

Immunotherapy, immunochemotherapy and chemotherapy for American cutaneous leishmaniasis treatment

Imunoterapia, imunoquimioterapia e quimioterapia no tratamento da leishmaniose tegumentar americana

Wilson Mayrink¹, Ana Cristina de Carvalho Botelho^{1,3}, Paulo Araújo Magalhães^{6†}, Sebastião Mariano Batista^{1†}, Antonio de Oliveira Lima^{7†}, Odair Genaro^{1†}, Carlos Alberto da Costa⁴, Maria Norma de Melo¹, Marilene Susan Marques Michalick¹, Paul Williams¹, Magno Dias², Waleska Teixeira Caiaffa⁸, Evaldo do Nascimento¹ and George Luiz Lins Machado-Coelho²

ABSTRACT

The first choice of treatment for American cutaneous leishmaniasis is the pentavalent antimonial drug. Although it has been shown that this treatment is mostly effective and indicated, some disadvantages should be taken into account such as side effects, long term treatment inconveniences and counter-indication for patients suffering from cardiopathy, nephropathy; yet, aging, pregnancy and other conditions. With the advent of the vaccine anti-American cutaneous leishmaniasis as a prophylactic measure, studies on therapy using the vaccine associated or not with other drugs have been performed by many investigators and it is currently among the alternative treatments and prevention measures for American cutaneous leishmaniasis. In conclusion, the association between antimony and vaccine (immunochemotherapy) showed the same cure rate when compared with the standard treatment (100%) and it was also able to reduce the salt volume in 17.9% and treatment length from 87 to 62 days, decreasing side effects.

Key-words: American cutaneous leishmaniasis. Treatment. Immunotherapy.

RESUMO

O tratamento de primeira escolha para leishmaniose tegumentar americana é o antimonial pentavalente. Embora este tratamento seja na maioria das vezes efetivo e indicado, devem ser consideradas as desvantagens tais como efeitos colaterais, longa duração do tratamento e contra-indicação para cardiopatas, nefropatas, idosos, grávidas e outras condições. Com o advento da vacina antileishmaniose tegumentar americana para fins profiláticos e terapêuticos, associando-a ou não a outros fármacos, muitas pesquisas têm sido desenvolvidas, sendo a vacina a principal entre os atuais recursos no tratamento e prevenção da leishmaniose tegumentar americana. Em conclusão, a associação do antimônio com a vacina (imunoquimioterapia) apresentou o mesmo índice de cura em relação ao tratamento padrão (100%), e ainda reduziu o volume do sal em 17,9% e o tempo de cura significativamente, de 87 para 62 dias; conseqüentemente, reduzindo os efeitos colaterais.

Palavras-chaves: Leishmaniose tegumentar americana. Tratamento. Imunoterapia.

1. Departamento de Parasitologia do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, Belo Horizonte, MG. 2. Departamento Farmácia da Escola de Farmácia da Universidade Federal de Ouro Preto, Ouro Preto, MG. 3. Centro Pesquisas René Rachou da Fundação Oswaldo Cruz, Belo Horizonte, MG. 4. Departamento de Parasitologia da Faculdade de Farmácia da Universidade Federal de Minas Gerais, Belo Horizonte, MG. 5. Departamento de Ciências Biológicas da Universidade Federal de Ouro Preto, Ouro Preto, MG. 6. Superintendência de Campanha Sanitária do Ministério da Saúde. 7. Fundação Ataulfo Paiva, Belo Horizonte, MG. 8. Departamento de Medicina Preventiva e Social da Faculdade de Medicina da Universidade Federal de Minas Gerais, Belo Horizonte, MG.

† *In memoriam.*

Supported by FAPEMIG, SMSC, CNPq, UFMG, CISMRC

Address to: Dr. Wilson Mayrink. Rua Equador 140/201, Bairro São Pedro, 30330-390 Belo Horizonte, MG, Brasil

Tel: 55 31 3287-3038

e-mail: wilsonmayrink@uol.com.br

Recebido para publicação em 2/7/2004

Aceito em 23/11/2005

Leishmaniasis is a disease caused by several digenetic protozoan species of the genus *Leishmania*, which affect humans and different domestic and sylvatic animal species. The disease may arise in different forms and the most important are: American visceral leishmaniasis (AVL) or American kala-azar and American cutaneous leishmaniasis (ACL). The latter, is primarily a disease that affects sylvatic animals. Leishmaniasis is transmitted to humans through the female bite of female haematophagous insect vectors of the genus *Lutzomyia*, which are known, in Brazil, as 'birigüi', 'mosquito-palha' or 'cangalhinha'.

ACL is a polymorphic disease of skin and mucous membranes with single or multiple ulcerative lesions (simple cutaneous form), nodular lesions (diffuse form) or mucocutaneous lesions (mucocutaneous form), affecting nasopharynx regions simultaneously with or just after an initial cutaneous infection. Such variation is closely related to the patient's immunological status and the *Leishmania* species involved. ACL constitutes an important health problem mainly because of its difficult prevention. The World Health Organization (WHO) estimates around 1-2 million annual new cases annually⁷⁸.

Up to 1912, leishmaniasis treatment had been unsatisfactory. Row⁶² reported results on the curative properties of promastigote forms, from *Leishmania* culture, used as a vaccine in the treatment for Oriental sore; this therapy was then abandoned due to a new systemic treatment with antimonial drugs introduced that year by the Brazilian physician Gaspar Vianna⁷⁶. This investigator used emetic tartar at 1%, which proved to be more efficient than the vaccine.

Emetic tartar has been the only therapeutic alternative for leishmaniasis for many years and because of its toxicity, administration difficulties and unsatisfactory efficacy (frequent recurrences), it was, at last, replaced with pentavalent antimonial drugs^{21,25}. Despite its counter-indications for elderly patients and other conditions, such as cardiopathy, nephropathy and pregnancy, the pentavalent antimonial drug represents the standard therapy for leishmaniasis nowadays.

