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Antischistosomal effects of cyclosporin A[1]

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Abstract

Administration of cyclosporin A, a new selective immunosuppressive agent, to mice infected with *Schistosoma mansoni* resulted in a significant reduction in the number of mature and immature male and, to a greater extent, female worms. With lower, subeffective, doses a reduction in hemoglobinase activity and protein content of female schistosomes is produced. Evidence available so far suggests that the antischistosomal effects of cyclosporin A are mediated through a stimulation of host mechanisms directed against the parasite.

Introduction

Recently, it was found that the reduced hepatic drug metabolism capacity in experimental schistosomiasis is dependent upon an immunological host reaction, consisting in the formation of granulomas around schistosome eggs deposited in the liver. In athymic nude mice the granulomatous response in the liver is much smaller (CHA et al., 1980) [1]. This is paralleled by a markedly smaller depression in hepatic microsomal drug metabolism. Therefore, it was of interest to determine whether or not a similar correlation is demonstrable by means of a selective immunosuppressive compound, cyclosporin A (CS-A) [2]. However, unexpectedly, this did not prove feasible because administration of this agent produced antischistosomal effects, some of which are reported in this paper.

Materials and methods

Female, 5 to 7 week old Swiss-Webster CD-1 mice were infected each with 60 cercariae of *Schistosoma mansoni* (Puerto Rican strain) by tail immersion.

CS-A was kindly supplied by Drs J.F. Borel and E. Wiskott of Sandoz Ltd., Basle, Switzerland, either as a powder or, in sealed ampules, as a 10% solution in Miglyol 812. Immediately before use, an appropriate aliquot of the solution was suspended in 0.9% NaCl containing 5% mouse serum (P.J. Tutschka; personal communication). This suspension was administered subcutaneously at a daily dose of 25 mg/kg for either 3 or 5 subsequent days. The lower dosage schedule (total dose: 75 mg/kg) produced no, or only a marginal reduction in the number of worms. This dose was used to determine biochemical effects and their time course. With the 5-day schedule (total dose: 125 mg/kg) a significant decrease in the number of parasites occurred. An interval of at least 50 days was allowed to elapse between the last dose and the autopsy, when the average total number of live worms present in the mesenteric and portal veins and in the liver sinuses was compared with that of untreated controls [3, 4].

Powdered CS-A was dissolved in 20 vol. of Emulphor El 622 (kindly supplied by GAF Corporation, New York, N.Y.). This solution could be added to aqueous media yielding a final concentration of up to 1 m*M* without precipitation.

Determination of hemoglobinase activity. Denatured powdered hemoglobin (Sigma, St. Louis, Mo.) was used as the substrate. A 15% aqueous solution of this preparation was dialyzed overnight against 21 of a 10 m*M* sodium malate buffer (pH 3.85) at 4°. After determination of the hemoglobin content [5] the concentration was adjusted to 10% with the malate buffer. Aliquots of this solution were stored at −20°C. With this preparation, optimal hemoglobinase activity was observed at a concentration of 2% for the enzyme of male, and at 0.8% for that of female schistosomes. While it has been reported that schistosome hemoglobinase activity is enhanced in the presence of sulfhydryl compounds when globin is used as the substrate [6], enzyme activity was not affected by dithiothreitol, cysteine or other sulfhydryl compounds when hemoglobin served as the substrate.

After removal from the host, the parasites were placed in 75% horse serum containing 1×10^{-4} *M* carbaminoylcholine. Use of this acetylcholine analog stabilized hemoglobinase activity for at least 2 h at room temperature. Furthermore, because of the cholinergic effect [7] separation of the two sexes was facilitated greatly. The worms were homogenized in 0.05 *M* malate buffer (pH 3.85) (20 to 30 males, 15 to 20 females per ml) in ground glass homogenizers.

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The homogenate (50 µl) was incubated at 55°C[1] for 1 h, in a total volume of 0.3 ml containing final concentrations of 0.04 *M* malate buffer (pH 3.85) and 3% hemoglobin.

Samples without homogenate and other samples without hemoglobin served as controls. All incubation mixtures were run in duplicate. The reaction was stopped by addition of 0.30 ml of 5% trichloroacetic acid (TCA), followed by thorough mixing, incubation in an ice bath for at least 10 min and centrifugation at 8000 × *g* for 4 min. Determination of terminal amino acids in the supernatant was based on the ninhydrin method of MOORE and STEIN [8].

