*Biochemical Pharmacology*, Vol. 36, No. 22, pp. 3815–3821, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00


# TREATMENT OF SCHISTOSOMIASIS BY PURINE NUCLEOSIDE ANALOGUES IN COMBINATION WITH NUCLEOSIDE TRANSPORT INHIBITORS*

MAHMOUD H. EL KOUNI,† NORMA J. MESSIER and SUNGMAN CHA
Division of Biology and Medicine, Brown University, Providence, RI 02912, U.S.A.

(*Received* 18 *December* 1986; *accepted* 7 April 1987)

**Abstract**—In contrast to their effects on mammalian cells, the nucleoside transport inhibitors nitrobenzylthioinosine 5′-monophosphate (NBMPR-*P*) dilazep, benzylacyclouridine (BAU), and to a lesser extent, dipyridamole have no significant effect on the *in vitro* uptake of adenosine analogues by *Schistosoma mansoni* [el Kouni and Cha, *Biochem. Pharmac.* **36**, 1099 (1987)]. Coadministration of either NMBPR-*P* or dilazep with potentially lethal doses of tubercidin (7-deazaadenosine), nebularine or 9-deazaadenosine protected mice from the toxicity of these adenosine analogues. Dipyridamole caused partial protection, whereas BAU did not protect the animals from this toxicity. Toyocamycin caused delayed mortality (after 16 weeks) which could not be prevented by coadministration of NBMPR-*P*. In *S. mansoni* infected mice, treated with the combination of NBMPR-*P* and 9-deazaadenosine was not effective against the parasite. On the other hand, the combinations of NBMPR-*P* or dilazep with either tubercidin or nebularine were highly toxic to the parasite but not the host. Combination therapy caused a marked reduction in the number of pairing of worms. Effectiveness of combination therapy could also be noted by a drastic decrease in the number of eggs in the liver and small intestine. All eggs found were dead, indicating a direct effect on ovigenesis. Although dipyridamole was less effective than NBMPR-*P* or dilazep in protecting the host from the toxicity of tubercidin or nebularine, the combinations with dipyridamole produced similar significant therpeutic effects in animals that survived. Mice receiving the combination of tubercidin (or nebularine) plus NBMPR-*P* or dilazep, as well as those that survived the combination with dipyridamole, appeared healthy and were found to have normal size livers and spleens. These results suggest that highly selective toxicity against schistosomes can be achieved by coadministration of various nucleoside transport inhibitors with adenosine analogues.

Purine nucleotides are required by all living organisms for the synthesis of DNA, RNA and other metabolites. Purine nucleotides can be synthesized by *de novo* and/or by the salvage pathways. The *de novo* pathway utilizes simple precursors for the synthesis of various purine nucleotides. The salvage pathways, on the other hand, are reutilization routes by which the organism can satisfy its purine requirement from endogenous and exogenous preformed purines. Unlike their host, schistosomes lack *de novo* purine biosynthesis and depend on the salvage pathways for their purine requirements [1]. Therefore, these parasites can be selectively deprived of vital purines by blocking or interfering with the parasite purine salvage pathways. One way to intervene with the purine salvage pathways is to use one or more of numerous available purine analogues. However, few purine analogues have been tested as antischistosomal drugs because they are either not efficiently metabolized to the nucleotide level by the parasite or are also very toxic to the host [1–6].

An alternative strategy for depriving the schistosomes of purines is to use nucleoside transport inhibitors to block the uptake of purine nucleosides by the parasite. Recently, however, we reported [3, 6] that, in contrast to mammalian systems, the uptake of purine nucleosides by schistosomes is not inhibited significantly by the nucleoside transport inhibitors nitrobenzylthioinosine 5′-monophosphate (NBMPR-*P*‡), dilazep, benzylacyclouridine (BAU) and to a lesser extent by dipyridamole. Therefore, we reasoned that coadministration of a nucleoside transport inhibitor with a toxic purine nucleoside analogue would cause selective toxicity against schistosomes. This indeed was found to be the case when the nucleoside transport inhibitor NBMPR-*P* was coadministered with potentially lethal doses of tubercidin (7-deazaadenosine) to *Schistosoma mansoni*- and *S. japonicum*-infected mice. We were able to protect the host but not the parasite from tubercidin toxicity [3, 4].

