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SERUM ANTIBODY CHANGES AFTER REPEATED CHEMOTHERAPIC SERIES IN «PARASITOLOGICALLY CURED» PATIENTS WITH SCHISTOSOMIASIS MANSONI

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SUMMARY

Patients with *S. mansoni* previously treated with schistosomicidal drugs (Niridazole, Hycanthone or Oxamniquine) and considered "parasitologically cured" (PC) were again submitted to Niridazole or Hycanthone therapy (second phase). Passive hemagglutination, indirect immunofluorescence and immunodiffusion tests performed on sera collected before and after treatment (second phase) showed significant increases of antibodies titres and/or precipitin bands in 6 out of 9 (66.7%) patients, previously "cured" by one first series of Niridazole. As to 14 patients previously "cured" by one IM injection of Hycanthone, antibody changes were detected in only 4 patients (28.6%) at the second phase. Such results strongly suggest that Hycanthone is more schistosomicidal than Niridazole.

INTRODUCTION

To our knowledge, no systematic study on serum antibodies has been performed on previously treated and parasitologically cured schistosomotic patients, who were submitted to new series of chemotherapic drugs in order to investigate whether living worms still persisted in their organisms.

According to the criteria adopted by a WHO Group [11], a schistosomotic patient is considered as parasitologically cured (PC) when stool examinations are negative for a period of at least 6 months after treatment in the absence of re-exposure.

In a previous report [9] we showed that patients with active schistosomiasis and submitted to Hycanthone treatment presented significant increases of antibody titers by hemagglutination and immunofluorescent tests and in number of precipitin bands as compared to schistosomotic patients who received placebo (control group).

This paper presents the results of a serological study in some patients considered as PC who were again treated by new series of either Hycanthone or Niridazole.

MATERIAL AND METHODS

1) *Blood samples* — Sera were obtained from all patients before and after the second and fourth weeks of treatment, and kept at −70°C.

2) *Serological tests* — Passive hemagglutination (HAT) indirect immunofluorescence

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TABLE I

Antibody (Ab) changes after new chemotherapic series in parasitologically cured patients

Patients (*)	Series of chemotherapy					
	Second	Ab (**)	Third	Ab	Fourth	Ab
1. SMS	Hycanthone	+				
2. OPR	Niridazole	−	Hycanthone	−		
3. APR	Niridazole	−	Hycanthone	−		
4. RA	Niridazole	+	Hycanthone	+	Hycanthone	−
5. EP	Niridazole	+	Hycanthone	−	Hycanthone	−
6. EMS	Niridazole	−	Hycanthone	+	Hycanthone	−
7. JAF	Niridazole	−	Hycanthone	+	Hycanthone	−
8. MMC	Hycanthone	−				
9. MLO	Niridazole	+	Hycanthone	+	Hycanthone	−
10. JCG	Hycanthone	−	Niridazole	−		
11. OVS	Niridazole	−				
12. JBS	Niridazole	−				
13. JSN	Hycanthone	+	Niridazole	+		
14. LSN	Hycanthone	−				
15. MGSN	Hycanthone	−				
16. CASN	Hycanthone	−	Niridazole	−		
17. HSN	Hycanthone	+	Niridazole	−		
18. MPR	Hycanthone	−				
19. MLDS	Niridazole	−				
20. DSG	Hycanthone	−				
21. NSP	Niridazole	+				
22. MJS	Niridazole	−				
23. CMES	Niridazole	+				
24. AHO	Niridazole	+				
25. VBC	Hycanthone	+				

(*) Patients 1 to 9 were parasitologically cured (first series) by Niridazole (Group I), nos. 10 to 23 by Hycanthone (Group II) and nos. 24 and 25 by Oxamniquine (Group III).
(**) Presence (+) or absence (−) of significant antibody changes.

(IFT) and immunodiffusion (IDT) tests were used as previously reported [5, 8].

3) *Patients and chemotherapy* — Twenty five patients were divided into 3 groups, as follows (Table I):

Group I – 9 Patients parasitologically cured (PC) by one first series of Niridazole (25 mg/kg of body weight for 7 days) were submitted to new series of Niridazole and/or Hycanthone.

Group II – 14 Patients PC by one intramuscular (IM) injection of Hycanthone (2.5 mg/kg) were submitted to another injection of Hycanthone and/or to a Niridazole series.

Group III – Out of 2 patients PC by one single IM injection of Oxamniquine (7.5 mg/kg) one was submitted to one IM injection of Hycanthone and the other patient to one series of Niridazole.

4) *Statistical analysis* — Log_{10} of reciprocal of titres were analysed by a discriminat-

