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COMBINATION THERAPY OF *SCHISTOSOMA JAPONICUM* BY TUBERCIDIN AND NITROBENZYLTHIOINOSINE 5′-MONOPHOSPHATE*

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Abstract—Coadministration of nitrobenzylthioinosine 5′-monophosphate (NBMPR-P) with high doses of tubercidin by i.p. injection into *Schistosoma japonicum* infected mice beginning 5 weeks post-infection was highly toxic to the parasite but not the hose. Combination therapy resulted in a striking reduction in the number of worms, and the few worms that could be found were stunted. Combination therapy also caused a drastic reduction in the number of eggs in the livers (from 86,500 to 2,800 eggs/liver) and intestines (from 2,200 to 74 eggs/cm^2), and 95% of eggs that were found were dead, indicating the termination of oviposition. Mice receiving the combination of tubercidin plus NBMPR-P appeared healthy and had normal size livers and spleens. These results demonstrate that by combining NBMPR-P with tubercidin high selective toxicity against *S. japonicum* can be achieved, as was shown previously with *S. mansoni*.

Recently, we have shown that, in contrast to mammalian cells, nitrobenzylthioinosine 5′-monophosphate (NBMPR-P‡), dipyridamole and dilazep [1, 2] do not inhibit the uptake of several nucleoside analogues, including tubercidin (7-deazaadenosine), in *Schistosom mansoni* [1, 2]. We have exploited this difference in nucleoside uptake between the parasites and the host and used these nucleoside transport inhibitors to protect the host from the toxicity of some antischistosomal nucleoside analogues [1, 2]. When we coadministered NBMPR-P with high doses of tubercidin, the combination was selectively toxic against the parasite [1]. Since it is known that the effectiveness of a drug against one species of schistosomes does not necessarily predict that the same is true for other species, we have tested the effects of combination therapy with tubercidin and NBMPR-P on *S. japonicum*. *S. japonicum* is endemic in Asia and the South Pacific and is the most resistant of all human schistosomes species to chemotherapy. A preliminary report has been presented [3].

MATERIALS AND METHODS

Chemicals. Tubercidin was obtained from the Sigma Chemical Co., St. Louis, MO; NBMPR-P was a gift from Dr. A. R. P. Paterson, Cancer Research Unit (McEarchern Laboratory), University of Alberta, Edmonton, Alberta, Canada.

Animals. Female CD1 mice (20 g) infected with *ca.* 50 cercariae of *S. japonicum* (Chinese strain) were obtained from Dr. Y. S. Liang, The University of Lowell, Lowell, MA.

Chemotherapy of infected mice. Mice were treated beginning at 5 weeks post-infection, a period sufficient for completion of gametogenesis, pairing of adult worms, and oviposition [4]. This was confirmed by quantitative examination of three mice before initiation of treatment (Tables 1–3). Mice were then treated with either tubercidin alone (5 mg/kg/day × 4), NBMPR-P alone (25 mg/kg/day × 4), or a combination of tubercidin plus NBMPR-P at these same doses. A second series of four daily injections was administered following a 10-day rest period. Drugs were dissolved in saline solution (0.9% NaCl) and administered by i.p. injection in volumes that were proportional to 0.2 ml/20 g body wt. The nontreated control group received saline only without the drugs.

Criteria of therapeutic effects. All surviving infected mice were killed at 9–10 weeks after infection, and the following variables were monitored: the weight and condition of the liver and the spleen, and the number, sex, copulatory status and morphology of worms recovered by hook dissection of the portal and mesenteric veins [5]. The number of eggs per liver was counted after overnight digestion at 37° in a solution of 1% KOH and 0.9% NaCl [6]. The number and developmental stage of eggs per cm^2 of the intestine were an average count from three 2-cm segments of the small intestine using the protocol described by Pelligrino *et al.* [7].

RESULTS

The results in Table 1 clearly demonstrate that the coadministration of NBMPR-P with tubercidin protected the animals from the lethal effect of tubercidin alone. All mice receiving tubercidin alone died

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‡ Abbreviations: NBMPR, nitrobenzylthioinosine or 6-[(4-nitrobenzyl)thio]-9-β-D-ribofuranosylpurine; and NBMPR-P, nitrobenzylthioinosine 5′-monophosphate.

Table 1. Effects of tubercidin and NBMPR-P on the survival of mice infected with *S. japonicum* and on the weight of liver and spleen*

Treatment	Number of mice surviving	Weight (g)	
		Liver	Spleen
Before treatment†		1.80 ± 0.10	0.20 ± 0.1
Saline controls	10/14	2.59 ± 0.42	0.60 ± 0.13
Tubercidin (5 mg/kg)	0/14		
NBMPR-P (25 mg/kg)	11/14	2.20 ± 0.62	0.44 ± 0.06‡
Tubercidin (5 mg/kg) + NBMPR-P (25 mg/kg)	14/14	2.08 ± 0.14‡	0.24 ± 0.08‡

*Animals received daily i.p. injections for 4 days beginning 5 weeks after infection with *ca.* 50 cercariae per mouse. A second series of four daily injections was administered after a 10-day rest period. Measurements were taken 9–10 weeks after infection. Values are mean ± S.D.

† Data obtained from three mice.

‡ Significantly different ($P < 0.01$) from saline controls.

