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Potentiation of the antitumor activity of cisplatin in mice by 3-aminobenzamide and nicotinamide

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Summary. 3-Aminobenzamide (3AB) and nicotinamide (NA), inhibitors of adenosine-ribose transferase (ADPRT), potentiated the antitumor activity of cisplatin (DDP) on Ehrlich ascites carcinoma in mice. The mean survival times of the mice increased from 21.2–37.0 days in DDP-treated groups to 47.0–54.6 days in mice treated with DDP plus NA or 3AB. These drugs also potentiated DDP antitumor activity on sarcoma 180, with the inhibition rates increasing from 12.4%–20.8% in groups treated daily with DDP to 29.8%–46.4% in those treated with DDP plus NA or 3AB; however, neither 3AB nor NA alone showed any antitumor activity. The single-dose lethality of DDP on mice was partially reversed by either NA or 3AB. The pathological study revealed that the morphologic changes in the proximal tubules 1 month after a single dose of DDP (10 mg/kg) were partially prevented by a single protective dose (5 mmol/kg) of NA or 3AB. Our results suggest that the combination of DDP with ADPRT inhibitors might be used clinically in the future.

Introduction

Cisplatin (DDP) is a potent cancer chemotherapeutic agent with wide clinical use in a variety of tumors such as testes cancer, ovarian cancer, lung cancer, and head and neck cancer. Unfortunately, however, its clinical use is limited by its nephrotoxicity [6].

Nicotinamide (NA) and 3-aminobenzamide (3AB) are inhibitors of adenosine-ribose transferase (ADPRT), the enzyme found in the nuclei of eukaryotes that carries out important biological functions in DNA repair, DNA replication, and cell differentiation [8]. It has been reported that NA and 3AB can enhance the antitumor activity of bleomycin in various animal tumors [1–3, 7]. The present experiments were designed to evaluate the effects of NA and 3AB on the antitumor activity as well as toxicity of DDP.

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Materials and methods

Materials. DDP powder and solution (10 mg/2 ml per ampule) were provided by Kunming Noble Metal Institute, Kunming, China; 3AB was donated by Toyko Kasei Chemical Co., Toyko, Japan; and NA was purchased from Shanghai Second Reagent Factory, Shanghai, China. DDP powder was dissolved in physiological saline immediately before use. DDP solution, used only in the toxicity study, was diluted with physiological saline at a final concentration of 1 mg/ml just before its administration. 3AB and NA were dissolved in physiological saline with or without DDP according to the administration schedule at a final concentration of 0.25 m*M*/ml. NIH mice were provided by the Animal House of our institute, and Kunming mice were provided by the Experimental Animal Laboratory of our university; all animals were maintained on a basal diet in our laboratory.

Antitumor study. Antitumor tests were conducted in NIH male and female mice weighing 20±3 g divided into several groups consisting of 8–10 mice each, according to the randomized block design after matching for age, sex, and weight. Once a week, sarcoma 180 and Ehrlich ascites carcinoma were transplanted s.c. and i.p., respectively, in our laboratory. The drugs were injected i.p. 24 h after the inoculation of 1×10^6 tumor cells. For the evaluation of their growth-inhibiting effect on sarcoma 180 in mice, the animals were sacrified 10 days after the inoculation, the tumors were isolated and weighed, the inhibition rate (IR) was calculated by the formula IR = (1-T/C)×100%, and the results were analyzed using Student's *t*-test.

In the study on mice bearing Ehrlich ascites carcinoma, the drugs were given i.p. daily for 7 days. The mean survival time was calculated from the day of tumor inoculation (survival periods lasting more than 60 days were recorded as 60 days). The percentage of increase in life span (ILS) was calculated according to the formula ILS = (T/C-1)×100%.

Lethality study in Kunming mice. Male Kunming mice (20±2 g) were randomly distributed into several groups of 10 mice each. The numbers of surviving mice were recorded daily for 30 days after the single-dose treatment (DDP alone or with NA or 3AB).

Pathological observation. At the end of the lethality experiments, the surviving mice (groups treated with 10 mg/kg DDP with or without NA or 3AB) were killed and the kidneys were removed and fixed in 10% formaldehyde. The kidney slices were processed in hematoxylin and eosin, and the stained sections were subsequently prepared for histopathological observation.

