

ORAL TREATMENT OF NEW WORLD CUTANEOUS LEISHMANIASIS WITH ANTI-MALARIAL DRUGS IN ECUADOR: A PRELIMINARY CLINICAL TRIAL

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Abstract: The current study was designed to evaluate anti-leishmanial activity of mefloquine hydrochloride (Mephaquin®) and artesunate (Plasmotrium®) which are currently being used as malarial drugs. A total of 17 patients (volunteers) with cutaneous leishmaniasis were treated with these drugs in this study. Of these subjects, 16 were treated by the oral administration of a total dosage of 1,500mg (1 Lactab® each for 6 days) mefloquine, 4.2mg/kg/day for 6 days, and if necessary the dosage was repeated with 3 weeks intervals. The majority of cutaneous lesions healed within 2 to 3 weeks after the commencement of mefloquine treatment, showing an average of 3.6 weeks of healing times with 100% cure rate. One slowly healing within 8 weeks after the commencement was observed; this case grew worse because of infection with *Tunga penetrans* at the late healing phase of leishmaniasis. The remaining one patient with an ulcer lesion was treated by the oral administration of a total dosage of 1,200mg (2 Lactab® each for 3 days) artesunate, i. e., 6.7mg/kg/day for 3 days, and the same dosage was repeated 2 weeks later. The lesion healed within 6 weeks after the commencement of artesunate treatment. In the present study, all the patients received mefloquine or artesunate were treated without admission, performing their normal daily activities. No specified adverse reaction was noticed.

INTRODUCTION

The pentavalent antimonials sodium stibogluconate and meglumine antimonate remain as the first choice of drugs in the clinical treatment of different types of leishmaniasis, such as cutaneous, mucocutaneous and visceral forms (Bryceson, 1980; WHO, 1990). Since the introduction of these antimonials more than 50 years ago, many investigations have been done in order to find more efficient treatment without side effect for the disease. No satisfactory effective new drugs, however, have been developed, though several important advances have been made (Berman, 1988; Croft, 1988). There is, therefore, still a need to search for a new drug that is fully effective and orally applicable for most clinical forms of Old and New World leishmaniasis.

To date, trials of search for new drugs and treatment have been performed *in vitro* and *in vivo* using experimental animals and/or volunteer patients with

leishmaniasis. In the present study we tried to treat cutaneous leishmaniasis patients with two types of anti-malarial drugs, mefloquine and artesunate, which are clinically being used. The current paper describes the anti-leishmanial activities of these drugs, based on the data obtained from clinical trials at endemic areas of Ecuador.

MATERIALS AND METHODS

In the present study, a total of 17 patients with cutaneous leishmaniasis were examined. They came from different endemic areas, Zhucay and Manta Real (Province of Cañar), Zapotal (Province of Guayas), Muisne (Province of Esmeraldas) and Caluma (Province of Chimborazo). Of these subjects, 16 were treated orally with a total dosage of 1,500mg (1 Lactab® each for 6 days) mefloquine (Mephaquin®, Mepha Ltd., Aesch-Basle, Switzerland; each Lactab® contains

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mefloquine hydrochloride corresponding to 250mg mefloquine base), i.e., 4.2mg/kg/day for 6 days, and if necessary the dosage was repeated with 3 weeks intervals after the end of initial treatment. In patients treated with mefloquine, 13 out of 16 were male and 3 were female, aged from 3 to 81 years. The remaining one patient (14 years old male) was treated orally with a total dosage of 1,200mg (2 Lactab® each for 3 days) artesunate (Plasmotrium®, Mepha Ltd., each Lactab® contains artesunate 200mg), i.e., 6.7mg/kg/day for 3 days, and the same dosage was repeated 2 weeks later. All the subjects were informed of the purpose of the study and gave permission for drug administration and repeated physical examinations. The patients received treatment during their daily activities without admission. Almost all the volunteers (patients) lived in mountainous and dense forest areas in which no transportation systems are available (Fig. 1). Their dwellings are located at very remote areas from our laboratory of health centers in each endemic area. All the volunteers were in a very poor economic condition. In such a field situation and an ethical consideration, no placebo treatment was performed in the current trial.

All the cutaneous leishmaniasis patients treated in this study were diagnosed by the demonstration of *Leishmania* amastigotes in smear specimens from the lesions. Treated patients received a follow-up physical examination every 2 or 3 weeks, and they were recorded photographically at the same time. When their dermal lesions were partially still active in the examination, an additional administration of the drug was made as mentioned above. During the treatment, the patients were asked for the presence of any complaint, such as vomiting, nausea, diarrhoea, fever and etc.

