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CHEMOTHERAPY OF EXPERIMENTAL SCHISTOSOMIASIS

III. Comparative drug activity of some antimonial compounds in mice experimentally infected with *Schistosoma mansoni*

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SUMMARY

The comparative drug activity of some antimonial compounds in mice experimentally infected with *Schistosoma mansoni* was determined using sodium antimony III bis-pyrocatechol 3:5 disulphonate ("Fouadin"), lithium antimony III thiomalate ("Anthiomaline"), potassium antimony III tartrate (tartar emetic), sodium antimonyl III gluconate ("Triostib"), antimonyl III EDTA and antimony III dimercapto succinate (TWSb). A perfusion technique was used for recovering the worms and the CD50 (dose which clears from worms 50 per cent of the treated mice) and the RD90 (dose which kills 90 per cent of the worms in the treated mice) were graphically determined through the probit method. Two therapeutic indexes — LD50:CD50 and LD50:RD90 — were obtained. "Fouadin" presented the highest indexes but in general the therapeutic indexes of the antimonial compounds were low and no great differences were detected.

INTRODUCTION

In spite of the actual existence of many active antimonial compounds, most of them in the market, only a few data have been published concerning the determination of their therapeutic indexes in experimental schistosomiasis. Although there may be some criticism on the applicability to man of experimental results, there is some evidence that this quantitative estimation of the drug activity may be applied to human therapeutics. Other data, such as the correlation between drug activity, toxicity and antimony contents of the different compounds may also be determined this way.

A comparison of the efficacy of some active antimonial drugs has been carried out by BANG & HAIRSTON [1] in guinea-pigs experimentally infected with *Schistosoma japonicum;* a ratio based on the number of worms in the controls and treated animals was determined. A therapeutic index based in the ratio of the LD50 to the CD50 (dose which clears half of the mice from worms) was used by SCHUBERT, GOLDBERG & SCHREIBER [7] in the study of some antimonial compounds on mice infected with *S. mansoni.* STANDEN [9], also using *S. mansoni,* employed the hepatic shift accumulating 75 per cent or more of the female worms (HS 75) as a critical dose and reported the critical levels of tartar emetic and sodium antimony gluconate needed to give HS 75 in a single dose. BERBERIAN, DENNIS & FREELE [2], studying Miracil D and some related thiaxanthones

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in mice infected with *S. mansoni*, determined a therapeutic index derived as the ratio of the ED50 (dose calculated through the probit method to kill 50 per cent of the worms) to the calculated 5-day LD50. HILL [6], also with experimental mansoni infections, used two therapeutic ratios, the LD50:CD50 and LD50:CD95, CD50 and CD95 being the doses that cure respectively 50 and 95 per cent of the mice.

The comparative activity of some antimonial compounds on mice experimentally infected with *S. mansoni* is reported in this paper, along with a discussion about the methods and techniques used for the determination of therapeutic indexes in experimental schistosomiasis.

MATERIAL AND METHODS

Infection of animals: mice were infected through cutaneous route with 100 cercariae. The general procedure was similar to that described by STANDEN [10]: mice weighing 16-18 g were infected, individually, in glass specimen-pars containing the suspension of living cercariae. To avoid the deleterious action of urine and faeces on the cercariae, the animals, prior to the infection, are allowed to stay in lukewarm water for 15 minutes in order to eliminate their excretion. The mice are exposed to the cercariae for 30 minutes and are intensively illuminated by lamps during their immersion in the water. About 20 per cent of cercariae develop into adult worms and practically 100 per cent of the surviving mice get infected.

Drugs used:

a) sodium antimony III bis-pyrocatechol 3:5 disulphonate ("Fouadin" Bayer);

b) lithium antimony III thiomalate ("Anthiomaline" Rhodia);

c) potassium antimony III tartrate or tartar emetic (crystalline sample);

d) sodium antimonyl III gluconate ("Triostib" Burroughs Wellcome);

e) antimonyl III EDTA (crystalline sample);

f) antimony III dimercapto succinate, sodium salt (TWSb/6, Hoffman La Roche) and potassium salt ("Sulfantimon", Fontoura-Wyeth).