Since the introduction of antimonial drugs for leishmaniasis therapy, which have also been shown to produce divergent results^{2 3 8 17 18 20 24 26 29 33 44 49 51 52 60 63 65 66 70 74}, several studies have been carried out in order to establish the best therapeutic plan.

With the advent of the ACL vaccine for prophylactic aims, idealized by Gomes²³, Pessoa and Pestana⁵⁵, modified by Mayrink *et al*^{35,36}, and proved by Antunes *et al*⁴ and Armijos *et al*⁵, its therapeutic use has been under study, whether alone or associated with other drugs; and it has also been regarded as one of the current treatments, as well as one of the available prophylactic measures for ACL^{14 22 28 35 39 43}.

Reports on the use of chemotherapy associated with immunotherapy have shown considerable efficacy in some cases of diffuse leishmaniasis^{37 71 72}.

Immunotherapy combined with the use of BCG, first performed by Convit¹⁴, showed cure rates and treatment length comparable with those obtained through standard treatments with Glucantime®.

The present work was aimed at: 1) using immunotherapy in ACL patients for whom antimonial drugs are restricted and; 2) using immunotherapy combined with chemotherapy (immunochemotherapy), since we observed that an immunological stimulus associated with a chemotherapy treatment leads to a reduction in antimony volume and treatment period. This complex provides substantial benefits to the patient, reducing their visits to the outpatient facility, thus, diminishing ambulatory expenses, among many other advantages, when compared to the standard therapy. This process is described in detail in the present work.

The current work is aimed at evaluating the clinical effects among different therapeutic plans and not at assessing the immunological aspects involved in such responses, which has been widely studied by other investigators^{7 10 12 15 16 19 42 47 58 59 75}.

PATIENTS AND METHODS

Population studied. A sample of 542 patients diagnosed with ACL, including males and females aged over five years old (Table 1), was randomly selected in a open assay from a group of subjects attending the Outpatient Facility Paulo Araújo de Magalhães in Caratinga, Minas Gerais. The present investigation and the protocols comply with the Research Ethics Committee of Instituto de Ciências Biológicas of Universidade Federal de Minas Gerais-ICB/UFMG.

Diagnosis. Individuals with typical cutaneous lesions were clinically diagnosed as being carriers of ACL. Clinical diagnosis was confirmed through positive parasitological examination and/or Montenegro skin test (MT).

Parasitological examination. Parasitological examination was confirmed by the presence of amastigote forms of the parasite in skin biopsies from the lesions. The examination was carried out by compressing the tissue from biopsy between two histological slides, after removing blood excess blood with filter paper. The material collected was fixed in methanol, Giemsa stained and, then, examined under light optical microscopy (magnification 1,000X).

Montenegro test. Montenegro antigen was produced under sterile conditions from cloned promastigote cultures of I-III-D *Leishmania (Leishmania) amazonensis*, which were killed, sonicated and kept in merthiolate solution at 1:10,000⁴⁰. A volume of 0.1mL of the antigen with 40µg/mL of nitrogen^{38 41} was injected into intradermal layer in the inner side of the arm. Positivity was indicated after nodule formation, 48 hours post test, without considering erythematous areas.

Table 1 - Demographic and clinical characteristics of the treated American cutaneous leishmaniasis patients.

Groups	Therapeutic scheme	Number of cases	Male gender (%)	Age average (years)	Malnutrition (%)	Disease period (month)	Number of lesions	Lesion (cm)	MT (cm)	MT+ (%)	Positive parasitological exam (%)
2	QT 0.5	29	58.6	23.4	13.8	2.1	1.1	2.6	7.8*	82.8	92.9
1	QT 1.0	39	61.4	30.6	4.7	2.4	1.3	4.9	5.2	81.8	84.1
3	IT serial	53	64.2	33.2	9.6	5.9	1.2	3.1	2.8	73.1	88.7
1	QT 1.0	35	51.4	30.8	2.9	4.2	1.3	3.2	3.9	85.3	87.9
4	QT 1.0	38	50.0	21.5*	NR	2.4	1.4	3.6	3.7	89.2	89.5
1	QT 1.0	49	59.2	31.0	NR	2.7	1.3	4.6	5.3	83.7	93.6
5	IQT 0.5	47	44.7	27.3	14.9	2.3	1.3	3.6	7.1	85.1	85.1
1	IQT 1.0	44	61.4	30.6	4.7	2.4	1.3	5.0	5.2	81.8	84.1
6	colliquation	47	68.1	27.7	14.9	3.0	1.4	3.4	4.8	72.3	87.0
7	IT and BCG monthly	58	70.7	26.0	7.0	3.3	1.4	3.7	5.6	81.0	89.7
8	BCG monthly	13	53.8	26.8	15.4	3.6	1.2	7.5	7.2	84.6	76.9
9	IT monthly	12	33.3	28.1	16.7	2.9	1.1	5.9	7.8	100.0	58.3*
1	QT 1.0	78	61.5	30.8	14.1	2.7	1.3	4.7	5.2	84.6	94.8
Total		542	59.3	28.6	10.8	5.3	1.3	4.1	5.2	82.4	89.0

QT 1.0: Standard Group , * Significant difference when compared with standard group. MT: Montenegro Test.

Vaccine. The vaccine was prepared by the Laboratory of Leishmaniasis of the Department of Parasitology, ICB – UFMG, using dead promastigote forms of *Leishmania (Leishmania) amazonensis*, strain IFLA/BR/67/PH8, in accordance with the technique proposed by Mayrink et al³⁶ under quality control patterns of the Pharmacy School of UFMG.