The evaluation of the results of treatment was made as described by El-On *et al.* (1986) but partially modified as follows: 1) rapidly effective (grade 1), no parasites detected in cutaneous lesions, followed by total healing within 1-3 weeks after the commencement of treatment; 2) less rapidly effective (grade 2), the same process (no parasites and total healing) occurring within 4-6 weeks after the commencement; 3) effective (grade 3), no parasites detected but healing within 7-8 weeks; 4) ineffective (grade 4), parasites still present in lesions and/or no clinical healing after 9 or 10 weeks of the commencement of treatment.

In the present subjects, species of *Leishmania* are not identified precisely, but our previous work indicates that *L. (Viannia) panamensis* is the most frequently identified organism by zymodeme and serodeme analyses, followed by *L. (V.) guyanensis* in the surrounding

regions (unpublished data).

RESULTS

The number of cutaneous lesions per person ranged from 1 to 4 with different size of lesions, ranging from 3 × 3 mm to 30 × 30 mm in diameter (Table). All the present patients had ulcerative lesions; the majority of these lesions were located on the exposed body surface, such as forearm, foot and face. The duration time of infection varied from one to 12 months (average: 3.6 weeks) at the time of the commencement of mefloquine treatment. Nine (56.3%) of the 16 patients healed within 3 weeks after the commencement of oral treatment of mefloquine, showing grade 1 category (Fig. 2A-C), and other 6 cases took 4-6 weeks (grade 2) for healing. Only one case (patient No. 3) took 8 weeks for complete healing, because of *Tunga penetrans* infection in the cutaneous lesion which had been at the late phase of healing (4 weeks after the commencement of mefloquine treatment). No ineffective case was found in this study using mefloquine; the cure rate showed 100%. In the present trial of treatment with mefloquine, age or sex of the patients, number and size of the lesions and duration time of the infection did not show any influence against the efficacy of drug. However, heavy bacterial infections and other infection such as *Tunga penetrans* were fully influential for the healing time, especially in lesions located on the lower extremities.

In a patient (14 years old male) treated with artesunate (Plasmotrium®), an ulcer (20 × 17mm) on the forearm healed within 6 weeks after the commencement of treatment (Fig. 3A-C) and no recurrence was found after 5 months (Fig. 3D). In comparison with mefloquine, however, artesunate showed a slow effectiveness on the healing of the dermal lesion, especially in the early phase (1 week later) of oral treatment.

All the present patients treated with mefloquine or artesunate lived and worked in a rural and humid area with hygienically bad conditions. There is no specified adverse reaction in the patients treated with the drugs, including skin eruptions or pruritus. Therefore, none of the subjects treated were withdrawn from the study because of adverse reactions.

DISCUSSION

For the treatment of most forms of New and Old World leishmaniasis, pentavalent antimonial compounds are still remain as the drugs of choice. These drugs are given intramuscularly or intravenously, and generally

Table. Clinical data on the parasitologically-positive patients with cutaneous leishmaniasis who received Mephaquin® therapy

Patient No.	Age	Sex	No. of lesions	Size of lesions(mm)	Site of lesions	Type of lesions	Duration time of infection	Times (wks) for healing
1	41	♂	1	35×25	forearm	ulcer	2M*	6
2	16	♂	2	20×15 5×5	nose cheek	ulcers	12M	3
3	8	♂	2	30×30 10×5	foot	ulcers	2M	8
4	12	♂	2	5×5 5×5	forearm	ulcers	3M	5
5	19	♂	1	20×10	forearm	ulcer	3M	5
6	81	♂	4	20×10 15×15 10×10 10×10	forearm shoulder	ulcers	2M	4
7	26	♂	3	20×20 10×10 10×10	forearm	ulcers	1M	4
8	17	♂	1	15×10	neck	ulcer	1M	2
9	4	♂	4	10×10 5×5 5×5 4×4	face	ulcers	5M	2
10	22	♂	1	20×10	forearm	ulcer	1M	2
11	3	♂	2	4×4 3×3	face	ulcers	8M	2
12	13	♀	1	15×5	forearm	ulcer	2M	3
13	22	♂	1	4×3	forearm	ulcer	3M	3
14	22	♀	1	30×10	foot	ulcer	8M	3
15	31	♀	1	20×10	front	ulcer	2M	2
16	14	♂	1	34×26	foot	ulcer	3M	6