Toxicity: mice weighing 18-20 g were used for the tests. All drugs were administered through intraperitoneal route, in the same volume, and mortality observed for 3 days. The LD50 was determined graphically by the probit method.

Assessment of drug activity: the infected mice were divided into groups of about 30 animals and the drugs administered intraperitoneally in doses differing from each other by a constant factor. The drugs were injected once a day for 5 consecutive days in two series starting respectively on the 35th and 45th days of infection. The mice were sacrificed 7 days after the last dose and the living worms recovered through perfusion of the liver and mesenteric veins following a modified technique described by BUTTNER [3].

The CD50 (dose which clears from worms 50 per cent of the treated mice) and the RD90 (dose which kills 90 per cent of the worms in the treated mice) are graphically determined from the logarithms of the doses and the probits of the percentages of cure and worm reduction. Two therapeutic indexes, LD50:CD50 and LD50:RD90 are obtained.

RESULTS

The results obtained from the determination of toxicity and therapeutic activity are shown in Table 1. As the Sb contents of each compound are different, the same data were expressed, in Table 2, as milligrams of Sb III per kilogram. Fig. 1 shows the results obtained from the determination of the CD50 and RD90 with "Fouadin".

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TABLE I

Therapeutic indexes obtained with antimony compounds in mice experimentally infected with *S. mansoni*.

Drug	% Sb	LD_{50} (mg/Kgm)	CD_{50} (mg/Kgm)	RD_{90} (mg/Kgm)	$LD_{50}:CD_{50}$ (mg/Kgm)	$LD_{50}:RD_{90}$ (mg/Kgm)
Sodium antimony III bis pyrocatechol 3:5 disulphonate (Fouadin).	13.5	1.120	370	350	3.0	3.2
Sodium antimonyl III gluconate ("Triostib").	30.0	115	70	68	1.8	1.6
Antimonyl III EDTA.	27.0	85	44	41	1.9	2.0
Lithium antimony III thiomalate ("Anthiomaline").	16.0	110	72	72	1.5	1.5
Potassium antimony III tartrate (Tartar emetic).	39.0	48	20	24	2.4	2.0

TABLE 2

Therapeutic indexes obtained with antimony compounds in mice experimentally infected with *S. mansoni*. Results expressed as mg of Sb III per Kilogram.

Drug	LD_{50} (Sb mg/Kgm)	CD_{50} (Sb mg/Kgm)	RD_{90} (Sb mg/Kgm)
Sodium antimony III bis pyrocatechol 3:5 disulphonate (Fouadin).	151	49	47
Sodium antimonyl III gluconate ("Triostib").	34	21	20
Antimony III EDTA.	22	11	11
Lithium antimony III thiomalate ("Anthiomaline").	17	11	11
Potassium antimony III tartrate (Tartar emetic).	18	7	9

In the experiences performed with antimony dimercapto succinate (TW Sb), in the doses of 300, 600 and 900 mg/Kg, no cures were observed in spite of a marked shift of the worms to the liver and a reduction of about 50% of the worm population in the series treated with the higher dose.