In this investigation, we tested whether or not the other nucleoside transport inhibitors, dilazep, dipyridamole and BAU, are as effective as NBMPR-*P* in improving the therapeutic index of tubercidin. We also tested the therapeutic effectiveness of the nucleoside transport inhibitors in combination with other potential antischistosomal purine nucleoside analogues which we have identified previously [6],

* This research was supported by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases and Grant AI-22219 from the NIAID, DHHS.

† Author to whom all correspondence should be addressed.

‡ Abbreviations: BAU, benzylacyclouridine [5-benzyl-1-(2′-hydroxyethoxymethyl)uracil]; and NBMPR-*P*, nitrobenzylthioinosine 5′-monophosphate (6-[(4-nitrobenzyl)thio]-9-β-D-ribofuranosylpurine 5′-monophosphate).

namely nebularine, 9-deazaadenosine and toyocamycin. A preliminary report has been presented [7].

## MATERIALS AND METHODS

*Chemicals.* NBMPR-*P* was a gift from Dr. A. R. P. Paterson, Cancer Research Unit (McEachern Laboratory), University of Alberta, Edmonton, Alberta, Canada; dilazep from Asta-Werke AG, Frankfurt, F.R.G.; 9-deazaadenosine from Dr. R. S. Klein, Sloan-Kettering Institute, Rye, NY; and toyocamycin and nebularine from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. Tubercidin and dipyridamole were purchased from the Sigma Chemical Co., St. Louis, MO. To increase the solubility of dipyridamole, the chloride salt was made by dissolving dipyridamole in methanol and saturating the solution with HCl.

*Animals.* Female CD1 mice (20–25 g) were obtained from Charles River Laboratories, Wilmington, MA.

*Life cycle maintenance.* The life cycle of *S. mansoni* (Puerto Rico strain) was maintained using mice as the primary host and *Biomphalaria glabrata* (Puerto Rico Strain 2) as the vector snail. Mice were exposed to 200 cercariae for 30 min by tail immersion [8]. Miracidia were prepared by hatching eggs from livers of 7–9 weeks infected mice [8, 9]. Groups of 60–70 young snails, 5 mm in diameter, were exposed to about 10 miracidia per snail for 3 hr at 26°. Cercariae were collected from shredding snails 5–12 weeks after infection.

*Chemotherapy of infected mice.* Mice infected with *ca.* 150 cercariae were treated at 5 weeks post-infection, a period sufficient for completion of gametogenesis, pairing of adult worms, and oviposition [10]. This was confirmed by quantitative examination of a sample of ten mice before initiation of treatment (Tables 2–4). Treated mice were given either a nucleoside analogue, tubercidin (5 mg/kg/day), nebularine (37 mg/kg/day) [11], 9-deazaadenosine (1 mg/kg/day) alone, or a nucleoside transport inhibitor (NBMPR-*P*, dipyridamole or dilazep at 25 mg/kg/day) alone, or a combination of a nucleoside analogue plus a nucleoside transport inhibitor at these same doses. Animals received a daily injection of the drug mixture for 4 days. Animals treated with combinations containing tubercidin or nebularine received a second series of four daily injections of the nucleoside analogue plus a nucleoside transport inhibitor following a 10-day rest period [3]. 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 weight. The non-treated control group received saline only without the drugs.

*Criteria of therapeutic effects.* Infected mice that survived were killed at 9–10 weeks after infection, and the following variables were monitored: the number, sex, copulatory status and morphology of worms recovered by portal perfusion [12] or by hook dissection of the portal and mesenteric veins [13], as well as the weight of the liver and the spleen. The number of eggs per liver was counted after overnight digestion at 37° in a solution of 1% KOH and 0.9% NaCl [14]. The number and developmental stages of eggs per $cm^2$ of the intestine were an average count from three 2-cm segments from the small intestine [15].