The ninhydrin reagent was prepared as follows: 6 g of ninhydrin were dissolved in 75 ml of methyl cellosolve under a stream of nitrogen (delivered by means of a capillary pipette). Thereafter, 900 mg hydrindantin (reduced ninhydrin), another 75 ml of methyl cellosolve and 50 ml of 4 *M* sodium acetate buffer (pH 5.5) were added, the stream of nitrogen being continued throughout. The solution was stored in a tightly stoppered dark polypropylene bottle at room temperature. It was prepared freshly every 3 days. All reagents were obtained from Pierce Chemical Co., Rockford, Illinois. The reagent (0.5 ml) was added to 0.30 ml of the TCA supernatant, or to an aliquot thereof diluted to a total volume of 0.30 ml with 5% TCA. A reagent blank (0.30 ml of 5% TCA) and a standard (0.075 µmol l-leucine in 5% TCA) were run concurrently. The mixtures were heated in test tubes, covered with marbles, in a boiling water bath for 15 min and subsequently placed in an ice bath for 10 min. Thereafter 2.5 ml of 50% ethanol were added, the samples were thoroughly mixed and the optical density was determined at a wavelength of 570 nm. After correcting for the optical densities of the samples without enzyme and of those without hemoglobin, activity was expressed in terms of µmol equivalents of free amino nitrogen produced per hour, either per worm or per mg protein. Activities were found to be linear with time and enzyme concentration. In a total of 80 samples, hemoglobinase activities (in µmol) of the males varied between 0.213 (±0.056) per worm/h and 3.30 (±1.11) per mg protein/h. The corresponding values were 0.394 (±0.127) per worm and 22.6 (±4.02) per mg protein for the females.

Protein was determined by PETERSON's [9] modification of the method of LOWRY et al. [10].

Results

Daily subcutaneous administration of CS-A (25 mg/kg) for 5 successive days to mice infected with *Schistosoma mansoni* resulted in a reduction in the number of mature and immature worms (Table 1). Female mature worms (Group 1) proved more sensitive to the effect of this drug than fully developed males.[2] Furthermore, when the same dosage schedule was used it proved more effective against the early, immature, stages (Groups 4 and 5).

[1] At this temperature hemoglobinase activity was maximal, 2.8 times higher than at 37°.

[2] In the case of *S. mansoni* following cercarial infection 35 to 40 days are required for full maturation and egg production.

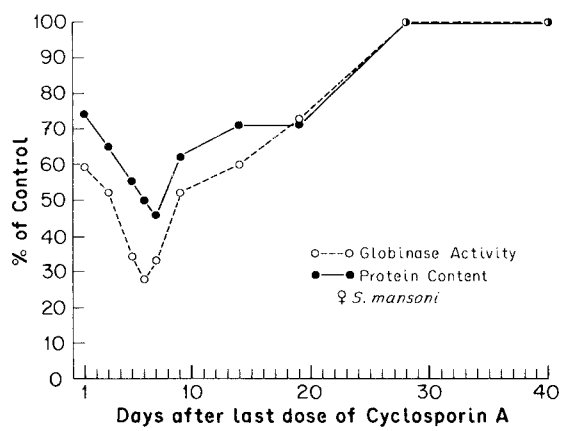
Table 1
Antischistosomal effects of cyclosporin A administered to mice infected with *Schistosoma manson*[a].

Group #	Number of days after infection when treatment was begun	% Reduction in number of worms	
		Male	Female
1	56	38	62
2	33	21	30
3	23	39	65
4	12	76	86
5	0[b]	69	72

[a] Cyclosporin A (25 mg/kg) was administered subcutaneously to mice infected with *S. mansoni,* daily for 5 successive days. The mice were sacrificed after an interval of 50 to 60 days after the last dose and the number of worms was compared with that found in untreated mice infected on the same day with the same group of cercariae.

[b] Treatment began 2 days before infection and continued for another 3 days after infection.