BCG. BCG, used as coadjuvant (BCG, Calmete Bacillus - Guerin da Fundação Ataufo Paiva, Rio de Janeiro, RJ), was reconstituted by dilution in 1mL solution (100µg/mL).

Antimonial N-methylglucamine. The pentavalent antimonial (Sb^v) used here was N-methylglucamine (Glucantime®; Rhodia, São Paulo, Brazil), comprising 85mg pentavalent antimonial per mL.

Therapy. Group 1 (Standard treatment) Chemotherapy I (QT 1.0). This group consisted of 245 patients treated with Glucantime®. It was slowly administered through intramuscular route, at a daily dose of 1mL/5kg body weight, without exceeding 10mL/day, for 10 days. After a 10-day interval, this treatment was repeated. This period of 10 days of treatment plus 10 days of interval was named *one series of treatment* and it is carried out, and repeated until complete clinical cure of the patients is achieved.

Group 2. Chemotherapy II (QT 0,5). This group comprised 29 patients under treatment with Glucantime®, at a daily dose of 0.5mL/5kg body weight. The administration was performed as described above and the maximum daily dose did not exceed 5mL/day.

Group 3. Serial immunotherapy (IT serial): in this group, 53 patients, underwent subcutaneous administration of the vaccine (nitrogen total concentration: 360µg/mL) administered to the top outer forearm, according to the method proposed by Mayrink³⁵ as follows: first day- 100mL; second day- 200µL; third day- 300µL; fourth day- 400µL and from the fifth to the tenth day- 500µL.

After a 10-day interval, daily doses of 500mL were administered.

Group 4. Immunochemotherapy I (IQT 1,0). In this group, 38 patients were administered anti-ACL vaccine through subcutaneous route, as described for Group 3, simultaneously with Glucantime® through intramuscular route at a dose of 1mL/kg body weight, as used for Group 1.

Group 5. Immunochemotherapy II (IQT 0.5). A group of 47 patients were given subcutaneous anti-ACL vaccine, as described for Group 3, simultaneously with Glucantime® through intramuscular route at a dose of 0,5mL/5kg body weight, as used for Group 2.

Group 6. Colliquation (BCG + Glucantime®). A group of 47 patients were administered 100µg intradermal BCG after 15 to 20 days of stimulation with the onset of colliquation, Glucantime® was used at a dose of 1mL/5kg body weight, as described for Group 1.

Group 7. Monthly immunotherapy with BCG (100µg) (IT and BCG monthly). Fifty-eight patients were submitted to monthly intradermal doses of an association of 0.6mL of vaccine with 100µg BCG until clinical cure was achieved, without exceeding a period of five months under treatment.

Group 8. Monthly BCG. Thirteen patients were treated with monthly intradermal doses of 100µg of BCG, administered into the deltoid muscle.

Group 9. Monthly immunotherapy (IT monthly). In this group, 12 patients were treated with monthly doses of 0.6mL vaccine.

Therapeutic failure. It should be noted that each therapeutic plan was maintained until complete clinical cure was achieved; if cure was not effected after two series, the plan was, then, substituted for the standard therapy (serial chemotherapy with Glucantime®).

Cure criterion. The cure criterion was clinical, i.e., total healing of cutaneous lesions and epithelization of the skin.

Statistical analysis. Variance analysis for treatment period analysis, adjusted by age, disease period, and MT area was used⁷³. A significance level of 95% was taken into account.

RESULTS

A total of 542 patients with positive ACL diagnosis were divided into 9 treatment groups. The demographic and clinical characteristics of the groups studied are shown in Table 1. The frequency of male gender was 59.3% and the mean age was 28.6 years old (ranging from 5 to 86 years old). No significant relationship regarding the gender and age of the patients was found among the groups tested, including the standard group. Malnutrition, defined by body mass index ($BMI = \text{weight}/\text{height}^2$), was observed in 10.8% of the patients, which was shown to be similar for all the groups under study (Table 1).

The period of ACL disease, defined as the time taken between the initial observation of the lesion, reported by the patient, and clinical diagnosis, ranged from 10 days to 43.4 months with an average of 5.3 months. The number of lesions varied from 1 to 3, and their mean diameter was 4.1cm. The mean MT area, which was shown to be positive in 82.4% of

patients, was 5.2cm. The parasitological exam was positive in 89% of patients. No relationship between the number of lesions, disease period, and area of ulcers was observed among patients (Table 1).

The treatment period of the groups under study and the volume of antimony necessary to achieve cure are shown in Table 2.

The average length of treatment in days for clinical ACL cure was: 94.6 for Group 1; 81.0 for Group 2; 172.8 for Group 3; 64.7 for Group 4; 105.5 for Group 5; 91 for Group 6; 177.2 for Group 7; and 159.5 and 226.7 days for Groups 8 and 9, respectively.

The therapy plans used in Groups 1, 4, 5, and 6 achieved clinical cure in 100% of the patients with varying periods (days) of 94, 64, 105 and 91 days, respectively.

Group 3, under immunotherapy, showed 98.1% of cured patients, despite its increased treatment length when compared with the standard group.

The average number of series, comprising all therapies, ranged from 3.3 to 11.3.

Table 2 - Therapy plans under study for the treatment of American cutaneous leishmaniasis patients.