## RESULTS

*Toxicity of toyacamycin and 9-deazaadenosine in mice and protection by NBMPR-P*

We have shown previously that administration of NBMPR-*P* (25 mg/kg/day) i.p. together with tubercidin (5 mg/kg/day) for 4 days protects mice from the toxicity of tubercidin even when the same regimen is repeated twice [3] or three times (unpublished results) at 10-day intervals. To determine the dose and regimen for toyocamycin and 9-deazaadenosine, the toxicity of different doses of these compounds was studied, and the degree of protection by coadministration of NBMPR-*P* (25 mg/kg/day) was determined. Table 1 presents the results of these experiments. A single i.p. injection of 10 or 20 mg/kg toyocamycin (Regimen A) caused 100% mortality. Coadministration of NBMPR-*P* neither protected the animals from toyocamycin-induced death nor did it improve the mean survival time (MST) of the animals that died. Toyocamycin was also lethal when mice were treated for 4 days with 1.6, 1.8, 2.0 and 2.2 mg/kg/day followed by another series of four daily injections after a 10-day rest period (Regimen B). Incomplete protection (40% survival) by NBMPR-*P* was observed only at the lowest dose of toyocamycin used in this regimen (1.6 mg/kg/day). Although NBMPR-*P* did not prevent lethality at higher doses of toyocamycin in regimen B, it markedly lengthened the MST of the animals that died. Treatment with toyocamycin for 2 days followed by a 12-day rest period and then another 2 days of treatment (Regimen C) proved to be less toxic. Coadministration of NBMPR-*P* at the high dose of tomocamycin (2.2 mg/kg/day) resulted in complete protection against toyocamycin-induced death when survivorship was monitored at 5 weeks after initiation of treatment. However, late mortality, beginning at 16 weeks after initiation of treatment, was observed among the mice that received Regimen C using toyocamycin alone (1.6 and 2.0 mg/kg/day) or toyocamycin (1.6, 2.0 and 2.2 mg/kg/day) plus NBMPR-*P*. Many mice were found to have extensive abdominal adhesions. These results show that toyocamycin has very delayed toxic and lethal effects in mice. Therefore, the use of toyocamycin in chemotherapy should be avoided.

With respect to 9-deazaadenosine, all doses in the regimen used (Regimen D, four daily injections) were lethal when used alone. Complete protection by NBMPR-*P* was observed when it was coadministered with the lower doses (0.625 and 1.0 mg/kg/day) of 9-deazaadenosine (Tables 1 and 2).

*Protection by different nucleoside transport inhibitors against toxicity of tubercidin and nebularine in mice.* Coadministration of NBMPR-*P* at a dose of 25 mg/kg/day with tubercidin (5 mg/kg/day) for 4 days provides protection against the toxicity of tubercidin even when that regimen is repeated after a 10-day rest period [3, 4]. Therefore, to determine whether or not other nucleoside transport inhibitors

Table 1. Toxicity of toyocamycin and 9-deazaadenosine in mice and protection by NBMPR-*P*

| Analogue (mg/kg) | NBMPR-*P* (25 mg/kg) | Regimen* | No. of mice alive† at 5 weeks | No. of mice alive† at 21 weeks | MST‡ (days) | Day of first death |
|---|---|---|---|---|---|---|
| Toyocamycin | | | | | | |
| 10.0 | – | A | 0/5 | 0/5 | 6.6 ± 3.8 | 4 |
| 20.0 | – | A | 0/5 | 0/5 | 4.0 ± 0.0 | 4 |
| 10.0 | + | A | 0/5 | 0/5 | 4.4 ± 0.5 | 4 |
| 20.0 | + | A | 0/5 | 0/5 | 4.0 ± 0.0 | 4 |
| 1.6 | – | B | 0/5 | 0/5 | 9.2 ± 0.4 | 9 |
| 1.8 | – | B | 0/5 | 0/5 | 7.8 ± 1.6 | 6 |
| 2.0 | – | B | 0/5 | 0/5 | 4.4 ± 3.4 | 7 |
| 2.2 | – | B | 0/5 | 0/5 | 6.4 ± 3.2 | 7 |
| 1.6 | + | B | 2/5 | ND§ | 28.5 ± 2.1‖ | 27 |
| 1.8 | + | B | 0/5 | 0/5 | 12.2 ± 5.3 | 6 |
| 2.0 | + | B | 0/10 | 0/10 | 13.3 ± 9.2 | 6 |
| 2.2 | + | B | 0/5 | 0/5 | 10.0 ± 5.3 | 8 |
| 1.6 | – | C | 5/5 | 4/5 | 136.8 ± 20.6 | 100 |
| 2.0 | – | C | 5/5 | 4/5 | 125.4 ± 46.1 | 43 |
| 2.2 | – | C | 0/5 | 0/5 | 14.0 ± 3.7 | 8 |
| 1.6 | + | C | 5/5 | 4/5 | 132.6 ± 32.8 | 75 |
| 2.0 | + | C | 5/5 | 2/5 | 134.0 ± 14.1 | 116 |
| 2.2 | + | C | 5/5 | 2/5 | 111.3 ± 61.8 | 40 |
| 9-Deazaadenosine | | | | | | |
| 0.625 | – | D | 0/10 | 0/10 | 8.8 ± 1.9 | 7 |
| 1.00 | – | D | 0/4 | 0/4 | 7.4 ± 0.5 | 7 |
| 1.25 | – | D | 0/5 | 0/5 | 5.8 ± 1.1 | 5 |
| 2.50 | – | D | 0/5 | 0/5 | 4.0 ± 0.0 | 4 |
| 0.625 | + | D | 4/4 | ND | ND | ND |
| 1.00 | + | D | 4/4 | ND | ND | ND |
| 1.25 | + | D | 0/9 | 0/9 | 6.0 ± 2.4 | 4 |
| 2.50 | + | D | 0/9 | 0/9 | 4.0 ± 0.8 | 3 |