Earlier effects of a smaller cumulative dose of CS-A (25 mg/kg on each of 3 succeeding days) produced marked reductions in the activities of the hemoglobinase and of the protein content of adult female schistosomes. These changes persisted after cessation of the treatment; in fact, they became progressively more pronounced for a period of 6 to 7 days (figure). An interval of more than 19 days after the last dose of CS-A was required until hemoglobinase activities and protein levels returned to the



Effect of the administration of subeffective doses cyclosporin A on the hemoglobinase activities and the protein levels of female *S. mansoni.* CS-A (25 mg/kg) was injected subcutaneously on each of 3 succeeding days. Each point represents the average of at least six experiments. In no instance did the standard deviations exceed ±6.8%.

control values. These effects were limited to female worms; only slight or no such changes were observed in male worms. This may explain the less pronounced decrease in the number of male parasites following a larger total dose of the drug (Table 1).

In contrast to the effects of CS-A in vivo on hemoglobinase activities of female schistosomes, the drug did not inhibit hemoglobinase activities of either male or female schistosomes in vitro. Concentrations as high as 5×10^{-4} molar had no effect.

Treatment of mice infected with *Schistosoma mansoni* with CS-A was at least additive to the effects of subcurative doses of amoscanate, a new potent antischistosomal drug [11, 12] (Table 2). Therefore, it appears that the actions of the two compounds on the parasites are synergistic with each other.

Table 2
Synergistic antischistosomal effects of cyclosporin A with a subcurative dose of amoscanate[a].

Drug or drug combination	% Reduction in the no. of worms	% Mice with parasitologic cures
Cyclosporin A	11	0
Amoscanate	58	0
Cyclosporin A + Amoscanate[b]	100	100
Cyclosporin A + Amoscanate[c].	100	100

[a] Cyclosporin A was administered subcutaneously for 3 successive days at a daily dose of 25 mg/kg. A single oral dose of 3 mg/kg of amoscanate was used. Autopsies were performed 65 days after starting the treatment. Treatment was started 50 days after infection with 60 cercariae of *S. mansoni*.
[b] Amoscanate was administered on the same day as the first dose of cyclosporin A.
[c] Amoscanate was administered on the day of the last dose of cyclosporin A.

Discussion

CS-A has been shown to suppress selectively the early proliferative stages of certain T cell subpopulations [14, 15]. Probably, in this capacity CS-A inhibits the rejection of a large series of allografts in several species, including kidney [16, 17], and prevents the graft-versus-host disease in man [18]. If the antischistosomal action of CS-A is mediated via the host, it is conceivable that its specific immunosuppressive effect is associated with a stimulation of a defense mechanism directed against the parasite. Such an effect appears to be highly specific because many other biochemical properties of the worms, such as carbohydrate metabolism and glutathione levels remain unchanged (Bueding, Fisher and Dolan: unpublished observations). Additional evidence for the selectivity of the antischistosomal effects of CS-A is provided by a much greater sensitivity of the hemoglobinase activity of female worms, as contrasted with that of the males. Schistosome hemoglobinase has a high specificity for globin and hemoglobin [19]. Degradation of hemoglobin in red cells ingested by the parasites can provide a supply of peptides and amino acids critical for the functional integrity of the worms and for protein biosynthesis. In fact, incorporation of leucine contained in host hemoglobin into schistosome proteins has been demonstrated [20]. Thus, inhibition of hemoglobinase activity following administration of CS-A to the host could account for its antischistosomal action.

It appears unlikely that the antischistosomal action of CS-A is due to a direct effect of this compound, or of one of its metabolites, on the worms. CS-A has no inhibiting action on hemoglobinase activity of female schistosomes in vitro. Furthermore, administration of radioactively labelled CS-A results in the almost complete elimination of this undecapeptide and of its metabolites within 24 h (J. F. Borel: personal communication). Yet, reduction of hemoglobinase activity and of protein levels of female worms not only persists, but becomes even more pronounced for at least 6 days after the last dose. By contrast, if the worms are removed from their hosts 24 h after the last dose of CS-A and are transferred into a culture medium, hemoglobinase activities and protein levels of the females are the same as in control worms from untreated mice after only a single day (Belton, Hawkins, and Bueding: unpublished observations). Therefore, the antischistosomal effects of CS-A may be mediated through host defense mechanisms against the parasite. Their elucidation could provide significant information about protective immunity in schistosomiasis.

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