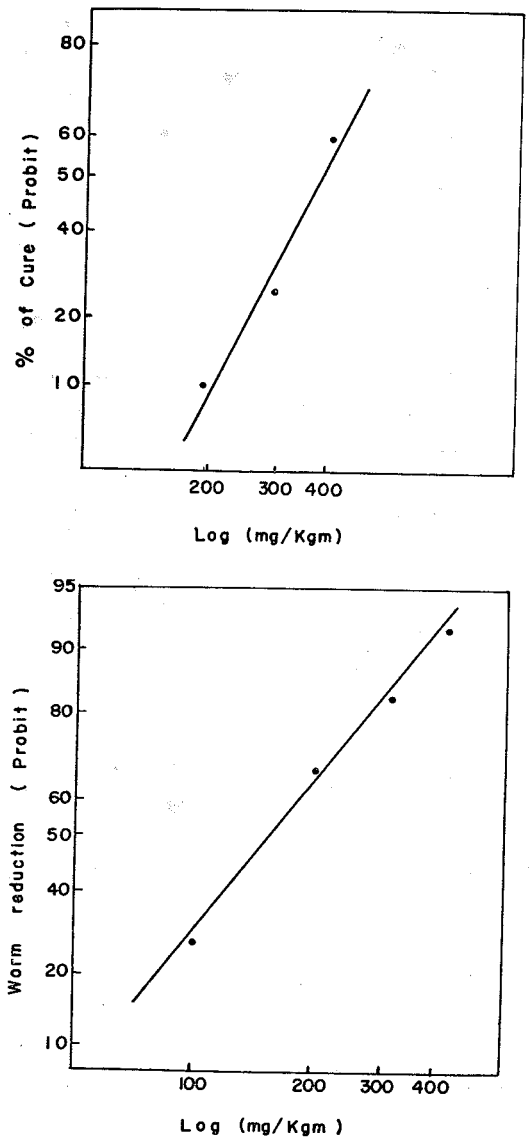

Fig. 1 — CD50 and RD90 in mice infected with S. mansoni *and treated with sodium antimony III bis-pyrocatechol 3:5 disulphonate ("Fouadin").*

DISCUSSION

For the assessment of drug activity in the case of the determination of comparative therapeutic indexes in experimental schistosomiasis, we need a reliable method for recovering living worms. Using the liver crush technique, a major trouble lies in the difficulty to distinguish the dead worms from living ones (STANDEN [9]). When, however, the sifht toward the liver is taken as a criterion for evaluating the drug comparative activity, it is not necessary to take into consideration whether the worms are dead or alive. This method, nevertheless, is used only to determine the initial effect of the drugs (STANDEN [7]) and, therefore, their healing action can merely be presumed from those data. The utilization of a perfusion technique permits the recovery of all living *S. mansoni;* worms already ensheathed by inflammatory tissue in the liver are not recovered. Previous experiences with treated animals showed that there was no significant difference between the number of worms obtained through perfusion on the 7th and 14th day after treatment.

The schedule of treatment was made in such a way as to avoid strong cumulative action and high mortality. With a two series schedule, a high percentage of cures and of worm reduction can be obtained.

Considering the great variation in the number of worms in mice infected with *S. mansoni,* even in untreated animals, indexes based on the percentage of cure seem to be more reliable and the known CD50 was selected. The CD95 used by HILL [6] was not adopted in our work because, at least with antimonial compounds, such and index could be often attained only by extrapolating the data. Following the suggestion of SCHUBERT & GOLDBERG [7], a second therapeutic index was determined based on the ratio of the LD50 to the dose which reduces 90 per cent the number of worms. In our experience the accidental variation in the number of worms in groups of infected mice has never been of such a great magnitude and a reduction of 90 per cent is quite reliable. According to the observations of SCHUBERT & GOLDBERG [7], the determination of the CD50 and RD90 through our method gives also similar results.

Tables 1 and 2 seem to show that the LD50 and the therapeutic values CD50 and RD90 have no correlation with the antimony contents, but that there is some correlation between the LD50 and the CD50 or RD90. It is interesting to remark that GOODWIN [5], studying the toxicity and trypanocidal activity of some organic antimonials, concluded that those two properties were "independent

of antimony content but have some correlation with one another and with the initial rate of excretion of the antimony". A study of the excretion rate of antimony compounds in experimental schistosomiasis would be highly desirable.

The results obtained with TWSb were quite surprising since no cures were achieved by us with such high doses as 900 mg/Kg, the therapeutic response being represented only by a reduction of the worm load. Considering the reported efficacy of this drug on man, such results can not be easily understood. We must emphasize, however, the lack of published data on the action of TWSb in mice experimentally infected with *S. mansoni*. FRIEDHEIM, SILVA & MARTINS [4] refer to data supplied by Luttermoser in guinea-pigs infected with *S. mansoni;* following Luttermoser, 15 per cent of the LD50 of TWSb produces the same reduction of the worms as 38 per cent of the LD50 of "Fouadin".

