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AMERICAN CUTANEOUS LEISHMANIASIS: DISAPPEARANCE OF AMASTIGOTES FROM LESIONS DURING ANTIMONIAL THERAPY

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SUMMARY

Observations were made on 10 patients under treatment with meglumine antimoniate (Glucantime) to determine the rate of disappearance of amastigotes from American cutaneous leismanial lesions during antimonial therapy. Before treatment, all patients had positive reactions to Montenegro antigen and amastigotes were detected in impression smears prepared from all lesions; promastigotes grew in NNN (PESSOA & MARTINS, 1982) cultures seeded with material aspirated from seven lesions; amastigotes were found in histological sections of biopsies taken from eight patients. The histopathological picture before treatment is briefly described. During the first 10 days of treatment with Glucantime, NNN cultures were consistently negative; amastigotes were detected in impression smears up to the fourth dose and in histological sections until the sixth dose of Glucantime; and some changes in the histopathological condition were noted in biopsies taken from two patients under treatment. The failure to detect parasites in lesions by routine diagnostic methods and in histological sections is an inadequate criterion for terminating therapy.

INTRODUCTION

Since 1965, we have treated about 2,000 cases of American cutaneous leishmaniasis with meglumine antimoniate (Glucantime). Some patients have been followed-up for as long as 15 years. The disease has not recurred in patients who completed the course of treatment, nor have any become reinfected.

Complete healing of lesions has been the criterion to end treatment. In view of the toxicity of antimonials, we have explored other methods to determine the best time to terminate administration of Glucantime and we have already reported (CHIARI et al., 1973) that the indirect fluorescent antibody test, using promastigotes as antigen, is a useful but not consistently reliable means of monitoring the progress of treatment. We now record observations, based on standard laboratory diagnostic methods, supported by histopathological studies on biopsies taken from patients before and during treatment, on the disappearance of amastigotes from lesions. Our results conflict with observations made by other investigators treating in other parts of the Americas with other therapeutic methods.

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MATERIAL AND METHODS

The 10 patients on whom observations were made were resident in the Rio Doce Valley, Minas Gerais, Brazil, and their ages ranged from one to 46 years.

After clinical inspection of a suspected leishmanial lesion, each patient was tested with Montenegro antigen (MELO et al., 1977), NNN (PESSOA & MARTINS, 1982) cultures were inoculated with material aspirated from a lesion, and incubated at 23°C and a biopsy was taken to prepare impression smears and histological sections.

Patients were given intramuscular injections of Glucantime at a rate of 60 mg/kg body weight up to a maximum daily dose of 3 g. The drug was administered on 10 consecutive days, followed by a 10-day pause before resuming treatment. This 10/10 day regime was maintained until patients were considered cured. The observations recorded here were made during the first 10-day period of treatment.

During treatment, NNN cultures were prepared from lesions of three patients who had received two doses of meglumine antimoniate (Glucantime), from three after the third dose, from one after the fourth, and from the remaining three after the fifth dose.

Biopsies, taken from lesions before treatment and on every day of treatment were divided to prepare impression smears and histological sections. Impression smears were fixed in methanol and stained with Giemsa. The other biopsied fragment was fixed in Bouin, embedded in paraffin wax, and 5μm thick sections were stained with haematoxylin and eosin.

Impression smears and sections were examined in their entirety before being considered negative.

RESULTS

Montenegro tests — All patients had positive reactions before treatment.

NNN cultures — Before treatment, promastigotes grew in seven cultures inoculated with material aspirated from lesions. All tissue aspirates during treatment were negative by culture.

Impression smears — Amastigotes were found in all smears prepared before treatment (see Table I). During treatment amastigotes were recorded up to the first (patients ACE, MFV), second (AMC, JFV, RRF), third (CAR) and fourth (MLR, GAF, AFS, AMG) dose of Glucantime.

TABLE I

Evidence of leishmanial infection before and during treatment with 30% n-methyl glucamine

Patient	Before treatment		During treatment *	
	Impression smear	Histological section	Impression smear	Histological section
ACE	+	+	1st	1st
MFV	+	—	1st	—
AMC	+	+	2nd	4th
JFV	+	+	2nd	2nd
RRF	+	+	2nd	3rd
CAR	+	—	3rd	—
MLR	+	+	4th	1st
GAF	+	+	4th	3rd
AFS	+	+	4th	5th
AMG	+	+	4th	6th

* Last dose giving parasitological evidence of the presence of amastigotes in a lesion.

