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Further studies on the effect of cyclosporin A on the course of *Paragonimus* infection in rats

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ABSTRACT

Paragonimus ohirai-infected rats were treated with cyclosporin A (CyA) at different times during the course of infection. CyA (5×80 mg/kg) affected the worm recovery, growth and maturation rates of *P. ohirai* with respect to control values. This tendency was most remarkable in animals treated 15 days and more after infection with CyA (groups B, +15 to +19 days; C, +25 to +29; D, +35 to +39 and E, +45 to +49). In group A (0 to +4), however, the drug did not affect markedly the growth and maturation of worms, although it significantly lowered worm recovery rates. CyA administration also affected normal migration of *P. ohirai* in the highly susceptible host (rat), when the drug was administered during the peritoneal and/or liver phase of infection. Thus, in this *P. ohirai*/rat model, CyA significantly reduced worm recovery rates, and affected the growth, maturation and migration of the worms depending on the time of administration.

KEY WORDS: *Paragonimus ohirai*, cyclosporin A, rat, immunosuppressants

INTRODUCTION

Cyclosporin A (CyA), a biologically active metabolite of *Tolypocladium inflatum*, has pronounced immunosuppressive properties (BOREL *et al.*, 1976). To date, reported opinions on the effect of the drug on parasitic infections have been conflicting and based on investigations in different host-parasite models. CyA alters the host's defence against parasitic infections by its immunomodulating capacity (BOUT *et al.*, 1984a, b; NILSSON *et al.*, 1985). The direct effect of the compound on helminths, such as *Schistosoma mansoni* and *Litomosoides carinii*, has been demonstrated *in vitro* (BUEDING *et al.*, 1981) and *in vivo (Mastomys natalensis)* (ZAHNER & SCHULTHEISS, 1987).

Consideration of the different effects of CyA in various host-parasite models and its potential for immunomodulation has led us to evaluate the effect of this drug on the course of *Paragonimus* infections in the final hosts. In our previous study (HASHIGUCHI & OKAMURA, 1988), the effect of CyA was examined in *P. miyazakii*-infected rats. In this case the host animal, the rat, is not as susceptible to *P. miyazakii* as cats or dogs. In the experimental model, administration of CyA 15 to 19 days post-*P. miyazakii* infection resulted in a significantly lower recovery rate of worms and cyst formation in the host's lungs than in controls. On the other hand, administration of CyA −1 to +3 days post-*P. miyazakii* infection enhanced the growth and maturation of the parasite with respect to control values. Thus, CyA affected the host-parasite relationship of *P. miyazakii* infection in an unsuitable host (rat), depending on the time of drug administration.

This study was designed to obtain further information on the effect of CyA on *Paragonimus* infections, using an experimental model of a suitable host-parasite relationship, viz., *P. ohirai* and the rat, a host which is highly susceptible to the parasite.

MATERIALS AND METHODS

Rats (strain WKA/Sea, Japan Clea Inc., Osaka; 7-week-old male, 220–240 g)

were kept under constant temperature conditions (22°C) and fed a commercially prepared diet. Water was available *ad libitum*. The animals were infected with *P. ohirai* metacercariae *per os*. The metacercariae were obtained from a sesarmid crab, *Sesarma holometopus dehaani*, collected at the estuary of Maruyama River, Kinosaki, Hyogo, Japan. Although all the materials were derived from naturally infected crabs from the field, the same batch of *P. ohirai* metacarcariae were used throughout the experiments.