Due to the lack of extensive comparative studies concerning therapeutical activity of antimonial schistosomicidal agents and considering the diversity of techniques used, an accurate comparison of the therapeutic indexes in experimental schistosomiasis mansoni is rather difficult to be made. SCHUBERT & GOLDBERG [7] and SCHUBERT, GOLDBERG & SCHREIBER [8], using some tri and pentavalent antimonials, concluded that "Fouadin" was the best of the antimonials used with an index 1. Comparing the minimal dose required to shift 75 per cent of *S. mansoni* females to the liver, STANDEN [9] found out that tartar emetic was more active than sodium antimonyl III gluconate. These scarce data are generally in agreement with our figures. From our experience, "Fouadin" presents the highest indexes but, in general, the therapeutic indexes of the antimonial compounds are low and no great difference can be detected in relation to the several compounds tested.

RESUMO

Um estudo comparativo da atividade terapêutica de alguns compostos antimoniais foi realizado em camundongos experimentalmente infectados pelo *Schistosoma mansoni*, usando-se o antimônio-pirocatecol-disulfonato de sódio ("Fuadina"), antimônio tiomalato de lítio ("Antiomaline"), tartarato duplo de potássio e antimônio (tártaro emético), gluconato de sódio e de antimônio ("Triostib"), antimônio III EDTA e antimônio dimercapto succinato de sódio (TWSb). Os vermes eram recuperados através de perfusão do fígado e veias mesentéricas. O CD50 (dose que cura metade dos animais tratados) e o RD90 (dose que reduz em 90% a população de vermes) eram determinados gràficamente por intermédio dos logaritmos das doses e dos probitos das percentagens de curas e redução do número de vermes. Dois índices terapêuticos, LD50:CD50 e LD50:RD90, foram obtidos. A "Fuadina" apresentou os mais altos índices terapêuticos, mas de um modo geral os índices obtidos foram baixos.

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REFERENCES

1. BANG, F. B. & HAIRSTON, N. G. — Studies on schistosomiasis japonica. IV. Chemotherapy of experimental schistosomiasis japonica. Amer. J. Hyg. **44**:348-366, 1946.

2. BERBERIAN, D. A.; DENNIS, E. W. & FREELE, H. W. — Chemotherapy of experimental *Schistosoma mansoni* in Swiss mice. Comp. rend. 5e. Congr. internat. Méd. trop. & Paludisme **2**:292-305, 1953.

3. BUTTNER, A. — Quelques données pratiques et observations sur le cycle evolutif expérimental de *Schistosoma mansoni* (Trématode, Plathelminthe). Bull. Soc. Pathol. éxot. **49**:1197-1211, 1956.

4. FRIEDHEIM, E. A. H.; SILVA, R. J. da & MARTINS, A. V. — Treatment of schistosomiasis mansoni with antimony-a,a'-dimercapto-potassium succinate (TWSb). Amer. J. trop. Med. & Hyg. **3**:714-727, 1954.

5. GOODWIN, L. G. — The toxicity and trypanocidal activity of some organic antimonials. J. Pharmacol. & exper. Therap. **81**: 224-234, 1944.

6. HILL, J. — Chemotherapeutic studies with laboratory infections of *Schistosoma mansoni*. Ann. trop. Med. & Parasitol. **50**:39-48, 1956.

7. SCHUBERT, M. & GOLDBERG, E. — Comparison of therapeutic values of several antimonials in experimental schistosomiasis mansoni in mice. Fed. Proc. **7**:254, 1948.

8. SCHUBERT, M.; GOLDBERG, E. & SCHREIBER, F. G. — Comparison of several antimonials in the treatment of experimental schistosomiasis mansoni in mice. Amer. J. trop. Med. **29**:115-127, 1949.

9. STANDEN, O. D. — Experimental schistosomiasis. III. Chemotherapy and mode of drug action. Ann. trop. Med. & Parasitol. **47**:26-43, 1953.

10. STANDEN, O. D. — Experimental schistosomiasis. II. Maintenance of *Schistosoma mansoni* in the laboratory, with some notes on experimental infection with *S. hematobium*. Ann. trop. Med. & Parasitol. **43**:268, 1949.

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