Groups	Therapy	Number of patients/ Number of cured cases	Cure percentage	Mean number of series ^a	Reduction of SB ^b volume (%)	Number of series of Glucantime in case resistance	Mean time of treatment until cure (day)
2	QT 0.5	29/29	100.0	4.2	50.0	-	82.2
1	QT 1.0	39/39	100.0	4.6	100.0	-	94.6
3	Serial	53/52	98.1	8.7	17.9	7.0	172.8
1	T 1.0	35/35	100.0	4.2	33.3	-	82.5
4	T 1.0	38/38	100.0	3.3*	7.7	-	64.7
1	T 1.0	49/49	100.0	4.8	100.0	-	94.8
5	T 0.5	47/47	100.0	5.4	100.0	-	105.5
1	T 1.0	44/44	100.0	4.8	100.0	-	94.6
6	Colliquation	48/48	100.0	4.7	-	-	91.0
7	IT and BCG monthly	58/27	46.6*	8.9*	-	3.6	177.2*
8	BCG monthly	13/4	38.8*	8.0*	-	4.2	159.5*
9	IT monthly	12/3	25.0	11.3*	-	4.3	226.7*
1	T 1.0	78/78	100.0	4.4	-	-	86.5
Total		542	89.9	5.7	-	3.9	113.1

1 QT 1.0: Standard Group, * Significant difference when compared with standard group.^a Adjusted by disease time, lesion area and area of Montenegro test.1/ QT 1.0: Glucantime 1,0mL/5kg body weight Standard Therapy, 2/ QT 0.5: Glucantime 0,5mL/5kg body weight, 3/ IT serial: Vaccine serial, 4/ IQT 1.0: Vaccine + Glucantime 1mL, 5/ IQT 0.5: Vaccine + Glucantime 0,5mL, 6/ Colliquation: BCG + Glucantime, 7/ BCG IT montly: BCG+ vaccine monthly, 8/ Monthly BCG, 9/ Vaccine Monthly immunotherapy

DISCUSSION

No individual aspect of the patients or possible correlations with age, gender, nutritional status and number of lesions was shown to have a significant difference through variance analysis among the groups under study.

Based on these results, immunotherapy (Group 3) may be an alternative treatment for those patients for whom Glucantime® is not indicated.

By associating the vaccine with the standard application of Glucantime® (Group 4), a reduction in the volume of antimony (17.9%) and treatment length for clinical cure (from 94.6 to 64.7 days) was observed, when compared with the standard treatment (Group 1).

In the case of immune-stimulation with vaccine, an enhancement in interferon γ and interleukin 2 (IL-2)

production was observed potentializing the response type Th1 by activating macrophages through INF γ production, in agreement with that shown by other investigators⁴⁵. This leads to the destruction of parasites by increasing oxygen radical levels, mainly nitric oxide. Furthermore, interferon α induces macrophages to produce IL 12, which acts on lymphocytes and "Natural Killer" (NK) cells, potentializing the immune response. These are ongoing findings in our works (data not shown).

When meglumine antimoniate volume was diminished by 50% to treat Group 2 patients, treatment length was shown to be as short (81 days) as that for Group 1, and cure rates were the same in both groups.

Association between the antimonial drug and immunotherapy (Group 4) showed a significant reduction in treatment length and also approximately 18% decrease in antimony volume, when compared with Group 1.

Immunotherapy associated with half the dose of meglumine antimoniate (Group 5) increased treatment length, in contrast, antimony volume was decreased by 33.3%, consequently, minimizing the side effects caused by Glucantime®.

Group 6, receiving BCG for 15 to 20 days before chemotherapy, showed a reduction of 7.7% in antimony volume, with no statistical significance, and a slight increase in treatment length for clinical cure in relation to Group 1.

The therapy comprising BCG and monthly immunotherapy (Groups 8 and 9) did not contribute to a reduction in the treatment period when compared with the standard treatment.

Considering the fact that other investigators only achieved 50.8% of clinical cure at a dose of 20mg/kg/day, either through intramuscular or intravenous route, for 20 days⁶⁰, it should be noted that our service achieved a cure rate of 100% with Glucantime® therapy (Group 1). Literature data has shown that treatment with meglumine antimoniate for 10 or 20 days, at a dose of 20mg/kg/day, achieves clinical cures of 61% and 67%, respectively⁵³.

Similar results to those obtained here with the standard treatment here (Group 1) were also achieved by our research team in 2002³¹ while treating 29 patients from the municipality of Araçuaí, State of Minas Gerais. These patients had no indication for treatment with the standard antimonial therapy, mainly due to the presence of electrocardiographic alterations (17), antimony allergy (4), pregnancy (6) and nephropathy (2). Among these patients, 10 underwent immunochemotherapy and another 19 immunotherapy. The cure rate proved to be 100% and the mean number of series up to clinical cure was 2.3 (ranging from 1 to 4 series), while for immunochemotherapy, this was 2.47 series, with similar variation for both therapies.

Considering that antimony may produce side effects, a concentration reduction in ACL treatments is quite important. However, appropriate therapy should be carried out in order to diminish likely recurrences of the mucous form⁶¹ as well as other kinds of recurrences that have been frequently reported^{1 32 46 48 49 54 57 67 77}.

Considering the broad range of therapies for ACL, there is no consensus among those aimed at seeking a reduction in the side effects of pentavalent antimonial drugs, there is no consensus. Azeredo-Coutinho and Mendonça⁶ compared two therapies: a continuous plan, as recommended by WHO⁷⁸, and a serial one. These investigators concluded that the cure rate obtained with the intermittent treatment (serial) proved to be significantly higher than that with the continuous plan. The cure criterion was defined by complete skin epithelization observed up to three months after the end of treatment. Surprisingly, the follow-up of the patients was greatly superior when they were submitted to the serial treatment. Most of the patients under continuous treatment did not attend the Outpatient Facility throughout the complete period of treatment. Such data corroborate our findings³⁴ concerning serial treatment, although there are divergences regarding clinical cure criteria. In the present study serial treatment was maintained until complete healing of the cutaneous lesions and the use of only three series was not followed.

Despite Machado-Coelho's³¹ reports on resistance, have been quite rare cases of resistance to Glucantime in chemotherapy treatment have been quite rare in the municipality of Caratinga. Patients from other regions usually seek this Out Patient Facility due to previous failed treatments, performed in other localities.

