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# THE EFFECT OF CHRONIC INGESTION OF ETHANOL ON MODULATION OF GRANULOMATOUS INFLAMMATION IN EXPERIMENTAL SCHISTOSOMIASIS IN MICE

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## SUMMARY

We studied the role of ethanol on the modulation of liver granulomata around *Schistosoma mansoni* eggs in mice. Albino mice, receiving 7% ethanol as the sole drinking liquid, at 60 and 90 days post-infection, presented smaller granulomata than controls did, when sacrificed at 120 days post-infection. No differences in diameters could be observed, when ethanol was given 4 months before up to 120 days after infection. The results suggested that modulation of schistosome granulomata by ethanol ingestion varies with time and duration of drug consumption.

**KEY WORDS**: Schistosomiasis mansoni; Ethanol; Modulation of granulomatous inflammation.

## INTRODUCTION

Chronic alcohol consumption and schistosomiasis mansoni are problems of Public Health in Brazil, as well as in many other parts of the World [3,8,10]. From an epidemiological viewpoint, alcoholism and schistosomiasis are frequently associated diseases. There are few studies carried out with humans showing this association. KASSANDA [6] observed a greater percentage of complications in the hepatosplenic form of the disease in human alcoholics in relation to non-alcoholics.

ORREGO et al [9] found a significant reduction of mean hepatic granuloma diameter in mice due to ingestion of ethanol. Their animals received ethanol administration from the 1st day of infection onward (carried out with 10 cercariae of a Puerto Rican strain of *Schistosoma mansoni*), and were killed 9 weeks after infection.

To observe the effects of ethanol on modulation of liver granulomata around eggs, we studied the constant administration of ethanol before and after infection of mice with *Schistosoma mansoni* cercariae (LE strain).

## MATERIAL AND METHODS

### *Animals*

We used female albino mice (*Mus musculus*), with an average body weight of 34.7 g. Each animal was infected subcutaneously with 15 *S. mansoni* cercariae (LE strain) shed by *Biomphalaria glabrata*. Albino mice (*Mus musculus*), as well as *S. mansoni* cercariae used in this experiment were obtained from the Schistosomiasis Research Unit laboratories, Federal University of Minas Gerais.

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The animals that received ethanol were housed and caged in the same way as the controls, but given 7% ethanol in water as the only source of drinking liquid.

The animals were divided into six groups as follows:

GROUP 1 - Twenty one animals were infected after 4 months of daily ingestion of ethanol, and sacrificed by cervical fracture at 60, 90, and 120 days after infection.

GROUP 2 - Twelve animals were infected and received ethanol 60 days later, and were sacrificed 90 and 120 days after infection.

GROUP 3 - Seven animals were given ethanol 90 days after infection, and were sacrificed at 120 days.

GROUP 4 - Twenty animals were infected and offered tap water throughout the experiment. They were sacrificed at 60, 90, and 120 days after infection.

GROUP 5 - Twenty three animals were given 7% ethanol solution from the beginning of the experiment onwards. They were sacrificed 6, 7, and 8 months later.

GROUP 6 - Twenty seven animals were given tap water, and sacrificed at the same time as Group 5.

All the six groups were fed on mouse chow (Purina R), *ad libitum*. A hundred grams of the chow provided 341.64 Kcal and had 24.0% of protein, 28.0% of fat, and 43.8% of sugars. The biochemical analysis of the Purina chow was performed by the Department of Biochemistry, Institute of Biological Sciences, Federal University of Minas Gerais, Brazil.

In order to evaluate whether the ingestion of ethanol and the ethanol plus schistosomiasis influenced the animals nutrition, 15 mice were individually caged in appropriate containers, receiving identical treatment as the ones from Groups 2, 5 and 6 (5 animals from each group) for comparison of food, ethanol and water ingestion. A mean of the daily ingested Kilocalories provided by ethanol and mouse chow was calculated, considering that 1.0g of ethanol provides 7.10 Kcal [7]. The p.a. ethanol (Merck) used was a concentration of 0.79 g/ml pure ethanol, so that the final solution (7% of p.a. Merck ethanol) had 0.393 Kcal per ml.

The animals nutritional status was evaluated by comparing their ingestion of chow and initial and final weights throughout the experimental period.

### *Histological Analysis*

A half of the liver from each mouse was fixed on Bouin's solution for 18 h, dehidrated, embedded in paraffin, cut into 5 μm sections and stained with hematoxilin and eosin. The other half was fixed in formalin for other studies not presented here [4].

The size of granulomata were evaluated by measurement of two perpendicular diameters of lesions around fresh single eggs, by means of a splitting eyepiece (10X, Ernst-Leitz, Wetzlar, Germany), adapted to a Zeiss microscope. Only granulomata containing eggs with eosinophilic staining of their miracidia were measured [9, 12].

At least 50 granulomata from each group were measured, and the mean granuloma diameter per group was determined.

TABLE 1

Mean granuloma diameter (μm) in the liver of mice infected with 15 *S. mansoni* cercariae (LE strain)

| GROUPS | Days of the sacrifice after infection | | |
|---|---|---|---|
| | 60 | 90 | 120 |
| 1 | 443.52 ± 36.90 (a) | 350.34 ± 20.19 (b) | 313.84 ± 44.68 (b) |
| 2 | - | 338.01 ± 18.31 (b) | 275.31 ± 25.74 (c) |
| 3 | - | - | 278.66 ± 13.80 (c) |
| 4 | 427.25 ± 7.65 (a) | 334.67 ± 36.24 (b) | 316.07 ± 33.39 (b) |

The same letters of the same column or line indicate that the values are statistically identical.

