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The effect of cyclosporin A on the course of *Paragonimus miyazakii* infection in rats

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ABSTRACT

The effect of the immunomodulatory fungal metabolite cyclosporin A (CyA) on the course of *Paragonimus miyazakii* infection in rats was studied. Administration of CyA 15 to 19 days post-infection resulted in a significantly lower recovery rate of worms and cyst formation in the host's lungs than in controls. Administration of CyA −1 to +3 days post-infection enhanced the growth and maturation of *P. miyazakii*, expressed as weight of worms and the number of worms with eggs in uteri with respect to control values. This study shows that administration of CyA to rats affects the host-parasite relationship, depending on the time of administration of the drug.

KEY WORDS: *Paragonimus miyazakii*, rat, cyclosporin A, infection course

INTRODUCTION

Cyclosporin A (CyA) is a biologically active metabolite of the fungus *Tolypocladium inflatum*, with pronounced immune suppressive properties and without marked myelotoxicity (BOREL *et al.*, 1976). In parasitic infections, CyA treatment has been shown to result in inhibition or enhancement of protozoan and helminthic infections. It is well known that in experimental schistosomiasis mansoni an infected animal develops a strong resistance against a challenge (secondary) infection. In this case, when animals (mice) were treated with CyA and then infected with *Schistosoma mansoni* cercaria, they revealed a significant worm reduction in both primary and secondary infections (BOUT *et al.*, 1984a, 1986; BUEDING *et al.*, 1981; NILSSON *et al.*, 1985). Furthermore, *Dipetalonema viteae* infections *per se* cannot reveal that animals were protected against infection, but in CyA treated *Mastomys natalensis*, 60% of the animals were free from the infection (BOUT *et al.*, 1984b). CyA also produced an antimalarial effect in mice infected with *Plasmodium berghei, P. chabaudi* and *P. yoelii* (NICKELL *et al.*, 1982; THOMMEN-SCOTT, 1981). Against *Toxoplasma gondii*, CyA showed an antimicrobial effect (MACK & MCLEOD, 1984). In leishmanial infection in BALB/c mice, CyA treatment enhanced host resistance to *Leishmania major* and suppressed the development of *L. tropica*-induced lesions in the genetically susceptible host (BEHFOROUZ *et al.*, 1986; SOLBACH *et al.*, 1986).

On the other hand, treatment with CyA exacerbated the course of *Trypanosoma cruzi* infection in outbred and inbred BALB/c mice (KIERSZENBAUM *et al.*, 1983; MCCABE *et al.*, 1985). Furthermore, *Giardia muris* infection in mice was accompanied by an increase in cyst output and a delay of the elimination phase when CyA was given to them (BELOSEVIC *et al.*, 1986).

Thus, in contrast to other immune suppressive drugs, CyA shows one type of effect on the course of parasitic infections with *Schistosoma, Dipetalonema, Plasmodium, Toxoplasma* and *Leishmania* and another type in *Trypanosoma* and *Giardia* infections. In our previous study, treatment with immunosuppressive drugs such as dexamethasone, prednisolone and hydrocortisone enhanced the susceptibility of rats to *Paragonimus miyazakii* by suppressing the host's immune responses (HASHIGUCHI & HIRAI, 1977).

Considering the different effects of CyA in various host-parasite models and its potential for immune modulation, we evaluated the effect of this drug on the course of *P. miyazakii* infection in the rat, a host which is not as susceptible to the parasite as cats or dogs. Animal paragonimiasis is a good experimental model to evaluate drug effects, because it allows us to assay host-parasite relationships based on various parasitological criteria, such as different degrees of the worm migrations and/or localization, worm development and maturation, and lung cyst formations, in addition to worm recovery rates in drug treated animals. The preliminary results reported here will be followed by further experiments, such as examination of CyA effects on: 1) different points of *P. miyazakii* infection course; 2) challenge infections.

MATERIALS AND METHODS

Rats

Male Jcl:Wistar rats (Japan Clea Inc., Osaka, Japan), 150 to 170 g and 7 weeks of age, were used. The animals were fed a standard pellet diet and given water *ad libitum*.

Parasites and mode of infection

Metacercariae of *P. miyazakii* were obtained from a potamonid crab, *Potamon dehaani*, collected in the endemic area of Tokushima, Japan (HASHIGUCHI & AGATSUMA, 1981). They were administered to the rats in normal saline *per os*, using a syringe with a slender vinyl tube. Each rat received 20 metacercariae. The animals were killed 60 days after infection.