* M : month.

cause serious adverse reactions, such as renal and liver disfunctions, nausea, headache and arthragia, in addition to pain at the injection site. For the treatment of Old World cutaneous leishmaniasis, El-On *et al.* (1986) reported topical application of paromomycin ointment, obtaining satisfactory cure rate as good as any currently used therapy. Recently, the therapy was also used for New World cutaneous leishmaniasis in Ecuador (Nona-ka *et al.*, 1992; Krause *et al.*, 1994) and Belize (Weinrauch *et al.*, 1993), confirming the results reported by El-On *et al.* (1986). However, such a topical treatment using ointment has limitations in its usage even in cutaneous leishmaniasis cases. Application of ointment would be only useful for relatively mild and simple lesions which are caused by *L. (L.) mexicana* groups. It is however not feasible for cutaneous disease forms caused by *L. (V.) braziliensis* (Weinrauch *et al.*, 1993) and mucocutaneous or visceral forms caused by other *Leishmania* agents.

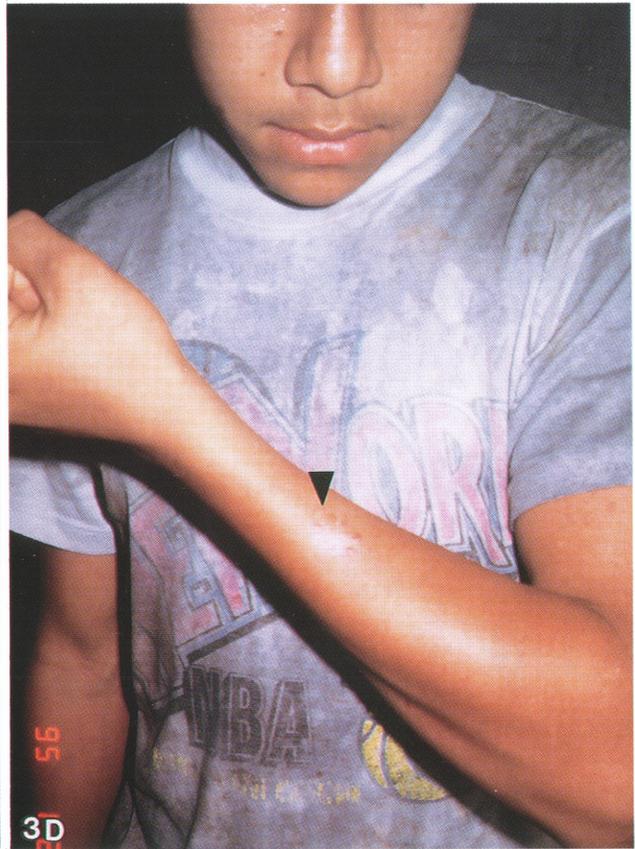
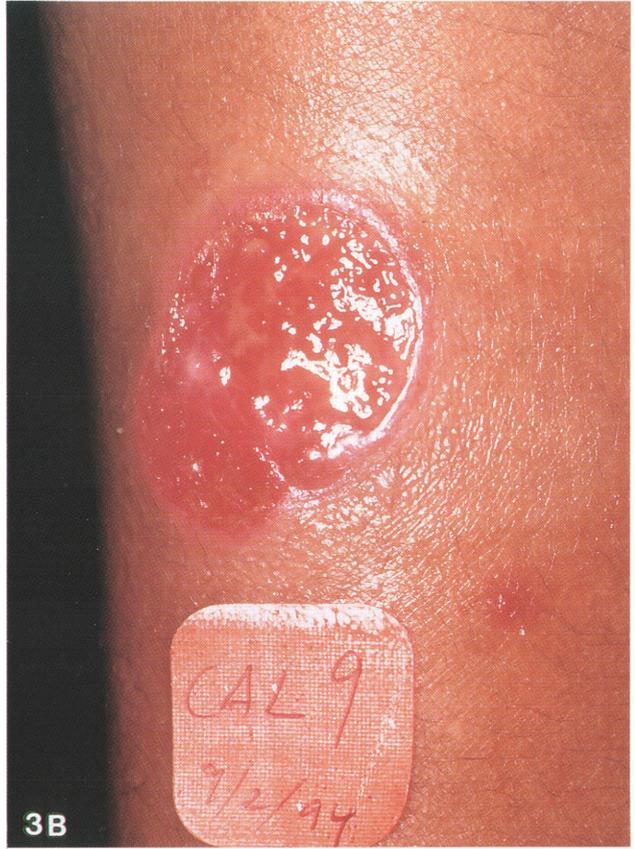
To date, various oral anti-leishmanial drugs, such as metronidazole, rifampicin, levamisole, ketokonazole, co-trimoxazole, dapsone and etc., have been used for different disease forms with variable results. In the present study, anti-malarial drugs, mefloquine and artesunate were selected for clinical trials in the continuing search for oral anti-leishmanial drugs. These drugs were found to be effective against malarial parasites in the Old and New World. Mefloquine, a long-acting quinine analogue is a schizonticide and destroys the erythrocytic, asexual forms of the *Plasmodium* parasites in man, and the mean elimination half-life of the drug is calculated as 21.4 days ranging from 15 to 33 days (Desjardins *et al.*, 1979; Schwartz *et al.*, 1980, 1982). According to Schwartz *et al.* (1980, 1982), maximum plasma concentrations are reached 2-12 hours after a single oral dose and plasma concentrations approaching 1 µg/ml are present after a dose of 1, 000mg of mefloquine. They also showed that similar