In the case of ACL recurrence, patients attended at our service are recommended to return to the Outpatient Facility of Caratinga, as well as those from other regions, who are addressed by different health units due to the popular knowledge on treatments of cutaneous lesions provided by this ambulatory in Caratinga. Some patients from different regions, who underwent the treatment, indicated by the WHO⁷⁸ (15mgSb/kg/day for 20 days and maximum daily dose of 15mL/day), sought our services due to disease recurrence or intolerance to the salt. Based on the WHO therapy, cure criterion is the complete healing of cutaneous lesions observed up to 3 months after the treatment end, which lasts 20 days with or without complete epithelization. Eight, from among these patients, sought our ambulatory and underwent further treatment with the antimony in a serial plan (a 10-day treatment + a 10-day rest) associated with the vaccine (0,5mL/day), simultaneously administered with Glucantime®. The Glucantime® daily dose was 1mL/5kg body weight without exceeding 10mL/day. This treatment varied from 2 to 4 series, which was only interrupted with clinical cure.

Several investigators have reported the side effects of Glucantime® used in different therapy plans^{27 26 32 45 51 64 66}. Considering the therapy plans of the current work, side effects such as nausea, asthenia, myalgia, arthralgia and abdominal discomfort were not observed throughout the follow-up every 20 days along with treatment until clinical cure. This might be due to the serial plan with 10-day intervals between administrations of N-methylglucamine antimoniate.

Due to the complexity involved in vector-host-parasite interactions, investigators have been faced with different therapeutic choices. No clear consensus exists among the various therapies available in literature^{9 11 13 29 79} and those presented here. One of the most serious problems is the lack of information regarding daily doses of antimony^{30 45 56 69}. Our therapy plans are in accordance with maximum daily dose of antimony (10mL - 850mgSb/day); thus, an intravenous treatment using 20mg/kg/day would take 1,400mgSb/day, or 16.47 mL, for a patient weighing 70kg, though most investigators do not mention such information.

The results from the patients under study and those from a casuistic of 6,900 cases, since 1965, provide strong evidence regarding the efficacy of the therapy used here. We have only observed 5 recurrence cases, which is frequently reported when the classic therapy recommended by the WHO^{1 45 48 50 54 67 68 77} is used.

The current work shows the possibility of decreasing antimony volume for ACL treatment, with minimal occurrence of the most likely side effects. It also offers an alternative immunotherapy with promising results for patients, for whom antimony use is not indicated.

ACKNOWLEDGEMENTS

We are grateful to the Secretaria Municipal de Saúde of Caratinga and the Consórcio Intermunicipal de Saúde of the Micro-região of Caratinga, Minas Gerais State, Brazil, and Mr. Jair Cecílio de Paula for his invaluable support during the field work.

