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SERUM ANTIBODY CHANGES AFTER CHEMOTHERAPY OF PATIENTS WITH SCHISTOSOMIASIS MANSONI. A STATISTICAL ANALYSIS

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SUMMARY

A significant increase of immunofluorescence (FAT) and hemagglutination (PHT) titers was observed after a single intramuscular injection of Hycanthone (2.5 mg/kg of body weight) in patients with schistosomiasis mansoni. FAT, PHT and immunodiffusion test showed to be a valuable tool for detecting early serological changes after treatment.

INTRODUCTION

Despite the great number of reports on the diagnostic value of serological methods (CAMARGO et al.[2]; HOSHINO-SHIMIZU[5]; KAGAN & PELLEGRINO[6]; MORRELL[9]) the limited study of humoral changes after chemotherapy of schistosomiasis mansoni has led to a few conflicting results. Some Authors described early evident increases of serum antibodies after treatment (CAPRON et al.[3]; DODIN et al.[4]; RAMOS-MORALES et al.[13]; SILVA[16]) while others pointed to a constant decrease (RIFAAT et al.[15]; TANAKA et al.[20]).

The use of different serological methods and a complete lack of uniformity in the time schedule in collecting blood samples might explain some of the observed discrepancies. Furthermore, since some Authors did not determine antibody levels (RIFAAT et al.[15]) they might not be able to detect any serological changes after drug therapy (SILVA[16]).

Lack of uniformity and consequent conflict in reported results are among the reasons which led a Group (OMS[11]; OMS[10]) from W.H.O. to recommend a detailed immunological study of schistosomotic patients under chemotherapy.

In order to evaluate the degree and frequency of antibody responses commonly observed after chemotherapy by the passive hemagglutination and immunofluorescence techniques (SILVA et al.[18]) we undertook a prospective controlled study aimed at the quantification of these changes.

MATERIALS AND METHODS

Patients

Forty patients were selected and each individual case was randomly allocated to one of 2 groups: Group I — 20 patients received placebo (Table I); Group II — 20 patients were submitted to treatment with a single intramuscular injection of Hycanthone in a dose of 2.5 mg/kg of body weight ($H_{2.5}$) (Table II). All patients had the hepatointestinal form of schistosomiasis, but

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2 patients in Group I and 5 in Group II had a palpable spleen (hepatosplenic form) (MEIRA [8]).

Blood samples

Sera were obtained from all patients before and after the 2nd and 4th weeks of treatment. Our previous experience (SILVA et al. [18]; SILVA [16]) showed that those intervals were the most adequate to detect post-chemotherapy changes of antibody titers by hemagglutination, immunofluorescence and immunodiffusion reactions. All sera were kept frozen at −70°C.

Since all patients were living in a non endemic area, no probability of reinfection was supposed to occur during this period of treatment.

Serological methods

Indirect fluorescence antibody test (FAT) and passive hemagglutination test (PHT) were performed as previously described (CAMARGO et al. [2]; HOSHINO-SHIMIZU [5]). Sera were submitted to serial doubling dilutions, beginning respectively from 1:20 and 1:40.

Immunodiffusion test (IDT) was basically the Ouchterlony method (OUCHTERLONY [12]), using microscope slides (SILVA & FERRI [17]).

All reactions were performed with worm antigens as previously described (CAMARGO et al. [2]; HOSHINO-SHIMIZU [5]; SILVA & FERRI [17]).

Statistical analysis

We defined as a positive effect any quantitative significant response in antibody titers elicited by chemotherapy. Defined that way, a positive result was considered as evidence of worm death, releasing such an amount of antigen as to produce a significant increase in antibody titers.

The pre-treatment phase was considered as period A, the second week following the administration of therapy (Group II) or placebo (Group I) as period B and the fourth week as period C.

Variations in PHT and FAT titers (variables X and Y) were considered simultaneously. Reciprocals of titers were preliminarly transformed into $\log_{10}$. Individual differences between period A and B or A and C were calculated and a choice of an adequate linear combination of both transformed variables was made in such a way as to maximize the chance of true classification of each individual case in one of the two alternative groups: a) cases with a positive effect and b) cases without a positive effect.

In this analysis the method of discriminating function was used (RAO [14]).

Global comparisons among groups or periods were performed by uni-variate analysis of variance of the transformed variables.

RESULTS

Statistical discrimination for individual patients between periods A and B and between A and C (Tables I and II) showed that a positive effect (significant increase of antibody titers) was observed, respectively, in 1 out of 20 patients from Group I and in 15 out of 20 patients from Group II (period B) and in 2 out of 20 patients from Group I and in 15 out of 20 patients from Group II (period C).

For the second week (period B), the discriminating function was: $L = 0.1050 \times + 0.0777$ Y. Variance analysis of its components showed $F_{calc} = 16.40$ ($0.01 F^{c}_{2.37} = 5.21$), significant at $\alpha = 0.01$.

For the fourth week (period C) the discriminating function was: $L = 0.0590$ X $+ 0.1051$ Y. Variance analysis of its components showed $F_{calc} = 12.83$ ($0.01 F^{c}_{2.37} = 5.21$), significant at $\alpha = 0.01$.

Thus, according to the discriminating functions, a significant increase of titers ($p < 0.01$) was observed in the second and fourth weeks after treatment with Hycanthone as compared to changes seen after placebo (Tables I and II).

No significant differences were found among titers obtained in periods B and C.

As to IDT, an increase of precipitin lines was observed in 16 out of 20 patients (80%).