Histological sections — Amastigotes were found in sections of eight biopsies taken before treatment (see Table). Sectioned material from two patients (MFV, CAR) was negative before and during treatment, though parasites were found in some impression smears prepared during treatment. Parasites were found in sections up to the first (ACE, MLR), second

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(JFV), third (RRF, GAF), fourth (AMC), fifth (AFS) and sixth (AMG) doses of Glucantime.

In general, sections prepared before treatment showed similar histological pictures. There was intense infiltration of diffuse inflammation of the papillary layer and, more markedly, in the reticular layer. The infiltrate was mainly composed of histiocytes and lymphocytes. In most cases, infiltration was diffuse but some lesions showed signs of the formation of tuberculoid granulomas with one or more Langhans'giant cells surrounded by histiocytes, lymphocytes and plasma cells. All lesions were necrotic and ulcerated due to secondary bacterial infections. The epithelium was characterized by acute acanthosis and parakeratosis which, in some lesions, presented an appearance of pseudoepithelioma.

No noteworthy differences were recorded in sections of biopsies taken during treatment except in two cases (JFV, RRF). The lesions from these two had less intense inflammatory reactions, more extensive fibrosis and a somewhat marked pseudocarcinomatous hyperplasia with the formation of horny pearls in the papillary and reticular layers.

DISCUSSION

Cutaneous and muco-cutaneous leishmaniasis occur in the Rio Doce Valley and there is evidence that several distinct forms of **Leishmania** exist in the area (MAYRINK et al., 1979). In view of this situation, we have previously proposed (MAYRINK, et al.) to adtopt the term **Leishmania** sp. until strains are characterized by biochemical methods. Of the strains studied to date, one belongs to the **L. mexicana** complex but does not correspond to any formally described subspecies, another is closely similar to **L. mexicana mexicana**, and a third belongs to the **L. braziliensis** complex but differs from described subspecies (LOPES, 1982).

The 10 patients were selected at random and there is no certainty that they were infected with the same parasites. The stocks isolated in NNN cultures before treatment have not yet been characterized biochemically.

We were not surprized by the failure, during treatment, to detect parasites by means of NNN cultures. In treating several hundreds of patients, we have noted that cultures are rarely positive after the first dose of Glucantime. Our experience with patients in the Rio Doce Valley contrasts with observations made elsewhere. KINNAMON et al. (1979) treated patients with two or three courses of Pentostam A and isolated parasites, by NNN culture, from clinically healed lesions. Similarly, MARSDEN et al. (1979) recovered living parasites from cured lesions following therapy with Nifurtimox.

Our failure to detect parasites by NNN culture from the very beginning of treatment might be related to the dosage level (60 mg/kg body weight) of meglumine antimoniate (Glucantime) normally administered. When, for various reasons it has been necessary to treat patients at a lower dosage rate, one of us (P.A.M.) has recorded recrudescence of apparently healed lesions. On the other hand, having followed-up many cases for several years without a single recrudescence, we are reasonably sure that no viable parasites remain in healed lesions of patients who complete the full course of treatment at the routine dosage rate.

MARSDEN et al. (1979) considered parasitological and histological evidence to be greatly important in assessing the progress of treatment for American cutaneous leishmaniasis but made no histological studies in their studies on the efficacy of Nifurtimox. In our experience, amastigotes could be detected in impression smears and/or sections only for 4-6 days during the first 10 days of treatment with Glucantime. The two methods for detecting amastigotes gave identical results in only two instances (ACE, JFV). In four cases, no parasites were found in sections when the corresponding smears were positive; in the other four cases, smears were negative when amastigotes were found in the corresponding sections. Because smears and sections were prepared from the same biopsed fragment, we think that the results indicate an uneven distribution of parasites in cutaneous leishmanial lesions, especially during the early phases of treatment. We are now making further observations on the distribution and viability, during treatment, of parasites in the deeper margins of ulcers, a well-known area of amastigote concentration.