Results

Experiments with sarcoma 180 in NIH mice

Table 1 shows that the antitumor effect of DDP was significantly enhanced by NA and 3AB (2.5 mmol/kg) in experiments I and II. Furthermore, this enhancement seemed to be more significant when the duration of the treatment was prolonged from 7 to 10 days; however, at the same dose, neither NA nor 3AB alone showed any inhibiting activity on the growth of sarcoma 180 in mice. We also investigated the intermittent combination therapy against tumor growth (Table 1, experiments III and IV). Treatment with DDP (1 mg/kg) plus NA or 3AB (5 mmol/kg given on days 1, 4, and 7) resulted in an increase in tumor growth inhibition over that using cisplatin alone. However, with the smaller dose of NA or 3AB (2.5 mmol/kg), no significantly enhanced effects for this combination appeared. However, when the treatment schedule was increased from 3 to 5 days (on days 1, 3, 5, 7, and 9 instead of 1, 4, and 7), 2.5 mmol/kg 3AB could still statistically increase the inhibition rate of DDP.

Experiments with Ehrlich ascites carcinoma in NIH mice

The same doses of these drugs were used to explore the effect of a combination of DDP with NA or 3AB on Ehrlich ascites carcinoma in NIH mice. In two separate experi-

Table 1. Effects of NA and 3AB on the antitumor activity of DDP on sarcoma 180 in NIH mice

Experiment[a]	Treatment[b]	Administration schedule	Tumor weight $\bar{x} \pm SD$ (g)	Inhibition rate (%)	P^c
I	NS		2.18±0.44		
	NA (2.5 mmol/kg)		2.27±0.46	−4.1	>0.5*
	3AB (2.5 mmol/kg)		2.30±0.52	−5.5	>0.5*
	DDP (0.4 mg/kg)		1.91±0.38	12.4	>0.1*
	DDP (0.4 mg/kg) + NA (2.5 mmol/kg)	i.p., qd×7	1.32±0.39	39.4	<0.001* <0.005**
	DDP (0.4 mg/kg) + 3AB (2.5 mmol/kg)		1.53±0.31	29.8	<0.002* <0.05**
II	NS		1.83±0.37		
	NA (2.5 mmol/kg)		1.85±0.25	−1.1	>0.5*
	3AB (2.5 mmol/kg)		1.72±0.39	6.0	>0.5*
	DDP (0.4 mg/kg)		1.45±0.23	20.8	<0.02*
	DDP (0.4 mg/kg) + NA (2.5 mmol/kg)	i.p., qd×10	1.08±0.19	41.0	<0.001* <0.001**
	DDP (0.4 mg/kg) + 3AB (2.5 mmol/kg)		0.98±0.21	46.4	<0.001* <0.001**
III	NS		2.20±0.37		
	DDP (1 mg/kg)		1.90±0.27	13.6	>0.05*
	DDP (1 mg/kg) + NA (2.5 mmol/kg)		2.04±0.35	7.2	>0.1* >0.1**
	DDP (1 mg/kg) + NA (5 mmol/kg)	i.p., qd on days 1, 4, and 7	1.42±0.24	35.5	<0.001* <0.002**
	DDP (1 mg/kg) + 3AB (2.5 mmol/kg)		1.59±0.37	27.7	<0.005* >0.05**
	DDP (1 mg/kg) + 3AB (5 mmol/kg)		1.20±0.18	45.5	<0.001* <0.001**
IV	NS		1.99±0.45		
	DDP (1 mg/kg)		1.63±0.33	18.1	<0.05*
	DDP (1 mg/kg) + NA (2.5 mmol/kg)		1.35±0.30	32.2	<0.002* >0.05**
	DDP (1 mg/kg) + NA (5 mmol/kg)	i.p., qd on days 1, 3, 5, 7, and 9	1.01±0.17	49.2	<0.001* <0.002**
	DDP (1 mg/kg) + 3AB (2.5 mmol/kg)		1.10±0.30	44.7	<0.001* <0.002**
	DDP (1 mg/kg) + 3AB (5 mmol/kg)		0.95±0.31	52.3	<0.001* <0.001**

[a] In experiments I, II, and IV, each group consisted of ten mice, in experiment III of nine mice
[b] NS, physiological saline
[c] * vs control; ** vs DDP

Table 2. Effects of NA and 3AB on the antitumor activity of DDP on Ehrlich ascites carcinoma