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TABLE I

Serum antibody levels before and after placebo as determined by passive hemagglutination (PHT), indirect fluorescence (FAT) and immunodiffusion (IDT) tests

Patients	Before (period A)			After placebo (periods B and C)					
				2nd week (B)			4th week (C)		
	PHT	FAT	IDT	PHT	FAT	IDT	PHT	FAT	IDT
1. ASS	640 (*)	80	1 (**)	640	160	1	640	80	1
2. ARL	640	80	0	640	80	0	320	80	0
3. ARO	320	80	0	320	80	0	160	80	0
4. ATS	40	10	0	40	10	0	160	20	0
5. CRX	2560	160	1	2560	160	1	2560	160	0
6. DDS	640	80	0	640	80	0	320	160	0
7. DMJ	2560	80	0	2560	80	0	5120	160	0
8. FAM	640	80	0	640	80	0	640	80	0
9. JRS	160	80	0	80	80	0	160	80	0
10. JEL	160	80	0	320	80	0	640	160	0
11. JO	320	80	0	320	160	0	320	80	0
12. LEN	40	40	0	160	80	0	320	160	0
13. MO	320	80	0	640	80	0	320	80	0
14. MFX	320	80	1	320	80	1	320	80	1
15. MS	80	40	0	80	40	0	160	40	0
16. MBV	80	80	1	160	80	1	160	80	1
17. NFN	160	40	0	80	40	1	80	40	1
18. PFN	2560	80	0	2560	80	0	5120	80	0
19. RCSR	320	80	0	320	80	0	320	80	0
20. VMD	320	40	0	160	20	0	640	80	0

(*) Reciprocal of titers

(**) Number of precipitin bands

TABLE II

Serum antibody levels before and after Hycanthone, as determined by passive hemagglutination (PHT), indirect fluorescence (FAT) and immunodiffusion (IDT) tests

Patients	Before (period C)			After Hycanthone (periods B and C)					
				2nd week (B)			4th week (C)		
	PHT	FAT	IDT	PHT	FAT	IDT	PHT	FAT	IDT
21. AMD	320 (*)	80	0 (**)	640	160	2	640	160	2
22. AFN	80	160	0	1280	160	1	5120	160	2
23. ABC	10240	160	2	81920	320	4	40960	640	3
24. ASN	160	80	0	2560	160	3	2560	320	3
25. ARS	80	20	0	320	80	1	320	160	1
26. ESN	80	80	0	640	160	1	1280	160	1
27. JJS	160	80	0	2560	320	2	2560	320	3
28. JGB	320	160	1	1280	320	2	1280	320	2
29. LVS	1280	80	0	2560	80	2	2560	80	2
30. MCCX	1280	40	0	5120	320	2	5120	320	2
31. MN	2560	80	0	10240	320	2	40960	320	2
32. MRS	160	40	0	320	160	0	640	160	0
33. MRS	640	160	1	2560	320	3	2560	320	3
34. MSP	160	40	0	320	80	1	320	80	1
35. MCC	320	160	0	2560	320	1	1280	320	1
36. NSF	160	40	0	80	40	0	160	80	0
37. OSS	2560	80	0	2560	80	0	1280	80	0
38. PHG	160	40	0	640	80	1	1280	80	1
39. VAS	40	20	0	1280	320	1	1280	320	0
40. ZVJ	320	40	0	640	80	0	1280	80	0

(*) Reciprocal of titers

(**) Number of precipitin bands

COMMENTS

Our results show that Hycanthone induces a significant increase of immunofluorescence and hemagglutination titers ($p < 0.01$) and of precipitin lines in patients with mansoni schistosomiasis.

AMBROISE-THOMAS [1] compared FAT titers obtained with sera from patients with schistosomiasis mansoni before and after treatment with Niridazole. The highest values for antibody titers were observed between the first and the third month after chemotherapy. During this period, however, only 5 to 12 patients were studied. Similar results were described by LE VIGUELLOUX et al. [7].

RIFAAT et al. [15] using cercariae as antigen neither mentioned such changes nor titrate the sera by FAT.

In spite of its great sensitivity, PHT was rarely used to detect antibody changes after chemotherapy. RAMOS-MORALES et al. [13] referred an increase of titers in 6 out of 9 patients treated by Niridazole but the magnitude of such increase was not mentioned.

The increase in FAT and PHT titers were previously observed by us (SILVA et al. [18]), particularly between the second and the fourth week after the use of Hycanthone or Niridazole. No report, however, mentioned a statistical analysis of serological changes observed after chemotherapy in comparison to those found after placebo.

Our results strongly suggest that changes of antibody levels are specifically due to drug treatment, as a consequence of worm death. It is worth mentioning that the administration of new chemotherapic series to previously treated patients with no viable eggs in the stools but still with positive serologic tests may or may not produce an increase of antibodies (SILVA [16]). Furthermore, no serologic changes were seen in 3 non-schistosomotic patients with hepatosplenomegaly who received Hycanthone (SILVA [16]).

As shown in Table I and II, IDT is also a valuable test to detect post-treatment antibody increase. Precipitin lines appear early in the follow-up, usually on the second week. According to our experience (SILVA et al. [18]; SILVA [16]), these changes are only preceded by an increase of eosinophil counts.

Summing up, PHT, FAT and IDT are a valuable tool for detecting early serological changes after treatment of mansonian schistosomiasis.

RESUMO

Alterações dos anticorpos séricos após quimioterapia de pacientes com esquistossomose mansônica. Análise estatística

Observou-se aumento significativo dos títulos de imunofluorescência e de hemaglutinação após injeção intramuscular única de Hycanthone (2,5 mg/kg de peso corporal), em pacientes com esquistossomose mansônica quando se comparou ao grupo não-tratado. As reações de imunodifusão, hemaglutinação e imunofluorescência mostraram-se de grande valor para detectar alterações precoces dos anticorpos após o tratamento.

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