

CHANGES IN PENTOBARBITAL INDUCED SLEEPING-TIME IN MICE INFECTED WITH *SCHISTOSOMA MANSONI* AND IMMUNOSUPPRESSED

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SUMMARY

To evaluate whether the intensity of the hepatic granulomatous response induced by *S. mansoni* eggs plays a role in drug metabolism, mice were infected with 40 cercariae and tested to assess the sodic pentobarbital induced sleeping-time. To decrease the inflammatory reaction the animals were irradiated with 400 Rad or received azathioprine, 20mg/kg, 3 times a week, for 4 weeks, respectively in or beginning in the 33th post-infection day. In infected animals receiving azathioprine the area of the hepatic granulomas was smaller and the sleeping-time was similar to that of non-infected ones (controls). In mice infected and irradiated the granuloma dimensions were similar to those of animals only infected, in these two latter groups of animals, the sleeping-time was more prolonged than that of the control animals. These results show that: 1) mice with unaltered hepatic granulomatous reaction show reduction in metabolism of sodic pentobarbital; 2) granulomatous response diminished by azathioprine does not interfere with the capacity of metabolism of the anesthetic drug.

KEYWORDS: *Schistosoma mansoni*; Immunosuppression; Sleeping-time; Drug metabolism; Granulomas

INTRODUCTION

In a previous work, COELHO et al. ⁷ showed that the sleeping-time induced by sodic pentobarbital was significantly prolonged in mice infected with *Schistosoma mansoni* and that the anesthesia time paralleled the worm load, suggesting a low hepatic metabolism of the anesthetic agent. In the same line of investigation, CHA & BUEDING ⁴ have shown that the activity of some hepatic microsomal enzymes was depressed in mice infected with *S. mansoni* and that the treatment of the parasitic infection was followed by reestablishing the

hepatic metabolic capacity. Further studies by CHA et al. ⁵, by using unisexual infection, and by CHA et al. ⁶, using thymectomized mice, have demonstrated the importance of parasite eggs and inflammatory response for the changes in the liver metabolism.

The present study is designed to confirm and to extend these latter informations, due to their possible significance in clinical setting. Changes in hepatic metabolic activity in schistosomotic people have practical inter-

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est, since a large number of these individuals receives anesthesia for various reasons or ingests toxic drugs, medical or not. If the hepatic metabolic capacity does become low in this disease, the dose of an anesthetic, for example, can be reduced. To attain the proposed goals, mice infected with *S. mansoni* were treated with an immunosuppressive drug (azathioprine) or with gamma irradiation, trying to decrease the granulomatous response. In order to estimate the potential of metabolizing chemical substances, the sleeping-time by sodic pentobarbital was evaluated.

MATERIALS AND METHODS

Adult female mice (Balb C) were subcutaneously infected with 40 cercariae of the LE (Belo Horizonte) strain of *S. mansoni*. This strain has been maintained in the laboratory of the "Grupo Interdepartamental de Estudos sobre Esquistossomose - Laboratório José Pellegrino (GIDE), Instituto de Ciências Biológicas, UFMG", by serial passages in *Biomphalaria glabrata* and hamsters (*Mesocricetus auratus*) for more than 30 years. The mice were divided into 6 groups, as follows: **Group 1.** Thirty mice infected with *S. mansoni* and treated with azathioprine, 20 mg/kg, orally, 3 times a week, for 4 weeks, beginning in the 33th day after infection; **Group 2.** Thirty mice infected with *S. mansoni* and irradiated in the 33th day after infection with 400 Rad (gamma rays); **Group 3.** Thirty mice infected with *S. mansoni*; **Group 4.** Twenty mice non-infected and treated with azathioprine, 20mg/kg, 3

times a week, for 4 weeks, from the 33th corresponding day of infection of animals in groups 1, 2 and 3; **Group 5.** Twenty mice non-infected and irradiated with 400 Rad in the 33th corresponding day of the infection in the groups 1, 2 and 3; **Group 6.** Twenty mice non-infected, non-irradiated and untreated.

In the 66th day post-infection (or corresponding to), the animals from the six groups were anesthetized with sodic pentobarbital (66.5 mg/kg). The sleeping-time was determined by the capacity of the animals to change from the dorsal position to the normal position.