REFERENCES

- Aebischer T. Recurrent cutaneous Leishmaniasis: A Role for Persistent Parasites? *Parasitology Today* 10: 25-28, 1994.
- Albanese G, Giorgetti P, Santagostinho L, Crippa D, Sala G. Cutaneous leishmaniasis. Treatment with itraconazole. *Archives of Dermatology* 125: 1540-1542, 1989.
- Alving CR, Steck EA, Chapman WL JR, Waits VB, Hendricks LD, Swartz GM JR, Hanson WL. Therapy of leishmaniasis: superior efficacies of liposome-encapsulated drugs. *Proceedings of the National Academy of Sciences of USA* 75: 2959-2963, 1978.
- Antunes CMF, Mayrink W, Magalhães PA, Costa CA, Melo MN, Dias M, Michalick MS, Williams P, Lima AO, Vieira JB. Controlled field of a vaccine against new world cutaneous leishmaniasis. *International Journal Epidemiology* 15: 572-580, 1986.
- Armijos RX, Weiget MM, Aviles H, Maldonado R, Racines J. Field trial a vaccine against new world cutaneous leishmaniasis in at-risk child population: safety, immunogenicity, and efficacy during the first 12 months of follow-up. *The Journal of Infectious diseases* 177: 1352-1357, 1998.
- Azeredo-Coutinho RBG, Mendonça SCF. Na intermitted schedule is better than continuous regimen of antimonial therapy for cutaneous leishmaniasis in the municipality of Rio de Janeiro, Brazil. *Revista da Sociedade Brasileira Medicina Tropical* 35:477-481, 2002.
- Barral-Netto M, Brodskyn C, Carvalho EM, Barral A. Human leishmaniasis @cytokines.Bahia.br. *Brazilian Journal of Medical and Biological Research* 31: 149-155, 1998.
- Bassiouny A, EL-Meshad M, Talaat M, Kutty K, Metawaa B. Cryosurgery in cutaneous leishmaniasis. *Brazilian Journal of Dermatology* 107: 467-474, 1982.
- Belazzoug S, Neal RA. Failure of meglumine antimoniate to cure cutaneous lesions due to *Leishmania major* in Algeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 80: 670-671, 1986.
- Bottrel RLA, Dutra WO, Martins FA, Gontijo B, Carvalho E, Barral-Netto M, Barral A, Almeida RP, Mayrink W, Locksley R, Gollob KJ. Flow Cytometric Determination of Cellular Sources and Frequencies of Key Cytokine-Producing Lymphocytes Directed Against Recombinant LACK and Soluble *Leishmania* Antigen in Human Cutaneous Leishmaniasis. *Infection and Immunity* 3232-3239, 2001.
- Bourée P, Anciaux ML, Taugourdeau. Antimoniate de methyl-glucamine et stibo-gluconate de sodium dans le traitement des leishmanioses. *Pathologie Biologie* 33: 607-610, 1985.
- Castes M, Tapia FJ. Immunopathology of American tegumentary leishmaniasis. *Acta Científica Venezuelana* 49: 42-56, 1998.
- Chulay JD, Anzeze EM, Koech DK, Bryceson AD. High-dose sodium stibogluconate treatment of cutaneous leishmaniasis in Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 77: 717-21, 1983.
- Convit J, Rondon Ulrich M, Bloom B, Castellanos PL, Pinardi ME, Castes M, Garcia L. Immunotherapy versus Chemotherapy in localized cutaneous leishmaniasis. *Lancet* 21: 401-404, 1987.
- Coutinho SG, Da-Cruz AM, Bertho AL, Santiago MA, De-Luca P. Immunologic patterns associated with cure in human American Cutaneous Leishmaniasis. *Brazilian Journal of Medical and Biological Research* 31: 139-142, 1998.
- Da-Cruz AM, Conceição-Silva F, Bertho AL, Coutinho SG. *Leishmania*-reactive CD4+ and CD8+ T cells associated with cure of human cutaneous leishmaniasis. *Infection and Immunity* 62:2614-2618, 1994.
- Dan M, Verrer E, el On J, Zuckerman F, Micheli. Failure of oral ketoconazole to cure cutaneous ulcers caused by *Leishmania braziliensis*. *Cutis* 38:198-199, 1986.
- El Darouti MA, alRubaie SM. Cutaneous leishmaniasis. Treatment with combined cryotherapy and intralesional Stibogluconate injection. *International Journal of Dermatology* 29:56-59, 1990.
- Elloso MM, Scott P. Expression and Contribution of B7-1 (CD80) and B7-2 (CD86) in the Early Immune Response to *Leishmania major* Infection. *Journal of Immunology* 162:6708-6715, 1999.
- Furtado T. Terapêutica de Leishmaniose Americana - Medicamentos de Escolha. *Revista Associação Médica de Minas Gerais* 25:108-113, 1974.
- Furtado TA. An appraisal of Chemotherapy of American Leishmaniasis. *Dermatology Ibero Latino Americano* III: 2557-2558, 1968.
- Genaro O, Toledo VP, Costa CA, Hermeto MV, Afonso LC, Mayrink W. Vaccine for prophylaxis and immunotherapy, Brasil. *Clinical Dermatology* 14: 503-512, 1996.
- Gomes LS. A intradermorreacção de Montenegro na Leishmaniose e outras pesquisas afins. *Brasil-Médico* 53: 1079-1087, 1939.
- Guderian RH, Chico ME, Rogers MD, Pattishall KM, Grogil M, Berman JD. Placebo controlled treatment of Ecuadorian cutaneous leishmaniasis. *American Journal of Tropical Medicine and Hygiene* 45: 92-97, 1991.
- Gundz K, Afsar S, Ayhan S, Kandiloglu AR, Turel A, Filliz EE, Ok UZ. Recidivans cutaneous leishmaniasis unresponsive to lipoma amphotericin B (AmBisone). *Journal European Academic Dermatology Venereology* 14: 11-13, 2000.
- Hepburn NC. Cutaneous leishmaniasis. *Clinical and Experimental Dermatology* 25: 363-370, 2000.
- Hepburn NC, Siddique I, Howie AF, Beckett GJ, Hayes PC. Hepatotoxicity of sodium stibogluconate therapy for American cutaneous leishmaniasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 88: 453-455, 1994.
- Hermeto MV, Genaro O, Costa CA, Toledo VPCP, Michalick MSM, Melo MN, Magalhães PA, Resende FS, Dias M, Williams P, Mayrink W. Immunotherapy for American Cutaneous Leishmaniasis in the State of Minas Gerais, Brazil, using Leishvacin®. Workshop Nacional sobre Leishmanioses, Recife, Memórias do Instituto Oswaldo Cruz, 1993.
- Herwaldt BL, Berman JD. Recommendations for treating Leishmaniasis with sodium stibogluconate (Pentostan) and review of pertinent clinical studies. *American Journal of Tropical Medicine and Hygiene* 46: 296-306, 1992.
- Koff AB, Rosen T. Treatment of cutaneous leishmaniasis. *Journal of American Academic Dermatology* 31: 693-708, 1994.
- Machado-Coelho GLL, Assunção R, Mayrink W, Caiaffa WT. American cutaneous leishmaniasis (LTA) in Southeast Brazil: space-time clustering. *International Journal of Epidemiology, Oxford* 28: 982-989, 1999.
- Marovich MA, Lira R, Shepard M, Fuchs GH, Kruezer R, Nutman TB, Neva FA. Leishmaniasis recidivans recurrence after 43 years: a clinical and immunologic report after successful treatment. *Clinical Infectious Diseases* 33: 1076-1079, 2001.
- Martinez S, Marr JJ. Allopurinol in the treatment of American Cutaneous Leishmaniasis. *New England journal of Medicine* 326: 741-747, 1992.
- Mayrink W, Botelho ACC, Michalick MSM, Melo MN, Machado-Coelho GLL. Imunoterapia, imunoquioterapia e Quimioterapia no Tratamento da Leishmaniose Tegumentar Americana. VII Reunião Anual de Pesquisa Aplicada em Leishmanioses. Uberaba, MG. *Revista da Sociedade Brasileira de Medicina Tropical* 35: 122-123, 2002.
- Mayrink W, Costa CA, Magalhães PA, Melo MN, Dias M, Lima AO. Responses to Montenegro Antigen After Immunization With Killed *Leishmania* Promastigotes. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 72: 676-676, 1978.
- Mayrink W, Costa CA, Magalhães PA, Melo MN, Dias M, Oliveira-Lima A, Michalick MSM, Williams P. A field trial of a vaccine against American Dermal Leishmaniasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 73: 385-387, 1979.
- Mayrink W, Magalhães PA, Michalick MSM, Costa CA, Lima AO, Melo MN, Toledo VPCP, Nascimento E, Dias M, Genaro O, Hermeto MV, Williams P.