Recovery of worms

The peritoneal cavity was opened, and the surface of the liver and peritoneal wall was inspected for haemorrhages. The peritoneal surface was flushed with normal saline and the washings were examined for free worms under a dissecting microscope. The internal organs were removed and washed with saline 5 times to recover free worms. The liver and lungs were examined for haemorrhages and worm cysts and then minced with scissors in petri dishes. To examine the minced liver and lungs for penetrating worms, the materials were incubated at 30°C for 12 hours in saline. The growth of *P. miyazakii* was evaluated in terms of their weight determined with an analytical balance (Mettler, Switzerland). Eggs in uteri and those deposited in lung cysts were examined in flattened worm specimens and wet smears of cyst contents, respectively.

Drug (CyA)

The commercially available formula, Sandimmun®; 50 mg CyA/ml solvent (Poly-[oxyethylen]-ricinus-oil [Cremophor EL®]); Sandoz Ltd., Basle, Switzerland, was used as stock solution throughout the experiment; it was administered subcutaneously into alternate thighs at a daily dose of 100 mg/kg of body weight at the first injection, thereafter 50 mg/kg for 4 consecutive days (5 consecutive days in total) in each experiment. Control animals received only the solvent.

Administration of CyA

Four to 5 rats in each group received CyA as follows: 1) −1 group, animals received 5 daily doses of CyA starting one day before *P. miyazakii* metacercariae inoculation through day 3; 2) +15 group, from day 15 post-infection through day

19; 3) control group, received solvent (Cremophor EL®) alone from day −1 through day 3.

Statistical analysis

The chi square test or Student's t test were used.

RESULTS

Worm recovery from the −1, +15 and control groups

Pertinent data are summarized in Table I. Worm recovery revealed a statistically significant difference between the +15 (19%) and control (54%) groups ($p<0{\cdot}001$, $\chi^2=22{\cdot}581$), but not between the −1 (43%) and control (54%) groups. The mean number of lung cysts per rat was 3·5 in the control, 3·4 in the −1 group and 0·8 in +15 group, showing a significantly lesser number of cysts in the +15 group than in the control ($0{\cdot}02<p<0{\cdot}05$, $t=3{\cdot}049$). When the migration of worms was compared among the −1, +15 and control groups, a greater number of worms in the control remained in the liver and peritoneal cavity of rats; the number of worms from these sites was 16 (37·2% of the 43 worms) in control, 5 (11·6% of 43) in the −1 group and 1 (6·7% of 15) in the +15 group. Recovery rate of worms from lung cysts per total recovery in each group was 81% in the −1 group, 40% in the +15 group and 60% in the control, demonstrating a marked low rate in the +15.

Worm growth in the −1, +15 and control groups

Weight (mg) of *P. miyazakii* and the eggs in uteri and those deposited in lung

TABLE I. *Paragonimus miyazakii* recovery from rats treated with CyA for 5 consecutive days; each given 20 metacercariae

Group*	Rat no.	No. of worms recovered from: peritoneal cavity	liver	pleural cavity	lung cysts	Total no. of worms (%)	Mean no. for group±S.D.	Mean no. of cysts (mean for group±S.D.)
Control	1		7		6	13 (65)	11·0±2·1	3
	2	2		1	6	9 (45)		3
	3	1	2		6	9 (45)		3
	4	1	3		8	12 (60)		5
	Total	4	12	1	26	43 (54)		14 (3·5±1·0)
−1	1	3		1	5	9 (45)	8·6±2·3	2
	2			1	8	9 (45)		4
	3	2		1	9	12 (60)		5
	4				7	7 (35)		3
	5				6	6 (30)		3
	Total	5		3	35	43 (43)		17 (3·4±1·1)
+15	1			2	2	4 (20)	3·8±1·7	1
	2			1	2	3 (15)		1
	3			2		2 (10)		0
	4		1	3	2	6 (30)		1
	Total		1	8	6	15 (19)		3 (0·8±0·5)

*Control animals received solvent alone from day −1 of *Paragonimus miyazakii* infection through day 3; −1 group received 5 daily doses of CyA starting one day before the infection, +15 group, from day 15 postinfection through day 19.