After the anesthesia test, 10 animals from each of the groups 1, 2 and 3 were perfused for adult worms, according to the procedure designed by PELLEGRINO & SIQUEIRA¹⁵, the remainder being sacrificed for histopathological study of the liver. This organ was fixed *in totum* in 10% formalin for 1 to 10 days and than sectioned. Five slices from different lobes were chosen from each animal and processed according routine procedures for paraffin embedding. Sections with 6 µm were stained with hematoxylin and eosin (HE).

Besides the histopathological analysis, the schistosomal granulomas were measured according to VON LICHTENBERG¹⁸. The greatest and the smallest diameter of all granulomas present in all the sections were measured, provided they were well individualized and should have a single egg inside. The area of the granuloma was calculated according to the formula of the ellipse area:

$A = D/2 * d/2 * \pi$, where D is the greatest and d the smallest diameter and $\pi = 3.1416$.

TABLE 1

Sleeping-time induced by sodic pentobarbital in mice infected or not with *S. mansoni*, with or without immunosuppression.

Groups	Number of animals	Anesthesia time (in minutes)
1. Infected + azathioprine	25	62.1 ± 39.4
2. Infected + irradiation	27	108.0 ± 54.0
3. Infected	29	104.0 ± 53.0
4. Non-infected + azathioprine	20	66.1 ± 23.5
5. Non-infected + irradiation	20	54.5 ± 23.4
6. Non-infected	20	64.0 ± 13.3

Student-Newman-Keuls test

1 x 2	p < 0.05	2 x 3	N.S.	3 x 5	p < 0.05
1 x 3	p < 0.05	2 x 4	p < 0.05	3 x 6	p < 0.05
1 x 4	N. S.	2 x 5	p < 0.05	4 x 5	N. S.
1 x 5	N. S.	2 x 6	p < 0.05	4 x 6	N. S.
1 x 6	N. S.	3 x 4	p < 0.05	5 x 6	N. S.

N.S. = not significant

TABLE 2

Area of hepatic granulomas in the three groups of *S. mansoni* infected mice

Groups	Number of measured granulomas	Area of granuloma (in mm ²)
1. Infected + azathioprine	737	0.070 ± 0.034
2. Infected + irradiation	663	0.093 ± 0.043
3. Infected	806	0.087 ± 0.037

Student-Newman-Keuls test

1 x 2	p < 0.05
1 x 3	p < 0.05
2 x 3	N. S.

N. S. = not significant

For statistical analysis the values of each variable were submitted to analysis of variance. To discriminate the significance of the differences found, the Student-Newman-Keuls test was applied.

Histological sections from the livers of the non-infected animals were also examined to detect possible morphological changes due to azathioprine treatment or to irradiation.

In order to know whether the parasite load has any effect on the anesthesia time, the animals were subdivided into subgroups according to the length of the anesthesia (animals with anesthesia time below 70 minutes or above 100 minutes).

RESULTS

Table 1 shows that in infected and azathioprine-treated mice the sleeping-time was not different from that of the non-infected animals. On the contrary, infected mice not-receiving azathioprine or infected and irradiated mice presented similar sleeping-time, which in turn was significantly longer than that found in the non-infected animals or in infected and azathioprine-treated ones.

The granuloma dimensions in the three groups of infected animals are seen in Table 2. The area of the granulomas in the infected azathioprine-treated group was significantly smaller in comparison with the groups infected and irradiated or only infected; between these two latter groups no difference was noted.

TABLE 3

Number of *S. mansoni* adult worms in the three groups of infected mice

Groups	Number of animals	Number of worms
1. Infected + azathioprine	10	24.9 ± 7.8
2. Infected + irradiation	10	22.3 ± 9.2
3. Infected	10	28.6 ± 6.8

F = 1.583 P = 0.223

No relevant qualitative morphological differences were observed in the granulomas in the three infected groups. Apart from granulomas, in all groups of infected animals there were intralobular inflammation and coagulative necrosis of hepatocytes, in general in proximity of the granulomas; the necrosis was more

frequent and slightly more extensive in irradiated or azathioprine-treated animals.

As seen in table 3, the number of worms was not different in the three groups of infected animals. In Table 4 it is shown the worm numbers in the subgroups of animals with sleeping-time below 70 minutes or above 100 minutes. There was no difference in the number of worms recovered from the animals in the different groups.