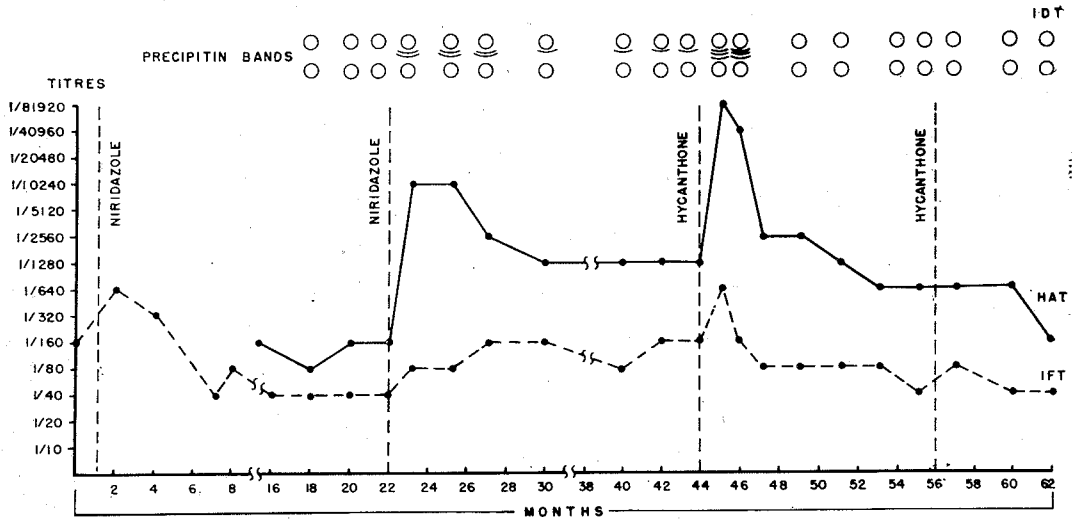
ing formula as previously reported [9]. For such analysis, only titres obtained from patients submitted to Hycanthone therapy were considered. For the other patients we considered as significant the titre elevations of two or more dilutions [5]. For all patients, an evident increase of one or more precipitin bands after therapy was also considered as indicative of worm death [8].

RESULTS

Significant antibody changes were observed in 6 out of 9 (66.7%) patients previously "cured" by one first series of Niridazole, and who were submitted to new chemotherapic series of Niridazol or Hycanthone (Table I).

As to 14 patients previously cured by one IM injection of Hycanthone, only 4 (28.6%) showed antibody changes after new series (Table I). Both patients PC by Oxamniquine showed similar changes after Niridazol and Hycanthone.

An illustrative case (patient no. 4) is shown in Fig. 1. No viable eggs were found either in repeated stool examinations or in rectal biopsies performed after each treatment. The patient was heavily infected before the first treatment.



PRECIPITINS (IDT), HEMAGGLUTINATION (HAT) AND IMMUNOFLUORESCENCE (IFT) TITRES AFTER FOUR CHEMOTHERAPIC SERIES IN PATIENT R. A. (No. 8), PARASITOLOGICALLY "CURED" AFTER THE FIRST SERIES.

COMMENTS

Our results strongly suggest that some patients considered parasitologically cured by the classical criteria [11] were still harboring living worms in their organisms. However, for its simplicity and objectivity, such criteria should be maintained. Furthermore, on the epidemiological point of view, the absence of viable eggs in the stools is very important as far as the interruption of the biological cycle of the parasites is concerned.

The immunological significance of the persistence of some living worms in the human organism is not fully established but it might be important to maintain some degree of immunity against reinfections (concomitant immunity [10]).

To our knowledge, the only practical way to demonstrate persisting live worms after

treatment is the use of serological tests with high sensitivity and specificity as the HAT, IFT and IDT [8]. Extracorporeal filtration of portal blood is a more direct method but it can be used only in surgical cases.

According to our results, Niridazole seems to be less schistosomicidal than Hycanthone. As a matter of fact, GENTILINI et al. [4] have also described an increase of precipitin bands after a new series of Niridazole in 3 "cured" patients with mansonian schistosomiasis. Such phenomenon was not observed in patients with *S. haematobium*. Those Authors concluded that the schistosomicidal activity of Niridazole is higher in the latter.

The evolution of antibodies in patient no. 4 (Fig. 1) and no. 9 (Table I) suggests that the *S. mansoni* worms were not completely destroyed even after the second series of Niridazole. Patient no. 9, however, did not take the drug regularly and the drug administration to patient no. 4 could not be checked because he was an out-patient. It is worth mentioning that 2 out of 3 patients (nos. 2, 3 and 8) "completely" cured after the first series of Niridazole were in-patients when treated. Thus, we might conjecture on the possibility of the irregular ingestion of Niridazole leading to an interruption of egg-laying without killing all the worms. So far, we have no evidence that such worms become resistant to the drug.

As far as Hycanthone is concerned, it seems to be more schistosomicidal, as fewer "serological rebounds" were observed. However, there is some evidence that *S. mansoni* worms which survived to Hycanthone may become resistant to that drug, not only in animals [1, 6] but also in humans [7]. Thus, a similar but extended study using different drugs in the second series should be performed. Finally, it should be mentioned that male worms are more susceptible to Hycanthone and Oxamniquine and female worms to Niridazole [2, 3].

RESUMO

Alterações dos anticorpos séricos após repetição da quimioterapia em pacientes esquistossomóticos "parasitologicamente curados"

Pacientes com *S. mansoni*, previamente tratados com drogas esquistossomicidas (Niridazol, Hycanthone ou Oxamniquine) e considerados "parasitologicamente curados" foram novamente submetidos a terapêutica com Niridazol ou Hycanthone (segunda fase).

Em soros coletados antes e após tratamento (segunda fase) executaram-se as técnicas de hemaglutinação passiva, imunofluorescência indireta e imunodifusão. Observou-se aumento significativo de títulos de anticorpos e/ou de arcos de precipitação em 6 de 9 pacientes (66,7%) previamente "curados" com uma primeira série de Niridazol.

Quanto aos 14 pacientes previamente curados com uma injeção intramuscular de Hycanthone, as alterações de anticorpos foram detectadas em 4 pacientes (28,6%) na segunda fase.

Tais resultados sugerem que o Hycanthone é mais esquistossomicida que o Niridazol.

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