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Effect of thymidine on the toxicity and antitumor activity of *Cis*-diamminedichloroplatinum (II)

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Summary. *The effect of thymidine (TdR) on the preclinical toxicity of cis-diamminedichloroplatinum (II) (DDP) was investigated in the BDF_1 mouse and the Sprague-Dawley rat. The effect of TdR on the antitumor activity of DDP was investigated using the ascites P388 murine leukemia model. TdR at 500 mg/kg consistently decreased the recovery of body weight after DDP treatment IP, but did not affect the lethal toxicity of DDP to non-tumor-bearing mice or those with the P388 murine leukemia. This effect was greatest when TdR was injected 30 min prior to DDP and at higher doses of DDP. A 500-mg/kg dose of TdR did not affect the antitumor activity of DDP 5 mg/kg administered on days 1, 5, and 9. Treatment of rats with TdR 500 mg/kg according to various schedules of timing relative to a 5-mg/kg dose of DDP did not consistently affect the DDP-related loss in body weight or nephrotoxicity at day 3. Pretreatment of mice with TdR 1,500 mg/kg 30 min prior to DDP 5 mg/kg (every 4 days × 3) resulted in a slower recovery of body weight, which became more pronounced with increasing doses of DDP. Pretreatment of ascites P388-bearing mice with TdR 1,500 mg/kg increased the number of early deaths when mice were treated with DDP 5 mg/kg (days 1, 5, and 9). These data suggest that the cytotoxicity of DDP is increased by TdR only at higher doses of either drug, but that the antitumor activity against P388 murine leukemia is not affected.*

Introduction

Considerable interest has been generated in the use of thymidine (TdR) in combination chemotherapy to increase the efficacy of antitumor drugs. TdR decreases the toxicity of methotrexate [7] but increases the toxicity of 5-fluorouracil [5, 8], ftorafur (FT) [3], and 1-β-D-arabinofuranosylcytosine (ara-C) [4] in vivo. Drewinko [2] has demonstrated that TdR increases the cytotoxicity of *cis*-diamminedichloroplatinum (II) (DDP) and ara-C to the LoVo cell line in vitro. Co-administration of TdR also increases the toxicity of FT to P388 murine leukemia and the BDF_1 host [3]. This study was designed to evaluate the effect of TdR on the preclinical toxicity and antitumor activity of DDP.

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Methods

Male BDF_1 mice and Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA) were used in the experiments. Upon arrival the animals were housed in plastic colony cages with woodchip bedding and allowed at least a 7-day acclimation period before use. They were given food and water ad libitum from the time of arrival throughout the experiment. DDP and TdR were administered as saline solutions, the combinations being administered via separate syringes. For all treatments except the high-dose TdR (HDTdR, 1,500 mg/kg), drug solutions were administered at 0.1 ml/10 g body weight. For HDTdR, the volume was 0.3 ml/10 g body weight.

Host toxicity. To determine the effect of TdR on the lethal toxicity of DDP, normal mice were pooled in a common cage, weighed, and randomly assigned to treatment groups so that the weight range of the five to seven mice in each group was less than 3 g. The average weight of the group on the day of treatment was used to determine the total volume of drug solution administered per mouse. Controls were treated with normal saline. Mice were weighed and observed for lethality for 30 days. The dose of DDP was escalated by 0.2 log increments, with and without TdR, until 100% toxicity was observed.

To determine the effect of TdR on the nephrotoxicity of DDP 5 mg/kg normal rats were assigned to groups at random, weighed individually, and treated with either DDP or DDP and TdR at 500 mg/kg IP at the intervals indicated. Three rats were treated according to each of the four regimens.

Antitumor activity. Mice were inoculated IP with 10^6 P388 murine leukemia cells from the laboratory stock maintained by weekly inoculation of 10^6 cells per DBA_2 mouse. On day 1 after tumor implant the mice were weighed and distributed as previously described for toxicity studies. Treatment was administered on days 1, 5, and 9, the volume of drug solution being determined as previously described. The experiment was terminated when all mice had died or at 45 days after the last treatment. Mice surviving longer than 45 days were considered 'cured', and were not included in the calculation of % T/C. The % T/C was calculated from the mean survival time for drug treated mice (T) and saline-treated controls (C).

Results

The effect of TdR 500 mg/kg on the lethal toxicity of a single dose of DDP is shown in Table 1. Although the lethal toxicity

Table 1. Lethal toxicity of DDP alone or in combination with TdR 500 mg/kg

Dose (mg/kg) DDP	Simultaneous[a, b]		Pretreatment[a, c]	
	Saline	TdR	Saline	TdR
8	0/5	1/5	0/6	0/6
13	0/5	1/5	1/6	1/6
21	2/5	5/5	6/6	6/6
25	5/5	5/5	–	–

[a] (Number of mice dying)/(number of mice treated)
[b] DDP and saline or TdR 500 mg/kg were administered simultaneously IP at 0.1 ml drug solution per gram body weight
[c] Saline or TdR 500 mg/kg was administered IP 30 min before DDP injection