- Immunotherapy as treatment of American cutaneous leishmaniasis: preliminary studies in Brazil. *Parasitologia* 34: 159-165, 1992.
38. Mayrink W, Melo MN, Costa CA, Coutinho SG, Hermeto MV, Genaro O, Toledo VPCP, Guerra H. Multinational development of standart skin test antigen in American: preliminary results in the Minas Gerais state, Brazil. 2A. Reunião Anual sobre Pesquisa Básica em Doença de Chagas. Caxambu – MG. Memórias Instituto Oswaldo Cruz 88: 226 1993.
 39. Mayrink W, Michalick MSM, Melo MN, Nascimento E, Magalhães PA, Costa CA, Oliveira-Lima A, Dias M. Tratamento da Leishmaniose Tegumentar utilizando vacina. *Annals Brazilian Dermatology* 66: 55-59, 1991.
 40. Mayrink W, Resende FS, Costa CA, Hermeto MV, Genaro O, Toledo VPCP, Michalick MSM, Melo MN, Nascimento E, Williams P, Oliveira-Lima A. Relatório de Atividades no Ambulatório de Leishmanioses, Caratinga, Minas Gerais, Período 1989-1992. Setor de Leishmanioses - Departamento de Parasitologia - Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, 1993.
 41. Melo MN, Mayrink W, Costa CA, Magalhães PA, Dias M, Williams P, Araujo FG, Coelho MV, Batista SM. Padronização do antígeno de Montenegro. *Revista Instituto Medicina Tropical de São Paulo* 19: 161-164, 1977.
 42. Mendonça SC, De Luca PM, Mayrink W, Restom TG, Conceição-Silva F, Da-Cruz AM, Bertho AL, Da Costa CA, Genaro O, Toledo VP. Characterization of Human T Lymphocyte-mediated Immune Responses Induced by a Vaccine Against American Tegumentary Leishmaniasis. *American Journal Tropical Medicine Hygiene* 53: 195-201, 1995.
 43. Michalick MSM, Mayrink W, Magalhães PA, Costa CA, Melo MN, Dias M, Williams P, Santos PH. Imunoterapia na leishmaniose tegumentar americana (LTA). In: Resumos do X Congresso da Sociedade Brasileira de Parasitologia, Salvador, BA, p. 119, 1987.
 44. Momeni AZ, Aminjaveheri M. Treatment of recurrent cutaneous Leishmaniasis. *International Journal of Dermatology* 34: 129-133, 1995.
 45. Mosman TR, Coffman RL. Th1 and Th2 cells: different functional properties. *Annals of Veterinary and Immunology* 7:145, 1989.
 46. Nadin A. Immunity to Cutaneous Leishmaniasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 78:848, 1984.
 47. Nascimento E, Mayrink W, Torres FV, Liu TY, Martins MT, Gazzinelli R, Fernandes AP, Genaro O, Costa CA, Toledo VPCP, Mc Master RW. Enhance Protection In Mice By Vaccination With Combined Parasite Recombinant Antigens (Rgp63 And Rp33) Against *Leishmania (Leishmania) amazonensis*. XXIII Congresso de Caxambu, MG. Memórias do Instituto Oswaldo Cruz 91: 163-163, 1996.
 48. Netto EM, Marsden PD, Llanos-Cuentas EA, Costa JM, Cuba CC, Barreto AC, Badaro R, Jonhson WD, Jones TC. Long-term follow-up of patients with *Leishmania (Viannia) braziliensis* infection and treated with Glucantime. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 84:367-370, 1990.
 49. Neva FA, Wyler D, Nash T. Cutaneous Leishmaniasis-a case with persistent organisms after treatment in presence of normal immune response. *The American Journal of Tropical Medicine and Hygiene* 28: 467-471, 1979.
 50. Oliveira-Neto MP, Mattos M, Souza CS, Fernandes O, Pirmez C. Leishmaniasis recidiva cutis in New World cutaneous leishmaniasis. *International Journal of Dermatology* 37: 846-849, 1998.
 51. Oliveira-Neto MP, Schubach A, Mattos M, Gonçalves-Costa SC, Pirmez C. Treatment of American Cutaneous Leishmaniasis: a comparison between low dosage (5mg/kg/peso and high dosage (20mg/kg/day) antimony regimens. *Pathologie Biologie* 45: 496-499, 1997.
 52. Oster CN, Chulay JD, Hendricks LD, Pamplin CL 3rd, Ballou WR, Berman JD, Takafuji ET, Tramont EC, Canfield CJ. American cutaneous leishmaniasis: a comparison of three sodium stibogluconate treatment schedules. *The American Journal of Tropical Medicine and Hygiene* 34: 856-860, 1985.
 53. Palacios R, Osorio LE, Gralalew LF, Ochoa MT. Treatment failure in children in a randomized clinical trial with 10 and 20 days of meglumine antimoniate for cutaneous leishmaniasis due to *Leishmania viannia* species. *The American Journal of Tropical Medicine and Hygiene* 64: 187-193, 2001.
 54. Passos VMA, Barreto SM, Romanha AJ, Krettl AU, Volpini AC, Costa MFFL. American cutaneous leishmaniasis: use of a skin test as a predictor of relapse after treatment. *World Health Organization* 78: 968-974, 2000.
 55. Pessoa SB, Pestana BR. A intradermo-reação de Montenegro nas campanhas sanitárias contra a Leishmaniose. *Arquivos de Higiene e Saúde Pública* 6:124-137 1941.
 56. Pirmez C, Fernandes O, Oliveira-Neto MP. A reação em cadeia da polimerase (PCR) antes e após tratamento na leishmaniose tegumentar. *Revista da Sociedade Brasileira Medicina Tropical* 33:58-59, 2000.
