Effect of SQ 14225, an inhibitor of angiotensin I-converting enzyme, on the granulomatous response to Schistosoma mansoni eggs in mice.

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Research Article

Murine schistosomiasis is a granulomatous disease associated with high serum and granuloma angiotensin I-converting enzyme (ACE) activity. SQ 14225, a specific competitive inhibitor of ACE, was administered to normal mice and mice infected with Schistosoma mansoni to determine whether this compound could inhibit granuloma ACE activity and modify the size of the granulomatous response to schistosome eggs. Peroral administration of SQ 14225 for 5 wk to infected mice with peak granulomatous responses decreased ACE activity in isolated liver granulomas. Treated mice demonstrated a decrease in granuloma size in the liver, colon, and ileum, and hydroxyproline concentration of isolated liver granulomas was increased. Mean diameters of synchronous pulmonary granulomas, induced by the pulmonary embolization of schistosome eggs into normal and sensitized mice, were decreased by a similar dose of SQ 14225. Withdrawal of SQ 14225 from unsensitized mice with 2-wk-old synchronous pulmonary granulomas induced an increase in inflammation. Infected, but not normal mice receiving SQ 14225 demonstrated reduced portal pressure, liver weight, and body weight. Both normal and infected mice experienced dipsogenesis, expanded intravascular volume, and increased serum ACE. These observations suggest that SQ 14225 can partially inhibit the granulomatous response to schistosome eggs and the pathological manifestations of schistosomiasis. It is possible that ACE has an inflammatory role in granulomatous inflammation.

Effect of SQ 14225, an Inhibitor of Angiotensin I-converting Enzyme, on the Granulomatous Response to *Schistosoma mansoni* Eggs in Mice

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ABSTRACT Murine schistosomiasis is a granulomatous disease associated with high serum and granuloma angiotensin I-converting enzyme (ACE) activity. SQ 14225, a specific competitive inhibitor of ACE, was administered to normal mice and mice infected with *Schistosoma mansoni* to determine whether this compound could inhibit granuloma ACE activity and modify the size of the granulomatous response to schistosome eggs. Peroral administration of SQ 14225 for 5 wk to infected mice with peak granulomatous responses decreased ACE activity in isolated liver granulomas. Treated mice demonstrated a decrease in granuloma size in the liver, colon, and ileum, and hydroxyproline concentration of isolated liver granulomas was increased. Mean diameters of synchronous pulmonary granulomas, induced by the pulmonary embolization of schistosome eggs into normal and sensitized mice, were decreased by a similar dose of SQ 14225. Withdrawal of SQ 14225 from unsensitized mice with 2-wk-old synchronous pulmonary granulomas induced an increase in inflammation. Infected, but not normal mice receiving SQ 14225 demonstrated reduced portal pressure, liver weight, and body weight. Both normal and infected mice experienced dipsogenesis, expanded intravascular volume, and increased serum ACE. These observations suggest that SQ 14225 can partially inhibit the granulomatous response to schistosome eggs and the pathological manifestations of schistosomiasis. It is possible that ACE has an inflammatory role in granulomatous inflammation.

INTRODUCTION

Angiotensin I-converting enzyme (peptidyldipeptidase, EC 3.4.15.1) (ACE),[1] which catalyzes the conversion of angiotensin I to angiotensin II and inactivates bradykinin (1–3), is increased in several human diseases. High ACE activity is detectable in serum (4–6) from some patients with sarcoidosis or leprosy (7), and is associated with the epithelioid cell of the sarcoid granuloma (8). Patients with Gaucher's disease, a nongranulomatous condition, also have increased ACE activity detectable in serum and in Gaucher cells (9–11). There are granulomatous conditions, however, which are usually not associated with high serum ACE activity, such as tuberculosis (4–7), Crohn's disease (12), and chronic berylliosis (13). The significance of ACE activity accompanying granulomatous reactions is unknown.

We have previously reported that isolated granulomas from mice infected with *Schistosoma mansoni* demonstrate ACE activity. Furthermore, there is enhanced ACE activity associated with a spontaneous decrease in the intensity of the granulomatous inflammation. This suggests that ACE may participate in modulation of the inflammation (14).