Figure 1 A dwelling site of inhabitants in an endemic area (Manta Real) surrounded by a dense forest where the present patients (volunteers) came from.

Figure 2A-C A cutaneous leishmaniasis lesion located on the lower extremity of a 22 years old female patient (No. 14) . A, An ulcer (34×26 mm) with typically elevated border and marked induration around the lesion, before mefloquine treatment. B, After 1 week of the commencement of oral administration of mefloquine, the lesion reduced slightly in size and showed a gradual disappearance of the ulcer border and induration. A marked epidermidalisation was observed on the surface of the lesion. C, After 3 weeks of mefloquine treatment, the lesion was completely covered by epidermis without induration.

Figure 3A-D A cutaneous leishmaniasis lesion located on the upper extremity of a 14 years old male patient. A, A lesion (20×17 mm) showing a typical ulcer border, before artesunate treatment. B, After 2 weeks of the oral administration of artesunate, the lesion showed a marked granulation and reduced ulcer border. C, The cured lesion completely covered by epidermis without induration, after 6 weeks of artesunate treatment. D, The lesion (arrow) after 5 months of the treatment, showing a typical scar.



maximum concentrations are present in the steady state after administration of 250mg (1 Lactab®) weekly; the concentration in the erythrocytes is almost twice as high.

In this study, using mefloquine, almost all cutaneous lesions healed within 6 weeks after the commencement of treatment showing 100% cure rate, in spite of a relatively low daily dosage (250mg/day) of the drug compared with the dosage used in malarial cases. The precise mode of action of mefloquine against *Leishmania* has not been determined, although it has been shown that more than 98% of the active substance against *Plasmodium* schizonts is bound to plasma proteins (Schwartz *et al.*, 1982). To some extent, the mode of action of mefloquine against *Leishmania* parasites might be similar to that found in malarial cases, affecting amastigote-macrophage interactions.

In oral treatment using artesunate, only one case was experienced in this preliminary trial, suggesting that the drug might remain as a candidate for future study. Artesunate, a preparation for the killing of erythrocytic stage of *Plasmodium* asexual form, reacts with intraparasitic heme in its mechanism of anti-malarial action (Meshrick *et al.*, 1991), but its mode of action against *Leishmania* parasites is still unknown precisely. According to Jiang *et al.* (1982), an advantage of artesunate administration is the speed of onset of action and inhibitory effect on the maturation of malarial parasites. On the other hand, in the present leishmaniasis case, it is likely that the drug has a tendency to act slowly as compared with mefloquine, especially at the early phase of treatment.

The current treatment with mefloquine or artesunate was done in the subjects who made their normal daily activities without admission. When a similar treatment using these drugs was performed in well-controlled subjects under admission, more rapidly healing would be found. Furthermore, a rapid healing might occur when antibiotics are used as complementary treatment to eliminate bacterial infections of cutaneous lesions. With regard to oral administration of the present drugs used, more suitable and effective dosage should be examined, in addition to their precise mechanism(s) of anti-leishmanial action.

In conclusion, the significant efficacy of anti-malarial drugs, mefloquine and artesunate against *Leishmania* following the oral delivery suggests that the novel anti-leishmanial activities of these drugs should be investigated further, and their potential as drugs for various clinical forms of leishmaniasis including visceral forms needs more study.

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