* A, a single i.p. injection; B, four daily i.p. injections repeated after 10-day rest period; C, two daily i.p. injections repeated after 12-day rest period; and D, four daily i.p. injections.

† Deaths were recorded daily up to week 21 from the first day of treatment.

‡ MST, mean survival time of mice that died. Recording ended at week 21 from the first day of treatment. Values are means ± SD.

§ ND, not determined.

‖ Calculated at week 5 from the first day of treatment.

would provide similar protection against the toxicity of tubercidin, the effects of 25 mg/kg/day dipyridamole, dilazep or BAU on the toxicity of tubercidin were investigated using the same time schedule of 4 days of treatment repeated twice with a 10-day interval. BAU did not protect against tubercidin toxicity (data not shown) and therefore was not studied further. The degree of protection against tubercidin toxicity by dilazep or dipyridamole is shown in Table 2. Table 2 also shows the lethality caused by nebularine and the effects of NBMPR-*P*, dilazep and dipyridamole.

The results in Table 2 demonstrate that coadministration of NBMPR-*P* or dilazep with tubercidin or nebularine completely protected the animals from the lethal effect of these adenosine analogues. All mice receiving tubercidin or nebularine alone died between day 3 and day 8 of treatment. In contrast there was no significant difference ($P > 0.05$ using $\chi^2$-test) in mortality among animals receiving either NBMPR-*P* alone, dilazep alone, the combination of NBMPR-*P* or dilazep plus tubercidin or nebularine, and controls receiving saline only. On the other hand, coadministration of dipyridamole produced only 50 and 76% protection against the toxicity of tubercidin and nebularine respectively (Table 2).

*Effects of combination therapy with purine nucleoside analogues and nucleoside transport inhibitors on schistosomiasis.* Tables 2–4 show the effects of various combinations of purine nucleoside analogues and nucleoside transport inhibitors on the different variables of infection under investigation. The combination of NBMPR-*P* with 9-deazaadenosine was ineffective against schistosomiasis. In contrast, other combinations involving tubercidin or nebularine proved to be highly effective.

Combinations with tubercidin or nebularine caused a striking reduction in the number and pairing of worms (Table 3). The worms recovered were stunted. The size of the stunted worms also made their recovery by hook dissection more difficult than by portal perfusion (Table 3). All untreated,

Table 2. Effect of purine nucleoside analogues and nucleoside transport inhibitors on survival and weight of liver and spleen of mice infected with *S. mansoni**

| Treatment | Number of mice surviving | Weight† (g) | |
|---|---|---|---|
| | | Liver | Spleen |
| Before treatment‡ | | 1.95 ± 0.29 | 0.24 ± 0.06 |
| Saline control | 45/57 | 3.65 ± 0.76 | 0.72 ± 0.18 |
| NBMPR-*P* (25 mg/kg) | 16/18 | 3.51 ± 1.18 | 0.84 ± 0.17 |
| Dipyridamole (25 mg/kg) | 21/30 | 3.90 ± 1.03 | 0.77 ± 0.27 |
| Dilazep (25 mg/kg) | 12/12 | 3.48 ± 0.59 | 0.57 ± 0.20 |
| Tubercidin (5 mg/kg) | 0/18 | | |
| + NBMPR-*P* (25 mg/kg) | 18/18 | 1.77 ± 0.24§ | 0.27 ± 0.15§ |
| + dipyridamole (25 mg/kg) | 22/44 | 2.21 ± 0.60§ | 0.37 ± 0.11§ |
| + dilazep (25 mg/kg) | 13/13 | 1.71 ± 0.33§ | 0.22 ± 0.06§ |
| Nebularine (37 mg/kg) | 0/8 | | |
| + NBMPR-*P* (25 mg/kg) | 5/5 | 2.22 ± 0.20§ | 0.25 ± 0.07§ |
| + dipyridamole (25 mg/kg) | 22/29 | 2.35 ± 0.46§ | 0.16 ± 0.10§ |
| + dilazep (25 mg/kg) | 12/12 | 1.92 ± 0.44§ | 0.19 ± 0.09§ |
| 9-Deazaadenosine (1 mg/kg) | 0/4 | | |
| + NBMPR-*P* (25 mg/kg) | 4/4 | 3.80 ± 0.42 | 0.82 ± 0.08 |