TABLE II. Growth and maturation of *Paragonimus miyazakii* (expressed in mg), eggs in uteri and eggs deposited in lung cysts, in rats treated with CyA for 5 consecutive days

Group*	Rat no.	No. weighed	Mean weight of worms±S.D.	No. with eggs in uteri (%)**	Eggs in*** lung cyst
Control	1	13	3·4±2·0	1 (8)	+++
	2	9	4·2±1·0	1 (11)	+
	3	9	4·1±2·1	1 (11)	+
	4	12	4·2±1·5	3 (25)	+++
	Summed	43	3·9±1·7	6 (14)	
−1	1	9	3·7±1·5	1 (11)	+
	2	9	4·3±1·2	3 (33)	++
	3	12	4·4±1·7	3 (25)	+++
	4	7	7·1±2·7	3 (43)	++
	5	6	4·3±1·0	2 (33)	+
	Summed	43	4·7±2·0	12 (28)	
+15	1	4	3·5±2·3	1 (25)	+
	2	3	4·3±1·5	0 (0)	−
	3	2	3·0±2·8	0 (0)	−
	4	6	3·2±1·9	1 (17)	+
	Summed	15	3·5±1·9	2 (13)	

*As in Table I.
**% of worms with eggs in uteri per total numbers recovered from each rat fed with 20 metacercariae.
***Eggs in wet smears were counted under ×100 magnification. +, 1 to 2 eggs; ++, three to nine eggs; +++, 10 eggs or more.

cysts in rats treated with CyA are shown in Table II. The worms recovered from the +15 group revealed slightly lower weight and fewer eggs in lung cysts as compared to the control, although the difference between these two groups was not statistically significant. There was a significant difference between the weight of worms from the −1 and +15 groups ($0{\cdot}02<p<0{\cdot}05$, $t=2{\cdot}090$), but no difference was recognized between the −1 and control groups. The number of worms with eggs in uteri was greater in the −1 group than in the +15 and control groups ($0{\cdot}01<p<0{\cdot}02$, $\chi^2=5{\cdot}907$).

Effect of CyA on rats

A direct effect of CyA on the rats was reflected by a decrease in body weight. In the control group there was no marked fluctuation of the body weight throughout the experiment. The animals in all of the treated groups showed decreased weight after the CyA treatment (145·0±12·9 g in treated versus 160·0±8·2 g in control, on the day 20).

DISCUSSION

The present study was designed to evaluate the effect of CyA on *P. miyazakii* infection in rats. The results showed that CyA treatment during the late phase of infection (+15 group) produced a lower worm recovery rate with respect to that of control values. The results also demonstrated that the growth and maturation of the parasite could be enhanced if the drug was given in the early phase of infection (−1 group). The precise reasons why CyA showed such different effects in connection with the time of drug administration in the early or late phase of infection with *P. miyazakii* remain unknown.

The persistence of CyA might be relatively short in rats. It has been shown that CyA is almost completely cleared within 96 hours in dogs (BEVERIDGE, 1982). CyA had no direct toxic effect on 15-day-old immature *P. miyazakii in vitro* at a concentration of 100 μg CyA/ml in normal saline (unpublished data); with this concentration a slight reduction of *S. mansoni* cercariae infectivity was noted by NILSSON *et al.* (1985).

The different effects of CyA in various parasitic infections noted above might be because the drug can modify the course of parasitic diseases according to the particular manner in which it affects the components of the immune system which contribute to the pathogenicity of, or host defense against, a given organism (KIERSZENBAUM *et al.*, 1983). In most of the models, however, the exact mechanism of action of the drug against the parasites remains an open question. It would be worthwhile investigating further the effects of CyA in different host-parasite models.

It is of interest to note that lung cysts observed were present in smaller numbers (0·75 on average) in the +15 group. Moreover, the worm recovery from lung cysts per total recovery in each group showed a markedly low rate in the +15 group, when compared with the other two groups (−1 and control). These are striking differences between the +15 group and the other groups, including the control.

When the rats infected with *P. miyazakii* were treated with other immunosuppressive drugs such as prednisolone, dexamethasone and hydrocortisone, the time of lung cyst formation was shortened remarkably and the number of cysts was greater compared to the untreated controls (HASHIGUCHI & HIRAI, 1977). In hydrocortisone treated rats infected with *P. westermani* the number of lung cysts was also over control values (TADA, 1967). These tendencies were also found in the rats treated with CyA during the early phase (day −1 through +3) of *P. miyazakii* infection in the present study.

Thus, CyA displayed an array of unexpected effects in the course of *P. miyazakii* infection, i.e., enhancement and suppression of the worm growth and maturation in the early and late treatment of host animals with the drug, respectively. Such an interesting phenomenon is designed to be examined more intensively in a future study.

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