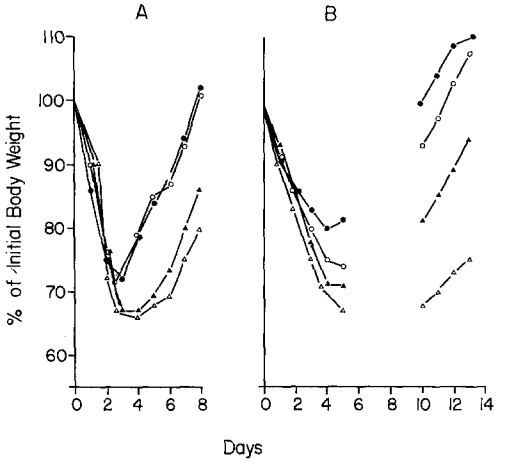

Fig. 1A, B. Body weight change for mice treated with DDP 8 *(circles)* and 13 *(triangles)* mg/kg alone *(closed symbols)* or in combination with TdR 500 mg/kg *open symbols)*. **A** Change after simultaneous DDP and TdR treatment; **B** change when TdR preceded DDP by 30 min. Treatment was daily for one course

Table 2. Effect of TdR on the antitumor activity of DDP against P388 murine leukemia in BDF_1 mice

Dose (mg/kg)[a]		Schedule TdR-DDP[b]	Mean survival[c] (days; ± SE)	% T/C	Wt loss[d]	
DDP	TdR				10	20
5	0	–	26.3 ± 0.5	255	83	106
5	500	S	27.4 ± 3.9	266	74	84
5	500	30	29.4 ± 1.5	285	74	77
5	500	60	27.6 ± 1.3	268	73	84

[a] DDP and TdR were administered IP on days 1, 5, and 9
[b] TdR was injected simultaneously (S) with DDP or 30 or 60 min before DDP
[c] Survival for saline-treated controls was 10.3 ± 0.2 days
[d] As percent age of body weight at initial treatment

of DDP alone varied between experiments, neither the simultaneous treatment nor the 30-min pretreatment with TdR significantly affected the lethal toxicity of DDP. Figure 1 illustrates the body weight loss and recovery as a percentage of the initial weight for mice treated with DDP at 8 and 13 mg/kg in combination with saline or TdR 500 mg/kg. Although the loss of body weight at day 3 was identical, the recovery time increased as the dose of DDP increased. Co-administration of TdR at 500 mg/kg resulted in a slight DDP-dose-related increase in loss of body weight, while pretreatment of mice with TdR 30 min before DDP resulted in a decreased recovery of body weight. This increased as the dose of DDP increased from 8 to 13 mg/kg.

To determine the effect of TdR 500 mg/kg on the antitumor activity of DDP, a schedule with treatment on days 1, 5, and 9 was chosen, TdR being administered simultaneously with or 30 or 60 min before a 5-mg/kg dose of DDP. TdR had no effect on the antitumor activity of DDP against ascites P388 murine leukemia (Table 2). Host toxicity, as measured by loss of body weight, was consistently greater at day 10 in mice that received 500 mg/kg of TdR in combination with DDP (74%, 74%, and 73%) than in those treated with DDP alone (83%). By day 20, the mice that had received TdR simultaneously with or 60 min before DDP had gained up to 84% of their initial weight, while mice treated with TdR 30 min before DDP had gained up to only 77% of their initial weight. Mice treated with DDP alone gained up to 106% of their initial weight by day 20.

The effect of TdR 500 mg/kg on the toxicity of DDP was further examined in the Sprague-Dawley rat. At day 3 after IP treatment, rats had lost 7%–8% of their initial body weight when DDP was given alone and 2%–12% when DDP was administered in combination with TdR. Histological examination of the kidneys showed that TdR had no consistent effect on the DDP-related kidney injury.

The values for BUN were elevated somewhat when TdR was administered either before or after DDP, but not when the drugs were administered simultaneously. BUN values did, however, tend to correlate consistently with the loss in body weight (Table 3).

The effect of HDTdR was investigated using a 1,500-mg/kg dose of TdR. This dose was chosen to give a 300 : 1 dose ratio (TdR : DDP) when mice were treated with DDP 5 mg/kg. Since the solubility of TdR dictates injection of a larger volume of drug solution, the SC route of administration was used; DDP was administered IP as before.

The toxicity of DDP 4 and 6 mg/kg preceded by a SC dose of saline or of TdR 1,500 mg/kg every 4 days × 3 is illustrated in Fig. 2. Although the loss of body weight of mice treated with DDP 6 mg/kg alone was greater, and the recovery somewhat slower, than for mice treated with 4 mg/kg, the body weight of both groups had recovered to above the original weight by day 28. The weight loss in mice pretreated with HDTdR 30 min before DDP 4 mg/kg was not different from that in mice treated with the same dose of DDP alone up to day 12. The recovery was slower but was complete by day 28, as in mice treated with DDP 4 or 6 mg/kg. Mice that received the 6-mg/kg dose of DDP 30 min after HDTdR, however, lost a greater proportion of their body weight after the last treatment and failed to recover to above 80% of the original body weight by day 28.