* All groups received a daily intraperitoneal injection 5 weeks after infection for 4 days. A second series of four daily injections was administered after a 10-day rest period. Measurements were made starting 9 weeks after infection.

† Mean ± SD.

‡ Data obtained from eight mice.

§ Significantly different ($P < 0.001$) from saline control.

NBMPR-*P*-, dilazep- or dipyridamole-treated mice showed characteristic symptoms and signs of schistosomiasis such as numerous egg granulomas, enlarged liver and spleen (Table 2), and a large number of eggs in all stages of development in the liver and small intestines (Table 4). In contrast, mice treated with the combination of tubercidin or nebularine plus NBMPR-*P* or dilazep and those that survived the dipyridamole combinations appeared healthy and had normal size livers and spleens (Table 2). There was a drastic reduction in egg numbers. The number of eggs in the liver was reduced from an average of 43,000 in untreated animals to approximately 4,000 eggs per liver. Similarly the number of eggs was reduced in the intestine from 680 to an average of 10 eggs per $cm^2$ of the intestine (Table 4). All recovered eggs were dead (Table 4), indicating that no new eggs were deposited subsequent to treatment. Furthermore, very few granulomas were detected in the livers of these animals. Sections of the livers showed lesions containing dead worms and what appeared to be a process of regeneration of normal hepatic tissue around old granulomas. Thus, combination therapy reduced the number and the progress of the primary pathological lesions associated with schistosomiasis.

## DISCUSSION

Our previous [3, 4] and present results clearly demonstrate that simultaneous administration of NBMPR-*P*, dilazep and to a lesser extent dipyridamole with either tubercidin or nebularine to *S. mansoni*- and *S. japonicum*-infected mice protected the host but not the parasite from the lethal effects of these nucleoside analogues. NBMPR-*P* dilazep, and dipyridamole inhibit nucleoside transport in mammalian cells [16–22] and thus protect such cells from the lethality of several toxic nucleoside analogues [11, 16, 19–21]. In contrast, neither NBMPR-*P* nor dilazep or, to a lesser extent, dipyridamole is effective in blocking the uptake of adenosine analogues by the schistosomes [3, 6]. Therefore, the combinations of nucleoside transport inhibitors with toxic nucleoside analogues produced highly selective toxicity against the parasite. This highly selective toxicity against the schistosomes supports our suggestion that the mechanism(s) of nucleoside transport in the parasites is different from that of their host [3, 6]. The lower efficacy of dipyridamole in protecting the host against the toxicity of the adenosine analogues could be attributed to the high affinity of dipyridamole for plasma proteins. It has been reported that upon i.v. administration of 20 mg dipyridamole to humans an average of 99.13 ± 0.24% of the drug is bound to plasma proteins with free drug levels ranging from 0.55 to 1.19% [23]. The results in Tables 3 and 4 also suggest that dipyridamole interfered with the therapeutic efficacy of tubercidin but not with that of nebularine. We have no explanation at the present time for this observation.

Although BAU inhibits nucleoside transport in mammalian cells [24] but not in schistosomes [6], the present results show it was ineffective in protecting the host from the toxicity of tubercidin. BAU ($K_i \simeq 150\,\mu M$) [24] is at least 1000-fold less potent as an inhibitor of nucleoside transport than the other nucleoside transport inhibitors examined in the present study [16, 17, 19–22]. Therefore, we conclude that BAU does not prevent the entry of the toxic tubercidin into host tissues as efficiently as NBMPR-*P* or dilazep.