Experiment[a]	DDP (mg/kg)	NA (mmol/kg)	3AB (mmol/kg)	No. of mice	Mean survival time $\bar{x} \pm SD$ (days)	ILS (%)	S60[b]	P[c]
1	0	0	0	8	13.4± 5.9		0	
	0	2.5	0	8	14.4± 3.6	7.5	0	>0.5*
	0	0	2.5	8	15.1± 3.1	12.7	0	>0.2*
	0.4	0	0	8	37.0± 6.1	176.1	0	<0.001*
	0.4	2.5	0	8	47.8± 9.1	256.7	1	<0.001* <0.02**
	0.4	0	2.5	8	47.0±11.1	250.7	2	<0.001* <0.05**
2	0	0	0	10	9.0± 2.4		0	
	0	5	0	10	9.3± 3.3	3.0	0	>0.5*
	0	0	5	10	9.9± 3.6	10.0	0	>0.5*
	0.4	0	0	10	21.2± 3.4	135.5	0	<0.001*
	0.4	5	0	10	54.6± 8.0	506.7	6	<0.001* <0.001**
	0.4	0	5	10	50.5±10.7	461.1	5	<0.001* <0.001**

[a] Drugs were given IP, qd × 7, in experiments 1 and 2
[b] Number of mice surviving longer than 60 days
[c] * vs control, ** vs DDP treatment alone

ments (Table 2), the mean survival times in DDP-treated groups were 37.0 and 21.2 days, with a percentage of increase in life span (ILS) of 176.6% and 135.5%, respectively, as compared with the control group. The addition of NA or 3AB at a dose of 2.5 mmol/kg in experiment I increased the mean survival times to 47.8 and 47.0 days, with an ILS value of 256.7% or 250.7%, respectively. In these groups, one tumor-bearing mouse in the DDP plus NA group survived more than 60 days, as did two in the DDP plus 3AB group. These enhanced therapeutic effects seemed to be more conclusive when the inhibitors were used at higher doses (5 mmol/kg): the mean survival time was 54.6 days and the ILS value was 506.6% in the NA plus DDP group, with six of ten mice surviving longer than 60 days; in the 3AB plus DDP group, these figures were 50.5 days and 461.1% with five of ten mice surviving longer than 60 days.

Table 3. Single-dose lethality study in Kunming mice

Treatment (IP)	Percentage of mice surviving after single-dose treatment			
	day 5	day 10	day 15	day 30
DDP (mg/kg)				
5	100	100	100	100
10	50	40	20	20
20	0	0	0	0
3AB (5 mmol/kg) + DDP (mg/kg)				
5	100	100	100	100
10	100	70	70	70
20	30	10	10	10
NA (5 mmol/kg) + DDP (mg/kg)				
5	100	100	100	100
10	90	90	80	80
20	10	10	10	10

Experiments using NA and 3AB as protection against DDP toxicity

A graded dose level of DDP (5, 10, and 20 mg/kg) was given i.p. simultaneously with a single protective dose of NA or 3AB (5 mmol/kg). Table 3 represents the experimental results of a 30-day observation, showing that NA or 3AB can reduce the acute lethality of DDP up to a dose of 10 mg/kg within 15 days.

By the end of the lethality experiments, the kidneys of surviving mice were removed for morphological observation. Those in the DDP-treated group (10 mg/kg) were macroscopically swollen and pale. The most significant features of microscopic renal lesions in this group were de-