The histological findings agree with published observations (BOGLIOLO, 1981; ROBBINS

& COTRAN, 1979). The fundamental changes were in inflammatory infiltration of variable intensity but were almost always well-marked, though diffuse and nonspecific, with a predominance of histiocytes, lymphocytes and plasma cells. Occasionally, the inflammation gave the appearance of a concurrent tuberculoid granuloma. Marked hyperplasia of the epidermis (acanthosis, hyperkeratosis, parakeratosis) was frequent. The extent and depth of ulceration varied and the histological picture was confused by the frequency of secondary bacterial infections. Because of the intensity of inflammation, it was often difficult to recognize amastigotes in sections prepared by routine histopathological methods (fixation in Bouin followed by staining with haematoxylin and eosin). Due to pyknosis, karyolysis, karryorrhexis and phagocytosis of nuclear remnants by macrophages, it was often difficult to identify amastigotes with certainty.

Despite the suggestion by MARSDEN et al. (1979) that histological evidence can be useful in monitoring the progress of treatment of American cutaneous leishmaniasis, our experience in dealing with patients from the Rio Doce Valley suggests that such evidence is of little practical value for routine purposes. Histological methods are time-consuming and, because of the complexities of the pathological processes involved in cutaneous leishmaniasis, complicated further by the high frequency of secondary infections, the services of an expert pathologist are required to interpret the results realistically.

Observations on histopathological modifications rather than the detection of parasites could well be of utility in following the progress of treatment. Our observations were limited to the first 10 days of treatment with Glucantime and, in this short period, we did not expect to find changes in the histopathological picture. In two cases, however, we obtained evidence of a diminution of inflammatory processes. We suggest, therefore, that a reversal of histopathological processes could be a useful means of monitoring therapy for American cutaneous leishmaniasis.

Our results suggests that a considerable reduction in the numbers of amastigotes occurs in the initial stages of treatment of lesions with Glucantime. Moreover, the failure to detect parasites by NNN culture when amastigotes could be found in impression smears and histological sections suggests impairment of the reproductive capacity of organisms surviving early contact with Glucantime. Parasitological evidence based on the detection of amastigotes in smears and/or sections is of dubious value as a method for monitoring treatment. The parasites detected by these methods might be morphologically normal but of diminished viability.

Although our evidence suggests the complete elimination of reproductive amastigotes within less than 10 days of treatment with Glucantime, we are not fully convinced that this is so. We have frequently noted recrudescence of lesions in patients who interrupted treatment during the first 10 days. We suspect that viable parasites persist in a lesion but cannot be detected by routine laboratory methods.

We conclude that clinical cure of a lesion is the best criterion for assessing treatment with Glucantime — at least in dealing with the forms of **Leishmania** that exist in the Rio Doce Valley of Minas Gerais. We join with MARSDEN et al. (1979) in emphasizing the importance of long term follow-up of patients.

RESUMO

Leishmaniose tegumentar americana: Desaparecimento de amastigotas das lesões durante terapêutica antimonial

Durante o tratamento com antimonial de N-metil glucamina (Glucantime) dez pacientes com forma cutânea de leishmaniose tegumentar americana foram observados, para se determinar o tempo de desaparecimento de amastigotas das lesões.

Antes do tratamento todos os pacientes apresentavam teste de Montenegro positivo e amastigotas em esfregaços realizados por apo sição de material biopsiado das lesões.

O isolamento de parasitos da lesão em meio de NNN foi positivo em sete pacientes e em oito os cortes histológicos mostraram presença de amastigotas.

Durante as 10 primeiras doses de glucantime todos os pacientes tiveram culturas negativas. Amastigotas foram detectadas em esfre-

gaços por aposição até a quarta dose e nos cortes histológicos até a sexta dose de Glucantime. Os aspectos histopatológicos antes do tratamento são brevemente descritos. Em apenas 2 pacientes observou-se durante o tratamento modificações histopatológicas dignas de nota: com o aparecimento de fibrose extensiva e hiperplasia pseudo carcinomatosa estes casos como os demais evoluiram para a cicatrização e cura das lesões.

Em vista dos resultados obtidos, os Autores concluem que a cura clínica das lesões, pelo menos nas formas de leishmanioses cutâneas no Vale do Rio Doce, é ainda o melhor critério para interromper o tratamento com antimoniato de N-metil glucamina, no esquema de tratamento utilizado no presente trabalho.

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