Compound SQ 14225 (E. R. Squibb & Son, Princeton, N. J.) is a competitive inhibitor of ACE which is effective after oral administration. This compound has potential application in the management of hypertension (15) and is an inhibitor of ACE activity in macrophages

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[1] *Abbreviation used in this paper*: ACE, angiotensin-converting enzyme.

cultured in vitro (16). In the present investigation, we tested the effect of SQ 14225 on murine schistosomiasis and determined whether this compound could inhibit granuloma ACE activity and alter the size of the granulomatous response to schistosome eggs. We determined that SQ 14225 not only decreased granuloma ACE activity, but also decreased granuloma size, increased granuloma hydroxyproline concentration, and modified disease manifestations.

METHODS

Animals used and methods of infection. Female CBA/J mice (Jackson Memorial Laboratories, Bar Harbor, Maine), 6–7 wk of age, were infected by subcutaneous injection of 25 cercariae of the Puerto Rican strain of *S. mansoni*. Perfusion of the portal system of infected animals yields 8–10 worm pairs.

Isolation of liver granulomas. Granulomas were isolated by modification of the method of Pellegrino and Brener (17). Subsequently, they were washed three times with Hanks' balanced salt solution, packed at 300 *g* to remove all supernate, and frozen at −70°C for periods up to 2 mo. Under these conditions, there is no change in ACE activity.

Isolation of schistosome eggs. With modification of the method of Coker and von Lichtenberg (18), schistosome eggs were isolated from the livers of mice infected 8 wk previously with 200 cercariae of *S. mansoni*.

Induction of synchronous pulmonary granulomas (19). Eggs were suspended in phosphate-buffered saline at a concentration of 8,000 eggs/ml, and 4,000 eggs were injected into the tail vein of 8-wk-old normal mice, using a tuberculin syringe with a 21-gauge needle. These eggs embolized to the lungs, inducing focal granulomas. Another group of normal mice was sensitized by intraperitoneal injection of 1,000 schistosome eggs 1 wk before intrapulmonary challenge.

Assay of ACE activity and protein concentration. To determine the ACE activity in isolated granulomas, granulomas were ground in a mortar with a pestle for 2 min (200 mg tissue/3 ml Hanks' solution) on ice, and subsequently sonicated at 0°C for 30 s. Protein concentration (20) and ACE activity were measured in these granuloma extracts.

ACE activity was determined by a spectrophotometric method (4). The substrate was hippuryl-L-histidyl-L-leucine. Results were expressed in serum as hippuric acid (nanomoles per minute) per milliliter and in tissue as nanomoles per minute per milligram protein.

Administration of SQ 14225. ACE inhibitor SQ 14225 was administered orally to animals in their drinking water. Drinking water containing the drug was changed twice weekly. ACE inhibitor activity was still present in drinking water after 4 d at room temperature. Infected animals received the drug from the 5th to the 10th wk of infection. Granuloma formation begins during the 5th wk of infection, and granuloma size peaks during the 10th wk of infection. The animals were housed usually three to a cage. Water consumption was recorded by groups. There were no apparent toxic reactions to the drug.

Determination of granuloma volume and hydroxyproline content. Mean granuloma volume was calculated from mean granuloma diameter. Diameter was measured in stained histologic sections of liver, ileum within 2 cm of the ileocecal valve, right half of colon, and/or lungs by a person who was unaware of the identity of the sections. Nonconfluent granulomas containing parasite eggs were located microscopically in the liver, lung, or lamina propria. Their diameters were measured with an image-splitting eyepiece (Vickers Instruments Inc., Woburn, Mass.) by determining the mean of two measurements made across perpendicular axes.

The hydroxyproline content of isolated liver granulomas was determined by the method of Neuman and Logan (21). The results were expressed as both hydroxyproline per moist granuloma weight and hydroxyproline per granuloma. Hydroxyproline per granuloma was determined by measuring the hydroxyproline content of a mass of 200 isolated granulomas per liver. Granulomas were counted with the aid of a dissecting microscope. The mean granuloma hydroxyproline concentration was calculated from the mean hydroxyproline per granuloma and from the mean granuloma volume.

Clinical parameters. Water consumption and weight were recorded weekly. Portal pressure was measured with a physiograph (Narco Bio-Systems, Inc., Houston, Tex.) (22). Blood volume was measured by bleeding animals through puncture of the ophthalmic venous plexus into a heparinized, siliconized, graduated cylinder. The total blood erythrocyte count was calculated from the blood volume and the erythrocyte count per cubic millimeter as determined with a hemocytometer.