Table 2. Effects of tubercidin and NBMPR-P on the number and copulation of worms*

Treatment	Number of worms			
	Per surviving mouse	Messenteric veins	Portal vein	*In copula*
Before treatment	21.0 ± 11.3	4.0 ± 5.7	7.0 ± 5.7	16.0 ± 11.3
Saline controls	28.8 ± 11.1	11.3 ± 3.8	17.5 ± 14.9	21.3 ± 1.9
NBMPR-P (25 mg/kg)	20.0 ± 5.7	7.5 ± 6.4	12.5 ± 0.7	19.0 ± 7.1
Tubercidin (5 mg/kg) + NBMPR-P (25 mg/kg)	2.5 ± 3.5†	0	2.5 ± 3.5	2.0 ± 2.8

* Animals were treated as described in Table 1. Worms were collected by hook dissection [5] 9–10 weeks after infection. Values are means ± S.D.

† Significantly different ($P < 0.01$) from saline control.

Table 3. Effects of tubercidin and NBMPR-P on number of eggs and stage of embryogenesis*

Treatment	Number of eggs†		% Eggs in stages of embryogenesis					
	Per liver	Per cm^2 of intestine	1	2	3	4	5	Dead
Before treatment	1,190 ± 1,090	85 ± 75	51	13	23	2	0	11
Saline controls	86,500 ± 10,600	2,200 ± 1,500	16	8	11	12	11	42
NBMPR-P (25 mg/kg)	84,000 ± 18,000	1,600 ± 500	16	12	15	3	4	50
Tubercidin (5 mg/kg) + NBMPR-P (25 mg/kg)	2,800 ± 4,000‡	74 ± 147‡	0	0	3	1	0	96

* Animals were treated as described in Table 1. Number of eggs per liver was estimated as described by Cheever [6]. Estimates of the number of eggs per cm^2 of intestine were made from egg counts in three 2-cm segments of the small intestine. Stages of embryogenesis of eggs in the intestine are those described by Pellegrino *et al.* [7].

† Mean ± S.D.

‡ Significantly different ($P < 0.01$) from saline controls.

between days 3 and 5 of treatment. Beginning at 8 weeks post-infection, mice from the groups receiving NBMPR-P or saline alone started to die from the infection. By 9–10 weeks post-infection, four out of the fourteen untreated, and three out of the fourteen NBMPR-P-treated mice had died. On the other hand, there was no mortality among the animals receiving the combination of NBMPR-P plus tubercidin, and these mice appeared healthy and had normal size livers and spleens. Furthermore, preliminary histological examination of kidneys and livers from these animals showed no evidence of the cytotoxicity associated with very high doses of tubercidin and NBMPR-P [8]. All surviving untreated or NBMPR-P-treated mice showed characteristic symptoms and signs of schistosomiasis

such as numerous egg granulomas and enlarged liver and spleen (Table 1).

Combination therapy also caused a significant reduction in the number of worms and, correspondingly, in the number of worms *in copula* (Table 2). The few worms that could be found were stunted. However, the sex of some of the worms was still identifiable, in contrast to the case with *S. mansoni* [1]. A large number of eggs in all stages of development were found in the liver and small intestines of untreated and NBMPR-P-treated animals (Table 3). In animals receiving the combination of tubercidin plus NBMPR-P, there was a drastic reduction in egg numbers from an average of 86,500 to 2,800 eggs per liver and from 2,200 to 74 eggs per cm^2 of the small intestine. Over 95% of all eggs found were dead, indicating that no new eggs were deposited subsequent to treatment. Furthermore, very few granulomas were detected in the livers of these animals.

DISCUSSION

NBMPR-P inhibits the transport of various nucleoside analogues, including tubercidin, in a variety of animal cells [9]. NBMPR-P exerts its effect after dephosphorylation to its active form, NBMPR, by cellular ecto 5′-nucleotidase (5′-ribonucleotide phosphohydrolase, EC 3.1.3.5) [10]. NBMPR inhibits nucleoside transport in mammalian cells by binding tightly but reversibly to the transport sites on these cells (for review, see Ref. 9).

In contrast to mammalian systems, neither NBMPR-P nor NBMPR interferes with the uptake of several nucleoside analogues, including tubercidin, by *S. mansoni* [1, 2], and coadministration of NBMPR-P with tubercidin resulted in a highly selective toxicity against the parasite but not the host [1]. The present results with *S. japonicum* confirm our earlier results with *S. mansoni* [1]. Since the combination therapy was as effective in *S. japonicum* as in *S. mansoni*, it is likely that NBMPR-P did not interfere with the uptake of tubercidin by *S. japonicum*, as was shown with *S. mansoni* [1]. This indicates that a sharp difference in the mechanism of nucleoside uptake exists between both species of schistosomes and their host, or that, in the parasites, cellular nucleoside transport systems may be NBMPR insensitive as in particular lines of animal cells [11, 12]. The inability of NBMPR-P or NBMPR to interfere with the uptake of tubercidin in schistosomes may arise from differences in the mechanism of nucleoside transport between schistosomes and their host. The recent success of the tubercidin plus NBMPR-P combination in the treatment of *Trypanosoma gambiense* [13] was attributed to the absence of NBMPR binding sites on the parasite [14]. Whether or not this is also the case in schistosomes remains to be determined.

The lack of kidney toxicity in treated animals is quite encouraging. This is particularly important in view of the finding of Kolassa *et al.* [8] that the combination of very high doses (LD_{90}) of tubercidin (45 mg/kg) and NBMPR-P (100 mg/kg) protect the livers of mice from tubercidin toxicity, but cause some kidney damage. In the present study we have used much lower doses of tubercidin (5 mg/kg) and NBMPR-P (25 mg/kg). These doses are non-toxic to the host [8] and produced excellent therapeutic results with no deleterious side effects on the treated animals, as evidenced by the lack of toxicity to the kidneys. Thus, it can be suggested that the combination therapy of tubercidin and a nucleoside transport inhibitor can be used safely in the treatment of schistosomiasis and other parasitic diseases.

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