Rats received 20 metacercariae each and were necropsied 60 days after infection. CyA (kindly supplied by Dr. J. Borel through Sandoz Ltd, Tokyo, Japan) was administered subcutaneously into alternate thighs at a daily dose of 80 mg/kg of body weight for 5 consecutive days in each experiment. Control animals

TABLE I. Recovery of *Paragonimus ohirai* 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 cyst	Total no. of worms (%)	Mean no. for group ±S.D.	No. of cysts (mean for group±S.D.)
Control	1			2	10	12 (60)		4
	2			4	9	13 (65)		5
	3			2	8	10 (50)		3
	4			4	8	12 (60)		3
	5			1	13	14 (70)		4
	Summed			13	48	61 (61)	12·2±1·5	(3·8±0·8)
A	1				3	3 (15)		1
	2			3	2	5 (25)		3
	3			1	2	3 (15)		1
	4			2	7	9 (45)		3
	Summed			6	14	20 (25)	5·0±2·8	(2·0±1·2)
B	1		2		1	3 (15)		2
	2		1	2		3 (15)		0
	3	7			7	14 (70)		4
	4		1	1		2 (10)		0
	5		1			1 (5)		0
	Summed	7	5	3	8	23 (23)	4·6±5·3	(1·2±1·8)
C	1	8		1	4	13 (65)		2
	2	1		3	2	6 (30)		1
	3	1		2		3 (15)		0
	Summed	10		6	6	22 (37)	7·3±5·1	(1·0±1·0)
D	1			5	2	7 (35)		3
	2			3	1	4 (20)		3
	3			5	2	7 (35)		3
	4			3	2	5 (25)		3
	5			1		1 (5)		0
	Summed			17	7	24 (24)	4·8±2·5	(2·4±1·3)
E	1				1	1 (5)		1
	2			1	8	9 (45)		5
	3				5**	5 (25)		3
	Summed			1	14	15 (25)	5·0±4·0	(3·0±2·0)

*Control animals received solvent alone from day −1 of *Paragonimus ohirai* infection through day 3; group A received 5 daily doses of CyA starting 0 day of the infection; group B, from day 15 postinfection through day 19; group C, from day 25 through day 29; group D, from day 35 through day 39; group E, from day 45 through day 49.

**Three of the 5 worms recovered were found to be dead.

received solvent (Tween 80) alone from day −1 of *P. ohirai* metacercariae infection through day 3. Three to 5 rats in each group received CyA as shown in Table I, viz., group A (0 to +4) received 5 daily doses of CyA starting day 0 of the infection; group B (+15 to +19), from day 15 postinfection through day 19; group C (+25 to +29), from day 25 through day 29; group D (+35 to +39), from day 35 through day 39; group E (+45 to +49), from day 45 through day 49. Worm recovery from rats, and investigations on worm growth and maturation were performed as described by HASHIGUCHI & OKAMURA, 1988.

RESULTS

In the current study, the CyA untreated (control) group showed higher values for worm recovery, cyst formation in host's lungs, and worm growth and maturation, when compared to CyA treated (A–E) groups.

TABLE II. Growth and maturation of *Paragonimus ohirai* (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	12	12·8±3·8	12 (100)	+++
	2	13	12·6±4·5	13 (92·3)	+++
	3	10	14·0±3·7	10 (100)	+++
	4	12	12·9±5·5	12 (100)	+++
	5	14	16·2±4·6	14 (100)	+++
	Summed	61	13·8±4·6	60 (98·4)	
A	1	3	13·0±1·0	3 (100)	++
	2	5	10·8±2·1	5 (100)	+++
	3	3	11·3±5·1	3 (100)	++
	4	9	14·1±3·9	9 (100)	+++
	Summed	20	12·7±3·5	20 (100)	
B	1	3	6·0±6·1	1 (33·3)	+
	2	3	5·3±2·5	2 (66·6)	+
	3	14	8·4±2·9	12 (85·7)	+
	4	2	3·0±1·4	0	−
	5	1	4·0	0	−
	Summed	23	7·0±3·6	15 (65·2)	
C	1	13	6·8±2·3	9 (69·2)	+
	2	6	6·7±2·0	0	−
	3	3	5·3±1·5	0	−
	Summed	22	6·6±2·2	9 (40·9)	
D	1	7	11·6±3·2	5 (71·4)	++
	2	4	10·8±3·3	3 (75·0)	++
	3	7	9·4±2·9	3 (42·9)	+
	4	5	5·0±2·0	0	−
	5	1	7·0	1 (100)	+
	Summed	24	9·3±3·6	13 (54·2)	
E	1	1	4·0	0	
	2	9	6·9±2·4	2 (22·2)	−
	3	2	6·0±1·4	0	+
	Summed	12	6·5±2·3	2 (16·7)	−

*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 10 eggs; ++, 11 to 50 eggs; +++, 51 eggs or more.