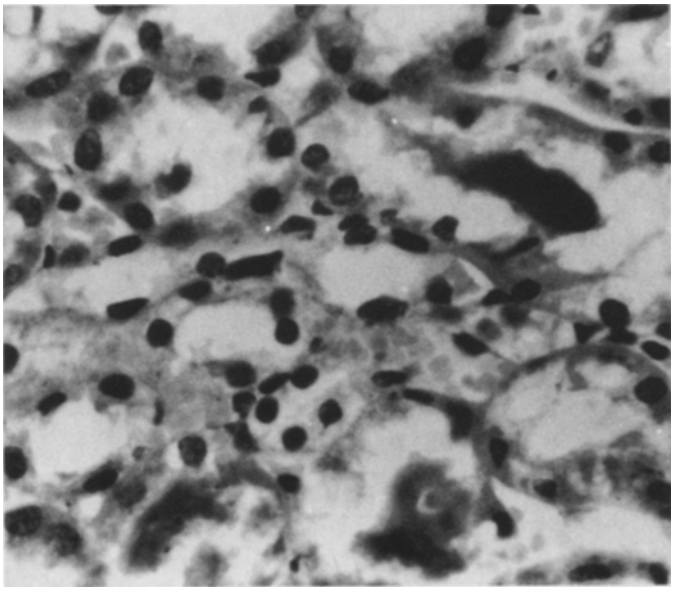

Fig. 1. Tubular necrosis and protein casts in the tubular lumens of the proximal tubules of mice treated with a single dose of DDP (10 mg/kg). ×400

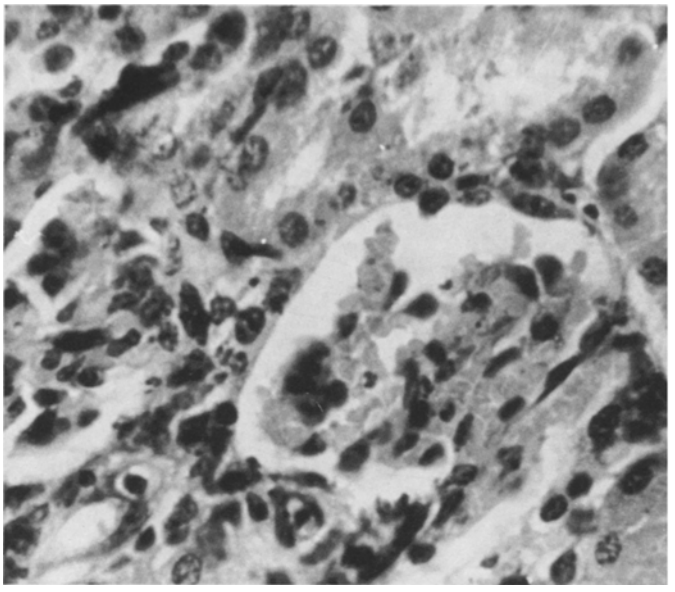

Fig. 2. Vacular and granular tubular degeneration in mice treated with a single dose of DDP (10 mg/kg). ×400

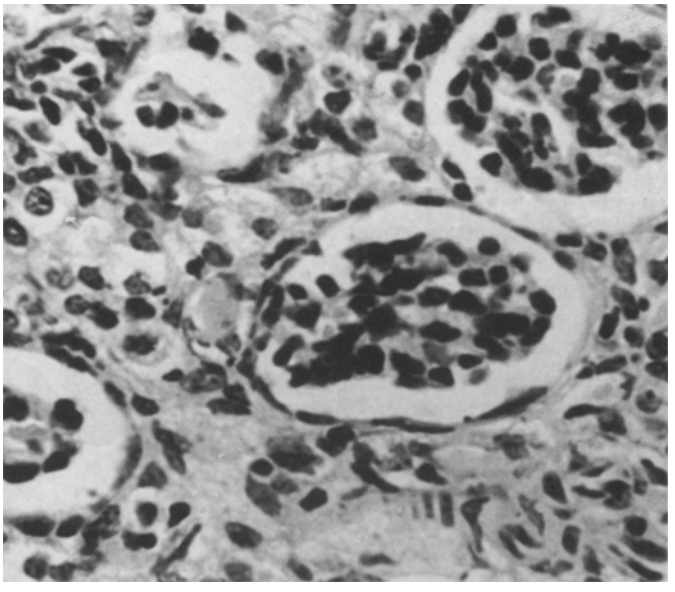

Fig. 3. Slight vacular and granular tubular degeneration in mice treated with DDP (10 mg/kg) plus NA (5 mmol/kg). ×400

generative changes in the proximal tubules, namely, tubular vacuolar and granular degeneration (Fig. 2). The eosinophilic protein casts could also be seen in the tubular lumens. Some proximal tubules exhibited necrosis with complete destruction of their structures (Figs. 1 and 2). In the combination treatment groups, there were only slight degenerative changes in the proximal tubules, but no protein casts or necrosis were found (Figs. 3 and 4).

