapidly excreted through the urinary system without binding to other biomolecules or entering cells [18, 40]. Furthermore, the diuretic action of STS [26] may also be related to reductions in renal toxicity. In fact, all rats given TRC excreted high volumes of urine after STS administration (data not shown).

In conventional TRC for i.p.-disseminated tumors, STS reduced the nephrotoxicity of DDP, but only when i.v. STS was given simultaneously with i.p. DDP [17]. When STS was given i.v. 10 min after 15 mg/kg i.p. DDP in the absence of AT-II, nephrotoxicity was obvious: BUN levels on day 4 after i.p. DDP were about 40 mg/dl. Thus, the administration of STS after DDP without AT-II failed to reduce DDP-induced nephrotoxicity.

The feasibility of an administration of STS 10 min after DDP in modified TRC can be explained by the pharmacokinetic data on Pt concentrations in the kidney (Fig. 3). At 10 min after i.p. DDP combined with AT-II-induced hypertension, Pt concentration in the kidney was three-fourths that observed without AT-II. Rosivall and Navar [33] have reported that the renal blood flow decreased by 30%–40% during AT-II-induced hypertension [33]; thus, the difference in Pt levels in the present study was assumedly caused by a transient decrease in renal blood flow during AT-II infusion. On the other hand, in modified TRC the Pt elimination curve after i.v. STS was almost equal to that in conventional TRC involving i.p. DDP plus concomitant i.v. STS. At 1 day after the i.p. injection of DDP, the low Pt retention by the kidney was obvious in the TRC groups compared with Pt concentrations in the group given i.p. DDP alone. Moreover, the serum total Pt concentrations at 1 day after 15 mg/kg i.p. DDP in the TRC groups were much lower than those in the group given 15 mg/kg i.p. DDP alone (data not shown).

These results suggest that even a 10-min postadministration of STS could protect rats from severe and irreversible nephrotoxicity, as the delivery of DDP to the kidney was transiently but significantly inhibited by AT-II. The BUN levels in the modified TRC groups remained as low as those in the conventional TRC groups, except that the modified TRC group given 20 mg/kg DDP showed a slight increase in BUN levels (Tables 2, 4).

Other side effects (loss of body weight, decreases in leukocytes) in the modified TRC group (15 mg/kg DDP) were practically equal to those in the group treated with 5 mg/kg i.p. DDP alone, despite the threefold DDP dose and the postadministration of STS.

The delivery of DDP to tumor tissue was probably much improved in modified TRC. With the administration of STS after DDP, the tumor cells were exposed to a high dose of active DDP for over 10 min. On the other hand, in conventional TRC, DDP in the tumor tissue was more or less inactivated at an early stage by the concomitant i.v. administration of STS because of the rapid diffusion of the latter into the peritoneal cavity. Howell et al. [13] have reported that measurable concentrations of STS occurred in the peritoneal cavity just after i.v. STS and that the intraperitoneal concentration of STS rapidly reached a level comparable with that seen in the serum. The survival of rats treated with conventional TRC was not prolonged by increasing the DDP dose, whereas the antitumor effect of modified TRC was much improved by increasing the dose

(Table 4). This indicates that i.p. DDP plus concomitant i.v. STS reduced not only DDP-induced nephrotoxicity but also the antitumor effect of the drug by the early neutralization of DDP.

Moreover, AT-II selectively enhances drug delivery to tumor tissue by a vasoconstricting action that selectively occurs in normal tissues but not in tumors [10, 36]; in the present study, drug delivery may have been favored by the fact that DDP entered the systemic circulation from the peritoneal cavity. This selective enhancement of drug delivery was suggested by the finding that rats treated with 5 mg/kg DDP plus AT-II survived much longer than those treated with 5 mg/kg DDP alone (Table 1).

In the clinical field, anticancer chemotherapy taking advantage of AT-II pharmacokinetics, so-called hypertension chemotherapy, has been used without serious side effects in Japan [20, 28, 35, 37]. Combination chemotherapy using DDP and STS is often prescribed to treat patients with peritoneally disseminated tumors [9, 12, 13, 25] because DDP has a rapid cytocidal effect and good activity against various cancers, particularly ovarian cancer [44]. Moreover, DDP can be given i.p. with few local complications, as has been reported with high-dose DDP (270 mg/m^2) injected into the human peritoneal cavity in combination with STS, with no local toxicity [12]. In our experiments, chemical peritonitis never occurred in rats given 20 mg/kg i.p. DDP. STS has long been clinically used at high doses for a renal function test [26] and for therapy in cyanide poisoning [5]. This combination therapy using DDP, STS, and AT-II for treating human peritoneally disseminated tumors warrants further attention.

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