CyA treated groups all demonstrated similar extremely low worm-recovery rates ranging from 15% to 37% in *P. ohirai*-infected rats (Table I). In B (+15 to +19) and C (+25 to +29) groups, however, migrating *P. ohirai* worms were recovered not only from the pleural cavity and lung cysts, but also from the peritoneal cavity and/or liver of rats. Severe macroscopical haemorrhages in the liver were most notable in the animals of B and C groups, suggesting that the migrating juvenile *P. ohirai* remained for the longest time in the liver. Some traceable lesions were also observed in animals of other treated groups. Host lung cysts were slightly fewer in mean number in groups B and C (1·2 in B and 1·0 in C) than in the other groups (2·0–3·8). It is noteworthy that three of the five worms from one rat (No. 3) of group E (+45 to +49) were dead.

The growth and maturation of *P. ohirai* in rats treated with CyA for 5 consecutive days are shown in Table II. Administration of CyA 0 to 4 days of post-*P. ohirai* infection in group A resulted in similar degrees of worm growth and maturation to control values. The remaining treated groups (B, C, D and E), however, contained markedly smaller worms and more often sterile flukes than controls and group A. In group D unexpectedly large worms were recovered.

DISCUSSION

The results show that CyA affects *P. ohirai* infection in the rat, a host which is highly susceptible to the parasite, causing a strong reduction in worm recovery, growth and maturation rates. This tendency was most notable in groups B (+15 to +19), C (+25 to +29), D (+35 to +39) and E (+45 to +49). The data obtained also demonstrates that the growth and maturation of *P. ohirai* in rats (including worm migration) could be affected by the time of CyA administration. A similar time-dependent effect of CyA was observed in our previous study, using *P. miyazakii* and the rat; although in this case rats were not so susceptible to the parasite (HASHIGUCHI & OKAMURA, 1988). In *P. miyazakii* infection in rats, CyA treatment during the early phase of infection enhanced worm growth and maturation, while drug treatment during the late phase significantly lowered worm recovery. In the current experiment, no significant difference was observed in worm recovery rates among CyA-treated groups. However, the recovery rates in treated groups were nearly one third of control values.

In groups B and C, 12 (52·2%) of the 23 worms and 10 (45·5%) of the 22 worms, respectively, were recovered from the peritoneal cavity and/or liver of rats at necropsy 60 days after infection. This result shows that CyA clearly interfered with the normal migration of *P. ohirai* in the susceptible host when the drug was administered during the peritoneal or liver phase of infection. In *P. ohirai*-infected rats, the parasite usually stays in the peritoneal cavity and/or liver until 20 or 25 days post-infection (OHKURA, 1963; HASHIGUCHI *et al.*, 1969; HASHIGUCHI, 1973). Thus, CyA administration for 5 consecutive days starting 15 or 25 days post-infection (group B or C) might affect the peritoneal and liver phase of *P. ohirai* in the rat.

Mean weights of *P. ohirai* in treated groups except group A were significantly smaller than control values. Normally, *P. ohirai* parasites in rats are able to reach maturity around 30 days onwards post-infection (HASHIGUCHI *et al.*, 1969; HASHIGUCHI, 1973), demonstrating nearly equal worm sizes with the present control values. Therefore, the small and dwarf sizes of worms recovered from rats in treated groups (especially in D and E) would be caused by CyA administration.

The current data, however, suggest that the low recovery, growth and

maturation rates of the worms in CyA-treated groups might be caused by a direct killing effect of the drug, but this is not certain. The precise mechanism (immunologic or anthelmintic) should be examined in future studies, by using CyA and its derivatives without immunological properties.

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