Statistical methods. Data were subject to the Student's *t* test to determine significant differences between groups. P values < 0.05 were considered significant.

RESULTS

Inhibition of ACE activity in isolated liver granulomas. Peroral administration of ACE inhibitor SQ 14225 for 5 wk decreased ACE activity in isolated liver granulomas from 10-wk infected mice with peak granulomatous responses (Fig. 1). Treatment, however, was associated with an increase in serum ACE activity in both infected and normal mice (Table I). ACE activity in homogenates of isolated liver granulomas from animals infected for 8 or 20 wk was inhibited 100% with the addition of SQ 14225 at 10 μM concentration.

Effect of SQ 14225 on the granulomatous response of mice with schistosomiasis. The inhibitory effect of SQ 14225 on granuloma ACE activity prompted us to determine whether inhibitor therapy would modify the granulomatous response. Inhibitor at 75 mg/kg per d

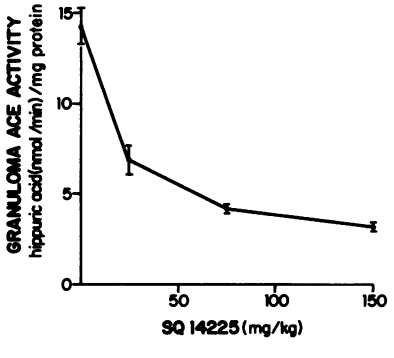

FIGURE 1 ACE activity in liver granulomas from mice receiving daily doses of SQ 14225 for 5 wk. Data are mean values ±SEM of six mice.

TABLE I

Effect of SQ 14225 Administration on Serum ACE Activity and on Granuloma Size and Hydroxyproline Concentration

	Infected mice*		Normal mice	
	Control	SQ 14225	Control	SQ 14225
Serum ACE activity (hippuric acid), *(nmol/min)/ml*	226±6.0	285.3±12.5‡	196.6±12.0	293.7±14.4§
Mean granuloma size, $mm^3 \times 10^{-4}$				
Liver	286.6±11.0	182.3±9.0§		
Colon	163.6±10.0	124.0±8.0§		
Ileum	31.0±1.5	25.0±0.5‖		
Liver granuloma hydroxyproline				
Hydroxyproline/granuloma wet weight, *mg/g*	26.5±1.5	33.5±1.0‡		
Hydroxyproline/granuloma volume, mg/mm^3	0.28±0.02	0.43±0.04¶		
Hydroxyproline/granuloma, $mg \times 10^{-3}$	6.53±0.78	7.55±0.85		

* 10-wk infected and normal mice received SQ 14225, 75 mg/kg per d per os for 5 wk prior to killing. Serum ACE activity and hydroxyproline data are mean values±SEM of six mice. Mean granuloma size data are mean values±SEM of 120 observations from six animals.

‡ $P < 0.001$.

§ $P < 0.0005$.

‖ $P < 0.005$.

¶ $P < 0.05$.

was administered over the 5th to the 10th wk of infection. Treated mice sacrificed at 10 wk of infection demonstrated a decreased mean granuloma size in the liver, colon, and ileum (Table I). Hydroxyproline concentration was increased in isolated liver granulomas (Table I). Similar therapy to normal mice did not affect liver hydroxyproline content (control vs. treatment groups; 1.09±0.04 and 1.17±0.06 mg/g liver weight, respectively).

Effect of SQ 14225 on synchronous pulmonary granulomas. Mean volume of synchronous pulmonary granulomas in normal and previously sensitized mice was decreased by SQ 14225 treatment, 75 mg/kg per day. Drug administration was instituted at the time of egg embolization. Withdrawal of the inhibitor after 2 wk of administration to normal, unsensitized mice with 2-wk-old synchronous pulmonary granulomas led to an increase in the granulomatous response (Fig. 2).