Discussion

The enhancing effect of NA or 3AB on DDP antitumor activity

The data presented in this paper demonstrate that NA or 3AB at nontoxic doses can potentiate DDP antitumor activity and reduce its acute lethality on mice. These finding are in general agreement with those thus far reported. Schein et al. [12] found in 1967 that NA pretreatment resulted in increased survival times in L1210 and P388 leukemic mice over that observed after streptozotocin treatment alone; a single dose of NA (500 mg/kg) partially prevented the lethality of a single dose of streptozotocin (225–425 mg/kg). In 1984, Erlichman et al. [5] reported that 3AB decreased the clonogenic survival of EMT/6 murine fibrosarcoma cells treated with DDP in vitro. However, there have been no reports on the enhancement of DDP antitumor activity or the reduction of DDP toxicity by either NA or 3AB in vivo. In the present study, neither NA nor 3AB at a dose range of 2.5–5.0 mmol/kg displayed anticancer activity, but they enhanced the therapeutic activity of DDP on sarcoma 180 as well as Ehrlich ascites carcinoma. Furthermore, we found that either NA or 3AB could circumvent the acute lethality of DDP. This is very encouraging, for as yet there has been no especially useful method for conquering the nephrotoxicity of DDP [6], although adequate hydration and osmotic diuresis can significantly reduce its overall clinical incidence [10, 11].

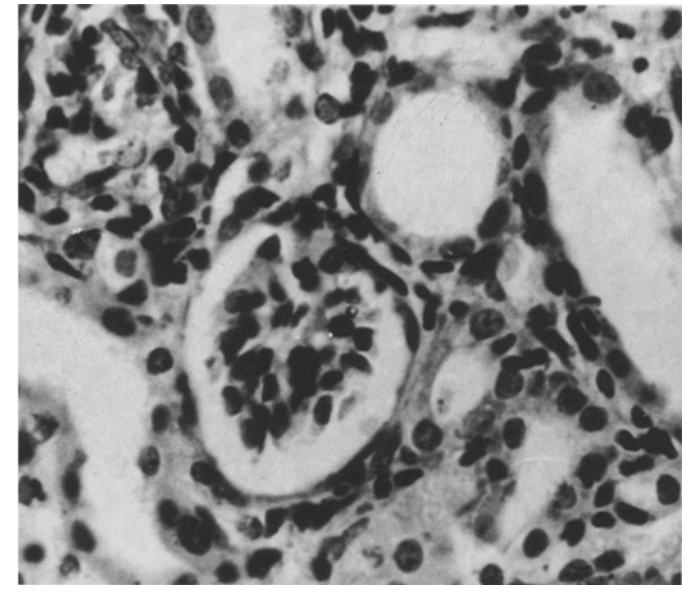

Fig. 4. Slight vacular and granular tubular degeneration in mice treated with DDP (10 mg/kg) plus 3AB (5 mmol/kg). ×400

Possible mechanisms involved in the enhancement of DDP antitumor activity by NA and 3AB

It is known that cisplatin can induce DNA cross-links and that the extent of such cross-links parallels its cytotoxicity as measured by soft agar cloning with various tumor lines [11]. Changes in DNA sedimentation and DNA alkaline elution profiles from DDP-treated cells proportional to the time following drug treatment have demonstrated DNA cross-link formation and their subsequent removal, which indicates that DNA cross-link repair might have occurred [14]. The DNA-repair ability and the magnitude of DNA damage in DDP-treated cells determine the cell's ultimate survival. Masuda et al. [9] have recently reported that increased DNA repair in DDP-treated human ovarian cancer cell lines was associated with induced resistance to the drug. On the other hand, 3AB and NA can inhibit DNA

excision repair in L1210 and In111R cells following treatment with dimethyl sulfate and streptozotocin by inhibiting the repair of DNA strand breaks [4, 13]. Thus, 3AB and NA might inhibit DNA cross-link repair induced by DDP; an investigation of this possibility is under way in our laboratory. The mechanisms by which 3AB and NA reverse the nephrotoxicity of DDP are at present difficult to explain, because the mechanism of DDP renal toxicity is not known. However, the latter question has challenged many scientists and should be answered in the future.

A drug that possesses no antitumor activity itself but can potentiate the antitumor activity and reduce the toxicity of conventional anticancer drugs should be of value in cancer chemotherapy. With these two qualifications, it is hoped that the combination of DDP and the ADPRT inhibitors 3AB and NA will be found useful in clinical cancer chemotherapy.

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