It has been reported previously that the protection by nucleoside transport inhibitors is not general to

Table 3. Effects of purine nucleoside analogues and nucleoside transport inhibitors on the number and copulation of worms recovered, by hook dissection or by portal perfusion, from mice infected with *S. mansoni**

| Treatment | Recovered by hook dissection | | | | Recovered by portal perfusion | |
|---|---|---|---|---|---|---|
| | Worms per surviving mouse | Mesenteric veins | Portal vein | No. *in copula* | Worms per surviving mouse | No. *in copula* |
| Before treatment | 33.9 ± 13.1 | 24.5 ± 7.8 | 9.4 ± 7.1 | 29.6 ± 12.1 | 45.0 ± 7.3 | 20.8 ± 10.2 |
| Saline control | 40.7 ± 8.5 | 33.8 ± 9.7 | 6.9 ± 5.4 | 36.1 ± 9.5 | 43.9 ± 11.3 | 25.9 ± 9.4 |
| NBMPR-*P* | 50.0 ± 16.8 | 40.2 ± 15.7 | 9.8 ± 5.7 | 47.3 ± 13.7 | 46.5 ± 5.5 | 33.1 ± 10.9 |
| Dipyridamole | 51.5 ± 13.1 | 43.5 ± 14.1 | 7.9 ± 3.6 | 46.9 ± 12.6 | 50.7 ± 14.7 | 35.7 ± 6.8 |
| Dilazep | 30.2 ± 10.8 | 23.8 ± 7.8 | 6.3 ± 4.3 | 28.3 ± 10.5 | 35.8 ± 26.7 | 17.3 ± 9.8 |
| Tubercidin + NBMPR-*P* | 3.3 ± 5.0† | 2.5 ± 4.1† | 0.8 ± 1.3 | 2.0 ± 3.2† | 13.5 ± 10.4† | 0.3 ± 0.8† |
| Tubercidin + dipyridamole | 17.1 ± 7.8† | 10.4 ± 7.2† | 6.8 ± 3.8 | 12.3 ± 7.4† | 26.5 ± 1.8† | 10.0 ± 0.9† |
| Tubercidin + dilazep | 4.4 ± 3.0† | 3.7 ± 2.6† | 0.7 ± 1.5† | 3.4 ± 2.3† | 21.6 ± 6.4† | 0.8 ± 1.0† |
| Nebularine + NBMPR-*P* | 17.3 ± 2.3† | 8.7 ± 2.5† | 8.7 ± 1.5 | 0.0 ± 0.0† | 25.6 ± 16.3† | 8.3 ± 13.1† |
| Nebularine + dipyridamole | 14.4 ± 5.1† | 6.4 ± 3.9† | 8.0 ± 2.6 | 2.9 ± 1.5† | 20.9 ± 15.6† | 4.5 ± 4.1† |
| Nebularine + dilazep | 14.2 ± 3.8† | 7.5 ± 3.2† | 6.7 ± 3.1 | 4.3 ± 3.0† | 14.2 ± 2.5 | 3.7 ± 2.3† |
| 9-Deazaadenosine + NBMPR-*P* | 60.0 ± 5.6 | 43.4 ± 10.8 | 16.7 ± 6.2† | 45.8 ± 6.2 | ND‡ | ND |

* Animals were treated with the same doses and the same regimen described in Table 2. Worms were collected starting 9 weeks after infection. Values are means ± SD.

† Significantly different ($P < 0.005$) from saline control.

‡ ND, not determined.

Table 4. Effects of purine nucleoside analogues and nucleoside transport inhibitors on the number of eggs and the stage of embryogensis from mice infected with *S. mansoni**

| Treatment | Number of eggs† | | % Eggs in stages of embryogensis | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Per liver | Per $cm^2$ of intestine | 1 | 2 | 3 | 4 | 5 | Dead |
| Before treatment | 1,400 ± 1,300 | 29 ± 25 | 39 | 27 | 16 | 3 | 2 | 13 |
| Saline controls | 43,050 ± 13,450 | 680 ± 215 | 9 | 8 | 16 | 6 | 15 | 45 |
| NBMPR-*P* | 40,650 ± 13,300 | 720 ± 215 | 6 | 5 | 8 | 5 | 12 | 44 |
| Dipyridamole | 55,400 ± 17,950 | 690 ± 200 | 6 | 8 | 16 | 7 | 13 | 51 |
| Dilazep | 52,600 ± 23,400 | 640 ± 225 | 8 | 10 | 14 | 7 | 14 | 48 |
| Tubercidin + NBMPR-*P* | 2,200 ± 2,100‡ | 4 ± 5‡ | 0 | 0 | 0 | 0 | 0 | 100 |
| Tubercidin + dipyridamole | 9,500 ± 5,000‡ | 25 ± 22‡ | 0 | 0 | 0 | 0 | 0 | 100 |
| Tubercidin + dilazep | 1,550 ± 1,100‡ | 6 ± 6‡ | 0 | 0 | 0 | 0 | 0 | 100 |
| Nebularine + NBMPR-*P* | 3,650 ± 1,150‡ | 10 ± 9‡ | 0 | 0 | 0 | 0 | 0 | 100 |
| Nebularine + dipyridamole | 4,400 ± 3,400‡ | 13 ± 14‡ | 0 | 0 | 0 | 0 | 0 | 100 |
| Nebularine + dilazep | 2,400 ± 1,100‡ | 13 ± 13‡ | 0 | 0 | 0 | 0 | 0 | 100 |
| 9-Deazaadenosine + NBMPR-*P* | 50,300 ± 22,150 | 420 ± 171 | 10 | 7 | 12 | 6 | 20 | 45 |