Physiological alterations induced by SQ 14225. Decrease of the size of the granulomatous inflammation associated with SQ 14225 administration prompted us to evaluate the effect of treatment on other pathological manifestations of schistosomiasis. Infected mice demonstrated decreased portal pressure, hematocrit, and liver weight while receiving SQ 14225 (Table II). These mice also consumed more water and gained less weight than comparable control animals (Fig. 3A). Normal mice receiving SQ 14225 also consumed more water and had decreased hematocrits, but demonstrated no alteration in portal pressure, liver, or body

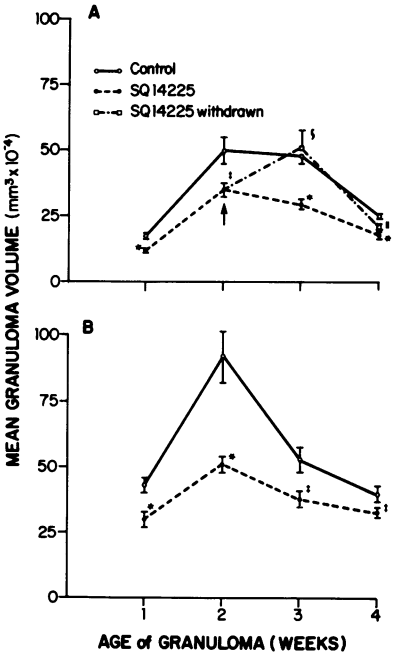

FIGURE 2 Effect of SQ 14225 treatment, 75 mg/kg per d, on the mean volume of synchronous pulmonary granulomas in (A) normal unsensitized and (B) sensitized mice. Inhibitor was discontinued in one group of unsensitized mice following 2 wk of therapy (arrow). Data are mean values±SEM of 120 observations from six mice. *, Treatment vs. control groups, $P < 0.0005$; ‡, treatment vs. control groups, $P < 0.01$; §, SQ 14225 withdrawal vs. treatment groups, $P < 0.005$; ‖, SQ 14225 withdrawal vs. control groups, $P < 0.0005$, SQ 14225 withdrawal vs. treatment groups, $P < 0.01$.

TABLE II
Physiological Alterations Induced by SQ 14225 Treatment

	Infected mice*		Normal mice	
	Control	SQ 14225	Control	SQ 14225
Portal pressure, cmH_2O	7.7±0.4	6.0±0.4‡	6.6±0.3	6.4±0.5
Liver weight, *g*	2.46±0.12	2.18±0.09‡	1.08±0.05	1.12±0.04
Spleen weight, *mg*	244.0±16.9	250.5±21.7	83.8±5.6	80.2±3.2
Hematocrit, %	39.9±0.6	35.7±1.4‡	42.9±0.6	36.8±0.5§
Blood volume, *ml*	1.42±0.08	1.63±0.03‖	1.31±0.04	1.50±0.04‖
Total blood erythrocyte count, $\times 10^6$			11.94±0.04	12.06±0.09
Leukocyte count, $cells/mm^3$			2,600±310	2,546±281

Values are mean±SEM. $n = 6$.
* 10-wk infected and normal mice received SQ 14225, 75 mg/kg per d per os, for 5 wk.
‡ $P < 0.01$.
§ $P < 0.001$.
‖ $P < 0.0005$.

weight (Table II, Fig. 3B). The decreased hematocrit was related to increased blood volume. Total blood erythrocyte and leukocyte counts and spleen weights were not altered (Table II).

DISCUSSION

The chronic granulomatous host response around schistosome eggs leads to liver fibrosis and portal hypertension (23). Though as yet unproven, suppression of the granulomatous response may reduce the pathologic manifestations and increase survival of heavily infected individuals. A variety of approaches have been tried to suppress the experimentally induced, schistosome egg granuloma, with varying success (24). However, no effective, safe, and practical means has been found for the suppression of the granulomatous response.

Peroral drug treatment inhibited ACE activity within the liver granulomas and decreased the size of the granulomatous response around schistosome eggs. Granuloma size was also decreased in the colon and ileum of infected mice. Similarly, synchronous lung granulomas, induced by the pulmonary embolization of schistosome eggs into normal or presensitized mice, also demonstrated a reduction in size.

There was a differential sensitivity of granulomas in various organs to ACE inhibitor treatment, the liver lesions being the most sensitive and the ileal granulomas showing relatively small though significant response to the drug. This is noteworthy because ileal granulomas are also smaller than their hepatic or colonic counterparts and do not undergo spontaneous modulation in size with chronic infection.[2]

Whether the drug diminished granuloma size by the specific inhibition of ACE activity or by a broader anti-inflammatory effect cannot be answered with certainty at present. SQ 14225 does not appear to be broadly anti-inflammatory because it did not suppress delayed