 57. Ramírez JL, Guevara P. Persistent Infections by *Leishmania (Viannia) braziliensis*. *Memórias Instituto Oswaldo Cruz* 92: 333-338, 1997.
 58. Reed SG, Scott P. T-cell and cytokine responses in leishmaniasis. *Current Opinion in Immunology* 5:524-531, 1993.
 59. Ribeiro-de-Jesus A, Almeida RP, Lessa H, Bacellar O, Carvalho EM. Cytokine profile and pathology in human leishmaniasis. *Brazilian Journal of Medical and Biological Research* 31:143-148, 1998.
 60. Rodriguez ME, In Guanzo P, Ramos A, Perez J. Treatment of cutaneous leishmaniasis with Co₂ laser radiation. *Revista Cubana Medicina Tropical* 42:197-202, 1990.
 61. Romero GA, Guerra MV, Paes MG, Macedo VO. Comparison of cutaneous leishmaniasis due to *Leishmania (Viannia) braziliensis* and *L. (V.) guyanensis* in Brazil: therapeutic response to meglumine antimoniate. *The American Journal of Tropical Medicine and Hygiene* 65: 456-465, 2001.
 62. Row R. Curative value of Leishmania culture vaccine in Oriental sore. *Brazilian Medical Journal* 9:540-541, 1912.
 63. Rzany B, Krutmann J, Goertler E, Schopf E. Cutaneous leishmaniasis: treatment with cryotherapy and intramuscular injections of Meglumine Antimoniate (Glucantime®). *Hautarzt* 41:98-101, 1990.
 64. Saenz RE, Narváez E. Evaluation of the effectiveness and toxicity of Pentostan and Glucantime in the treatment of cutaneous leishmaniasis. *Revista Medica do Panamá* 12:148-157, 1987.
 65. Saenz RE, Paz H, Berman JD. Efficacy of Ketoconazole against *Leishmania braziliensis panamensis* cutaneous leishmaniasis. *American Journal of Medicine* 89: 147-155, 1990.
 66. Saldanha ACR, Romero GAS, Hamann EM, Magalhães AV, Macedo VO. Estudo comparativo entre estibogluconato de sódio BP 88R e antimoniatado de meglumina no tratamento da leishmaniose cutânea: I. Eficácia e segurança. *Revista da Sociedade Brasileira Medicina Tropical* 32: 383-387, 1999.
 67. Saravia NG, Weigle K, Segura I, Giannini SH, Pacheco R, Labrada LA, Goncalves A. Recurrent lesions in human *Leishmania braziliensis* infection – reinfection? *Lancet*. 18: 398-402, 1990.
 68. Schewach-Millet M, Fisher BK, Semanh D. Leishmaniasis recidivans treated with sodium stibogluconate. *Cutis* 28: 67-68, 1984.
 69. Schubach A, Marzochi MC, Cuzzi-Maya T, Oliveira AV, Araujo ML, Oliveira AL, Pacheco RS, Momen H, Conceicao-Silva F, Coutinho SG, Marzochi KB. Cutaneous Scars in American Tegumentary Leishmaniasis patients: a site of *Leishmania (Viannia) braziliensis* persistence and viability eleven years after antimonial Therapy and clinical cure. *American Journal of Tropical Medicine and Hygiene* 58: 824-827, 1998.
 70. Sharquie KE, Al-Talib KK, Chu AC. Intralesional therapy of cutaneous leishmaniasis with sodium stibogluconate antimony. *Brazilian Journal Dermatology* 119: 53-57, 1988.
 71. Silveira FT, Mayrink W. Leishmaniose Cutânea difusa no Estado do Pará, Brasil: Registro do primeiro caso de cura da doença após associação de quimioterapia com imunoterapia. XXIX Congresso da Sociedade Brasileira de Medicina Tropical. *Revista da Sociedade Brasileira de Medicina Tropical* 26 (supl):229, 1993.
 72. Silveira FT, Mayrink W. Leishmaniose Cutâneo-Difusa No Estado do Pará, Brasil: Relato da Cura de Um Caso Depois de 24 Anos de Doença, Após Tratamento Combinado de Quimioterapia com Imunoterapia. In: Anais do XXXIII Congresso da Sociedade Brasileira de Medicina Tropical, p.129, 1997.
 73. Snedecor GW, Cochran WG. *Statistical Methods*, 8th edition. Iowa State University Press, Ames, Owa, 1989.
 74. Soto-Mancipe J, Grogl M, Berman JD. Evaluation of pentamidine for the treatment of cutaneous leishmaniasis in Colombia. *Clinical Infectious Diseases* 16: 417-425, 1993.

75. Tapia FJ, Dittamar GS, Sánchez MA. Inadequate epidermal homing leads to tissue damage in human cutaneous leishmaniasis. *Immunology Today* 5:160-165, 1994.
76. Vianna G. Tratamento da leishmaniose tegumentar por injeções intravenosas de tártaro emético. *In: Anais do 7º Congresso Brasileiro de Medicina e Cirurgia* 4: 426-428, 1912.
77. Weigle KA, Valderrama L, Santrich C, Saraiva NG. Recurrences of Tegumentary Leishmaniasis. *Lancet* 2:557-558, 1985.
78. World Health Organization – Division of Control of Tropical Diseases. Statistics as of April 1998. *Apud: Kenner JR, Aronson NE, Bratthauer GL, Turnicky RP, Jackson JE, Tang DB, Sau P. Immunohistochemistry to identify Leishmania parasites in fixed tissues. Journal of Cutaneous Pathology* 26:130-136, 1999.
79. Wortmann G, Miller SR, Oster C, Jackson J, Aronson N. A Randomized, Double-blind Study of the Efficacy of a 10- or 20- Day Course of Sodium Stibogluconate for Treatment of Cutaneous Leishmaniasis in United States Military Personnel *CID* 35: 261-267, 2002.