The effect of TdR 1,500 mg/kg on the antitumor activity of DDP at 5 mg/kg on days 1, 5, and 9 is shown in Table 4. HDTdR alone had no activity against the P388 murine leukemia. The % T/C when mice were pretreated with TdR 1,500 mg/kg was significantly ($P < 0.05$) lower than that when mice were treated with the DDP alone, while the number of 45-day survivors was about the same (3/7 vs 2/7). Early death

Table 3. Nephrotoxicity of DDP alone or in combination with TdR for Sprague-Dawley rats

Dose (mg/kg)		Rat No.	Schedule TdR treatment[a]	% Wt loss[b]	Histopathologic diagnosis[c]	BUN[d] (mg/dl)
DDP	TdR					
5	0	1		7	Nephrosis, multifocal^{++}	54
		2		8	Nephrosis, multifocal^{++}	
		3		7	Nephrosis^{++}	
				$\bar{x}$ = 7.3		
5	500	1	30 min	10	Nephrosis, focal^{+}	78
		2	prior	8	Nephrosis, focal^{+}	
		3		13	Nephrosis, multifocal^{+}	
				$\bar{x}$ = 15.5		
5	500	1	Simultaneous	5	Nephrosis, focal^{+}	56
		2		8	Nephrosis, focal^{+}	
		3		2	Nephrosis, focal^{+}	
				$\bar{x}$ = 5.0		
5	500	1	30 min	7	Nephrosis, focal^{+}	69
		2	after	12	Nephrosis, focal^{+}	
		3		8	Nephrosis, focal^{+}	
				$\bar{x}$ = 9.0		

[a] Schedule of TdR treatment in relation to DDP treatment. Both drugs were administered IP
[b] Wt loss at day 3 after treatment
[c] Lesions scored as follows: $^{+}$ = minimal; $^{++}$ = mild; $^{+++}$ = moderate; $^{++++}$ = severe
[d] Blood urea nitrogen, determined for combined serum samples on day 3 after treatment. The BUN for a control rat gaining 3.5% was 20 mg/dl

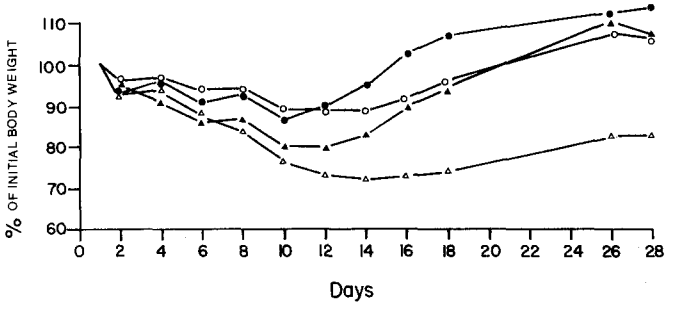

Fig. 2. Body weight change for mice treated with DDP 4 *(circles)* and 6 *(triangles)* mg/kg of DDP alone *(closed symbols)* or with TdR 1,500 mg/kg *(open symbols)*. TdR was administered 30 min prior to DDP. Treatment was given every 4 days for three courses

Table 4. Effect of high-dose TdR on the antitumor activity of DDP against P388 murine leukemia in BDF_1 mice

Dose[a]		Survival (days)	Survivors at 45 days	% T/C	Wt loss day 9[c]
DDP	TdR				
0	1,500	10, 10, 11 11, 11, 11	0	102	103
5	0	23, 27 28, 29	3	255	87
5	1,500	9, 10, 10 11, 26	2	126[b]	78

[a] DDP was administered IP, TdR was administered SC. For combination therapy, TdR was injected 30 min before DDP. The treatment was given on days 1, 5, and 9
[b] Survival of the saline treated controls was 10.5 ± 0.2 days
[c] As percent age of body weight at initial treatment

for the DDP-HDTdR group occurred in the absence of signs of tumor and the presence of severe weight loss (78% of the day-1 body weight).

Discussion

The antitumor activity of DDP 5 mg/kg was not affected by combination therapy with TdR 500 or 1,500 mg/kg. The % T/C was decreased when DDP was combined with TdR 1,500 mg/kg, while the number of 'cures' was the same, indicating an increase in host toxicity but not antitumor activity. The activity of DDP alone is similar to that previously reported [1], the optimal activity of DDP (% T/C = 345) against P388 leukemia being 4.5 mg/kg following treatment on days 1, 5, 9, and 13. The toxic dose of DDP to non-tumor-bearing mice is within the range reported earlier [6]. Administration of TdR at 500 or 1,500 mg/kg did not affect this lethal toxicity of DDP to normal mice. The toxicity of DDP to the BDF_1 mouse, as measured by a failure to regain body weight, was consistently increased by TdR. This effect was greater at the higher doses of DDP and when the TdR was injected 30 min prior to DDP.

TdR at 500 mg/kg had no consistent effect on body weight or on the nephrotoxicity of a 5-mg/kg dose of DDP in the Sprague-Dawley rat as measured by BUN or seen histologically.

These data suggest that the cytotoxicity of DDP is increased by TdR at higher doses of DDP and TdR, but that the antitumor activity against ascites P388 murine leukemia is not affected.

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