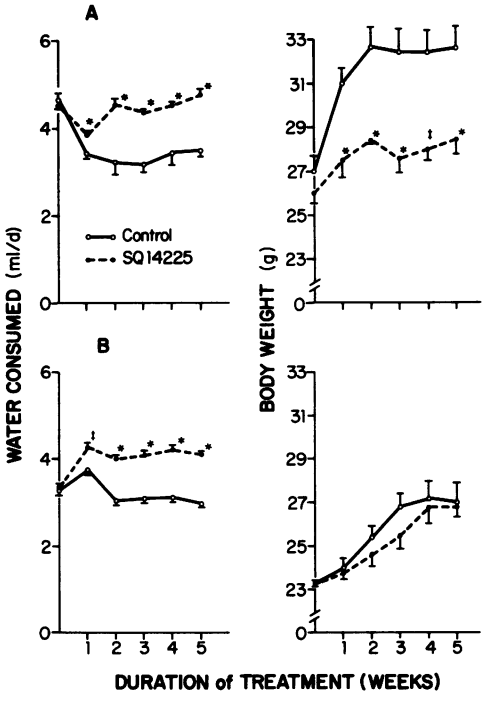

FIGURE 3 Mean water consumption and body weight of (A) infected and (B) age-matched normal mice receiving SQ 14225, 75 mg/kg per d for 5 wk. In (A), the inhibitor was administered from the 5th to the 10th wk of infection. Data points are mean values±SEM of 6–12 observations. *, Treatment vs. control groups, $P < 0.0005$; ‡, treatment vs. control groups, $P < 0.01$.

[2] Weinstock, J. V., D. L. Boros, and J. B. Gee. Submitted for publication.

tuberculin responses in guinea pigs sensitized to *Mycobacterium tuberculosis* (25). In addition, passive cutaneous anaphylaxis in rats was only weakly inhibited (26). Similarly, artificial hypersensitivity granulomas induced by pulmonary embolization of methylated bovine serum albumin-coupled Sepharose beads, which were devoid of ACE activity, were not suppressed (Weinstock, J. V., and D. L. Boros, manuscript in preparation).

The significance of enhanced ACE activity accompanying some but not all granulomatous diseases is currently unknown. Many patients with sarcoidosis and leprosy are shown to have increased ACE activity in the circulation (4–7, 27). The nine-banded armadillo develops lepromatous type leprosy which is also associated with increased serum ACE activity (28). Elevated serum ACE activity in patients with sarcoidosis is considered to be a reflection of the intensity of the granulomatous process, because activity returns to normal during remission or after corticosteroid therapy (29).

Our experiments, using a competitive inhibitor which caused a decrease in granuloma size, suggest a possible inflammatory role for ACE. What role this may be is unknown. Experiments using macrophage monolayers from dispersed granulomas indicate that ACE is secreted by macrophages.[3] This is consistent with previous observations associating ACE activity with granuloma macrophages (8, 10, 16). Thus, macrophages within the granulomas may engage in regulation of the chronic inflammatory response through the production and release of ACE.

SQ 14225 treatment of infected mice also resulted in increased granuloma hydroxyproline concentration but did not alter granuloma hydroxyproline content. This effect appeared to be specific to the granuloma since no increase in liver hydroxyproline concentration was detected in drug-treated normal animals. It is presumed that hydroxyproline concentration reflects granuloma collagen content. Thus, elevated hydroxyproline concentration may indicate either a direct drug effect on the fibroblasts or an indirect effect which somehow influences interaction between fibroblasts, lymphocytes, and/or macrophages (30–32) during fibrogenesis. It is also possible that altered cellular content and composition or changes in noncollagen, granuloma matrix account for these findings. Nevertheless, these data suggest that SQ 14225 treatment in some manner alters granuloma structure.

Administration of ACE inhibitor also decreased liver weight and ameliorated portal hypertension. This is presumably due to decreased liver granuloma size.

Both infected and normal mice receiving ACE inhibitor experienced dipsogenesis and expanded intravascular volume. Little is known pertaining to the effects of oral SQ 14225 on salt and water balance. Perhaps these phenomena are related to alteration of either the peripheral or intracerebral renin-angiotensin system (33, 34).

Thus, ACE inhibitor can partially inhibit the granulomatous response to schistosome eggs and modify pathological manifestations of murine schistosomiasis. This indicates a potential use for the drug as an anti-inflammatory agent in the therapy of some granulomatous diseases. It can also serve as a useful probe in the elucidation of the role of ACE in granulomatous inflammation.

[3] Weinstock, J. V., and D. L. Boros. Submitted for publication.

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