Group 1 - Ingestion of ethanol 4 months before infection up to the sacrifice day.
Group 2 - Ingestion of ethanol 60 days after infection onwards.
Group 3 - Ingestion of ethanol 90 days after infection onwards.
Group 4 - Schistosomiasis alone.

*Statistical Analysis*

Data were analysed by analysis of variance ($p < 0.05$).

## RESULTS

Table 1 presents the mean granuloma diameter of each group at the sacrifice day. In all the groups, there was a significant reduction of the mean diameter of the granulomata with time. Group 1 (ethanol given 4 months before infection onwards) did not show differences of mean granuloma diameter (443.52 μm, 350.34 μm, and 313.84 μm) at 60, 90, and 120 days after infection, respectively, in relation to controls (Group 4 = 427.25 μm, 334.67 μm, and 316.07 μm at 60, 90, and 120 days after infection, respectively). Groups 2 and 3 (ethanol given after infection) showed a significant reduction of the mean granuloma diameter 120 days after infection (275.31 μm and 278.66 μm, $p < 0.05$).

The cellular composition of the granulomata observed was similar as described in the literature [12, 13].

Mice that drunk ethanol ingested about 17.3% of the total calories/day as ethanol (Table 2). The caloric effects of ethanol apparently did not alter either the animals nutritional status, whether infected or not, or the relationship between the liver and body weight from each animal at the sacrifice day [4].

Statistically significant differences between the initial and final weights of mice could not be detected, so their nutritional status was considered stable throughout the experiment (Table 2).

## DISCUSSION

Modulation of granulomatous inflammation in the liver of mice with schistosomiasis has been extensively demonstrated [2, 13] since ANDRADE & WARREN'S study, in 1964 [1]. They observed that there is a spontaneous reduction of the granulomatous response with time of infection, and with improvement of the clinical and pathological aspects of the disease. The reduction is more evident at 6-8 weeks after infection, and the granulomata become considerably smaller by the 20th week.

We were able to observe modulation of the granulomatous inflammation in all the infected groups (mean granuloma diameter of 120 days smaller than those ones of 60 days) (Table 1).

ORREGO et al. [9] studied the interaction of chronic ingestion of ethanol in mice with schistosomiasis, and found a reduction of the mean granuloma diameter 9 weeks after infection, that corresponds to our time of sacrifice (60 days after infection). We did not find a reduction at this time, but we did at 120 days on the groups that ingested ethanol after infection, when compared to Group 4. In our experiment, the ethanol solution of 7% provided the animals with about 17-20% of the total calories consumed, which was less than the utilized by ORREGO et al. [9] (35% of total calories as ethanol). Possibly, the amount of ethanol ingested was important on the modification of the granulomatous response of mice observed.

The group of mice that consumed ethanol 4 months before infection onwards did not show any differences

TABLE 2

- Mean initial and final weights, food and ethanol ingestion per day by animals from Groups 2, 5 and 6*

| GROUP (7%) | WEIGHTS (g) | | CHOW | | ETHANOL** | | TKCAL/D |
|---|---|---|---|---|---|---|---|
| | INITIAL | FINAL | (g) | KCAL/D | (ml) | KCAL/D | |
| 2 | 38.20 | 37.60 | 44.38 | 10.80 | 7.00 | 2.73 | 13.60 |
| 5 | 34.72 | 34.78 | 54.88 | 13.40 | 5.83 | 2.27 | 15.66 |
| 6 | 31.06 | 31.36 | 61.28 | 14.95 | - | - | 14.95 |

* Experiment carried out with animals caged individualy.

** Given as 7% ethanol in water, as the only drinking liquid

Group 2 - Ingestion of ethanol 60 days after infection

Group 5 - Ingestion of ethanol throughout the experiment

Group 6 - Control

KCAL/D = Kilocalories ingested per day

TKCAL/D = Total Kilocalories ingested per day

on granuloma diameter, when compared to Group 4 (infection only). We believe that these mice became adapted to the condition of chronic consumption of ethanol.

Considering that the modulation of the granulomatous inflammation is probably the result of the interaction of subpopulations of T-lymphocytes (suppressor cells and effector cells), with a balance in favor of the suppressor subpopulation [2], it is possible that the ethanol may act on the function of these cells, either directly or indirectly. DEHNE et al. [5] observed that different doses of ethanol, when given continuously to mice, determined depression of cell mediated immunity, but his experiment lasted only 18 days.

Our study suggests that the granulomatous inflammatory reaction to *S. mansoni* in mice and its modulation are modified by the consumption of ethanol, depending on the time of consumption and/or if it is started before or after infection.

## RESUMO

**Efeito da ingestão crônica de etanol na modulação da resposta inflamatória granulomatosa no fígado de camundongos infectados por** Schistosoma mansoni

Estudamos a modulação da resposta inflamatória granulomatosa em torno de ovos de *S. mansoni* no fígado de camundongos albinos (*Mus musculus*), que receberam 7% de etanol como única fonte de líquido. Os animais que receberam etanol aos 60 e 90 dias após a infecção apresentaram granulomas menores do que os controles, quando sacrificados aos 120 dias após a infecção. Não houve diferença no diâmetro dos granulomas quando a ingestão de etanol foi iniciada 4 meses antes e prolongada até 120 dias após a infecção. Nossos resultados indicam que a modulação da inflamação granulomatosa varia de acordo com o tempo e a duração da ingestão do etanol.

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