TABLE 4

Number of *S. mansoni* adult worms and sleeping-time

Groups	Number of animals	Number of worms
<i>Anesthesia time < 70'</i>		
1. Infected + azathioprine	4	23.0 ± 7.0
2. Infected + irradiation	5	20.6 ± 6.8
3. Infected	5	28.4 ± 8.6
<i>Anesthesia time > 100'</i>		
1. Infected + azathioprine	6	26.2 ± 9.0
2. Infected + irradiation	5	24.0 ± 11.6
3. Infected	5	28.8 ± 7.0

F = 0.736 P = 0.606

The histopathological analysis of the livers in the non-infected animals demonstrated no changes related to the irradiation or to the toxic effect of the azathioprine.

DISCUSSION

The metabolism of sodic pentobarbital takes place mainly in the liver. For that reason, it is recommended to control its use in individuals presenting hepatic disturbances. The metabolism of barbiturates of short and medium duration involves oxidation of radicals linked to carbon 3, resulting in the production of alcohols, phenols, cetones or carboxylic acids ¹¹. Experimentally, studies of COELHO et al. ⁷, CHA ² and CHA & BUEDING ⁴ have shown a decrease in barbiturate metabolism in *S. mansoni* infected mice.

In the present study, it is demonstrated that azathioprine, when used at the dose of 20 mg/kg, 3 times a week, for 4 weeks, promotes immunosuppression in mice revealed by reduction in the area of hepatic granuloma around *S. mansoni* eggs. In these animals, the sleeping-time was not different from that of the animals non-infected with the parasite, indicating a normal metabolism of sodic pentobarbital. On the other hand, in infected mice with unaltered immunopa-

thological response the sleeping-time was increased, showing a reduction in activity of hepatic microsomal enzymes. So, an association between intensity of the granulomas reaction and degree of the pentobarbital metabolism could be established.

It is worthy to note that irradiation with 400 Rad was ineffective in reducing the intensity of the granulomatous reaction, measured by the area of the granuloma. These data are coherent with the increase in sleeping-time observed in these animals, which is similar to that of mice only infected, also indicating a decrease in metabolism of the anesthetic drug. PERROTO & WARREN¹⁶ also used gamma irradiation (450 Rad) to diminish the granulomatous response in mice, but have not found a significant reduction in the dimensions of pulmonary granuloma 32 days after inoculation of *S. mansoni* eggs into the tail vein.

The histopathological analysis of this investigation has shown that the more evident morphological change attributed to azathioprine is the reduction of granulomatous reaction. Other authors have also found a decrease in size of granulomas in athymic or immunosuppressed mice by drugs, including azathioprine^{1,2, 8-10, 12-14, 17}. The coagulative necrosis of hepatocytes seems to be related with the ischemia caused by embolization of eggs or worms rather than with the treatments employed.

The present results and similar data in the literature allow to suppose that schistosomotic patients can also present changes in drug metabolism. Therefore, clinical studies should be performed in order to evaluate the occurrence of such phenomenon in human beings.

RESUMO

Alterações no tempo de anestesia pelo pentobarbital sódico em camundongos infectados pelo *Schistosoma mansoni* e imunossuprimidos.

Para avaliar se a intensidade da reação granulomatosa hepática induzida por ovos de *S. mansoni* tem relação com o metabolismo de drogas, camundongos foram infectados com 40 cercárias e testados quanto ao tempo de anestesia pelo pentobarbital sódico. Para diminuir a resposta inflamatória usou-se irradiação com 400 Rad ou azatioprina na dose de 20mg/kg, 3 vezes por semana, por 4 semanas, respectivamente no

ou a partir do 33º dia pós-infecção. Nos animais infectados que receberam azatioprina a área dos granulomas hepáticos foi menor e o tempo de anestesia mostrou-se igual ao dos animais não infectados (controles). Nos animais infectados e irradiados as dimensões dos granulomas foram semelhantes às dos animais apenas infectados; nestes dois grupos de animais o tempo de anestesia foi maior que o dos animais controles. Esses resultados mostram que: 1) animais com reação granulomatosa inalterada apresentam diminuição da metabolização do pentobarbital sódico; 2) resposta granulomatosa hepática diminuída pela azatioprina não interfere na capacidade de metabolização da droga anestésica.

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