* Animals were treated with the same doses and the same regimen described in Table 2. Measurements were made starting 9 weeks after infection. Number of eggs per liver was estimated as described by Cheever [14]. Estimates of eggs per $cm^2$ of intestine were made from egg counts in three 2-cm segments of the small intestine. Stages of embryogensis are those described by Pellegrino *et al.* [15].

† Mean ± SD.

‡ Significantly different ($P < 0.001$) from saline control.

all toxic nucleoside analogues [19]. The present results also show that nucleoside transport inhibitors did not protect against the toxicity of all nucleoside analogues tested as exemplified by toyocamycin. Toyocamycin, at doses lower than its reported $LD_{50}$ [25], caused late mortality which was first observed at 16 weeks from initiation of treatment. This late mortality could not be prevented by any of the nucleoside transport inhibitors tested. Whether or not it may be possible to protect the host from toyocamycin toxicity by increasing the dose of the nucleoside transport inhibitor remains to be determined. A similar late manifestation of toxicity was not observed with the combination of tubercidin plus NBMPR-*P* (unpublished results).

The ineffectiveness of combination therapy with 9-dezaadenosine can be explained by the extremely high toxicity of this analogue to animal cells [26] but not to the parasite. Although 9-deazaadenosine ($10^{-4}$ M) is metabolized by schistosomes [6], this drug concentration does not affect the motility of schistosomules after 24-hr incubation *in vitro* [5]. Therefore, the dose of 9-deazaadenosine that was used safely in our combination therapy may be insufficient to kill the parasites. A similar argument can be made for the ineffectiveness of the combination of toyocamycin (2.2 mg/kg/day) with nucleoside transport inhibitors (25 mg/kg/day), using regimen C (Table 1), against schistosomes (unpublished results).

Although NBMPR-*P* is the best known inhibitor of nucleoside transport in mammalian systems [16, 17, 19], its usefulness in chemotherapy in humans remains to be determined. It is encouraging that other nucleoside transport inhibitors such as dilazep and dipyridamole are also effective in combination therapy. Dilazep and dipyridamole have been used in the clinic, the former in Europe and Asia, as coronary vasolidators or antiplatelet agents. The extensive clinical experience with these drugs, especially dipyridamole, favors their use in the combination therapy of schistosomes in humans.

In conclusion, the results presented here demonstrate that nucleoside transport inhibitors other than NBMPR-*P* can be used to protect the host but not the parasite against tubercidin toxicity. They also show that at least one other nucleoside analogue can replace tubercidin in the treatment of schistosomiasis by combination therapy. This, along with fact that histological examination and blood chemistry of animals receiving the combination of NBMPR-*P* plus tubercidin showed no evidence ofcytotoxicity (details to be published elsewhere), demonstrate a simple and inexpensive method by which nucleoside analogues can be made selectively toxic against schistosomes. Thus, the potency of purine nucleoside analogues against other species of human schistosomes (i.e. *S. japonicum*) [2, 4] as well as other parasites [2, 27–30] and the practicality and simplicity of combination therapy to achieve host protection, may provide an alternative chemotherapeutic approach for the treatment of schistosomiasis as well as other parasitic diseases. Indeed, a recent report showed that combination therapy is highly effective against malarial infections [31].

*Acknowledgements*—We are indebted to Drs. A. W. Senft, F. N. M. Naguib and R. E. Parks Jr. for critical reading of the manuscript. We thank Drs. A. R. P. Paterson, R. E. Klein, S. Chu and Asta-Werke AG, Frankfurt, F.R.G. for supplying compounds used in this study.

## REFERENCES

1. A. W. Senft and G. W. Crabtree, *Pharmac. Ther.* **20**, 341 (1983).
2. J. J. Jaffe, *Ann. N.Y. Acad. Sci.* **255**, 306 (1975).
3. M. H. el Kouni, D. Diop and S. Cha, *Proc. natn. Acad. Sci. U.S.A.* **80**, 6667 (1983).

4. M. H. el Kouni, P. M. Knopf and S. Cha, *Biochem. Pharmac.* **34**, 3921 (1985).
5. H. F. Dovey, J. H. McKerrow and C. C. Wang, *Molec. biochem. Parasit.* **16**, 185 (1985).
6. M. H. el Kouni and S. Cha, *Biochem. Pharmac.* **36**, 1099 (1987).
7. M. H. el Kouni and S. Cha, *Pharmacologist* **25**, 147 (1984).
8. C-L. Lee and R. M. Lewert, *J. infect. Dis.* **99**, 15 (1956).
9. P. M. Knopf, T. B. Nutman and J. A. Reasoner, *Parasitology* **41**, 74 (1977).
10. J. A. Clegg, *Expl Parsit.* **16**, 133 (1965).
11. A. R. P. Paterson, J. H. Paran, S. Yang and T. P. Lynch, *Cancer Res.* **39**, 3607 (1979).
12. S. R. Smithers and R. J. Terry, *Parasitology* **55**, 695 (1965).
13. A. W. Senft and G. W. Crabtree, *Biochem. Pharmac.* **26**, 1847 (1977).
14. A. W. Cheever, *Bull. Wld Hlth Org.* **39**, 328 (1968).
15. J. Pellegrino, C. A. Oliveria, J. Faria and A. S. Cunha, *Am. J. trop. Med. Hyg.* **11**, 201 (1962).
16. A. R. P. Paterson, N. Kolassa and E. Cass, *Pharmac. Ther.* **12**, 515 (1981).
17. P. G. W. Plagemann and R. M. Wohlhueter, *Curr. Topics Membr. Transp.* **14**, 225 (1980).
18. V. J. Pohl and N. Brock, *Arzneimittel-Forsch.* **24**, 1901 (1974).
19. A. R. P. Paterson, E. S. Jakobs, E. R. Harley, C. Cass and M. J. Robins, in *Development of Target Oriented Anticancer Drugs* (Eds. Y. C. Cheng, B. Goz and M. Minkoff), p. 41. Raven Press, New York (1983).
20. A. R. P. Paterson, E. S. Jakobs, E. R. Harley, N-W. Fu, M. J. Robins and C. E. Cass, *Regulatory Functions of Adenosine* (Eds. R. M. Berne, T. W. Rall and R. Rubio), p. 203. Martinus Nijhoff, The Hague (1983).
21. A. R. P. Paterson, E. Y. Lau, E. Dahlig and C. E. Cass, *Molec. Pharmac.* **18**, 40 (1980).
22. P. G. W. Plagemann and M. Kraupp, *Biochem. Pharmac.* **35**, 2559 (1986).
23. C. Mahony, K. M. Wolfram, D. M. Cocchetto and T. D. Bjornsson, *Clin. Pharmac. Ther.* **31**, 330 (1982).
24. K. H. Lee, M. H. el Kouni, S-H. Chu and S. Cha, *Cancer Res.* **44**, 3744 (1984).
25. M. Saneyoshi, R. Tokuzen and F. Fukuoka, *Gann* **56**, 219 (1965).
26. R. I. Glazer, K. D. Hartman and M. C. Knode, *Molec. Pharmac.* **24**, 309 (1983).
27. W. B. Eubank and R. E. Reeves, *Am. J. trop. Med. Hyg.* **30**, 900 (1981).
28. P. O. J. Ogbunude and C. O. Ikediobi, *Acta trop.* **39**, 219 (1982).
29. D. J. Hupe, *A. Rep. med. Chem.* **21**, 247 (1986).
30. H. V. Scott, A. M. Gero and W. J. O'Sullivan, *VI International Congress of Parasitology, Brisbane, Australia 1986 ICOPA VI—Handbook*, 225 (1986).
31. A. M. Gero, E. M. A. Bugledich, G. V. Brown and A. R. P. Paterson, *VI International Congress of Parasitology, Brisbane, Australia 1986 ICOPA VI—Handbook*, 136 (1986).