Department of Microbiology and Immunology

RE: Weinrauch

November 20, 1993

Director
Science Program
U.S.-Israel CDR Program
Room 720 SA-18
Washington D.C. 20523
U.S.A.

RE: Grant No. DPE-G-SS-7047-00

Dear Sir,

On April 30, 1992 a draft of our final report on "Chemotherapy of cutaneous leishmaniasis in Belize" was sent to you for approval. Since then no comments to this report were received. Please find enclosed a copy of the above mentioned report together with two papers published recently on this topic.

Looking forward to hear from you.

Sincerely yours

Joseph El-On, Ph.D.        Louis Weinrauch, M.D., F.A.A.D.

cc: Boston

DEC 20 1993

cc: Mr. David Molinex, American Attache
U.S. Embassy, Hayarkon St. 3, Tel-A
CHEMOTHERAPY OF CUTANEOUS LEISHMANIASIS IN BELIZE

Grant No. DPE-5544-G-SS-7047-00

FINAL REPORT

BELIZE
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ISRAEL
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Jerusalem and Belize City, December 1991
ABSTRACT

Strains of *Leishmania mexicana* isolated from Belizean patients were found to be highly susceptible to paromomycin sulphate (PR) treatment. This compound, at 100 µg ml⁻¹, destroyed 85-99.5% of *in vitro* cultivated *Leishmania* promastigotes within 4 days of exposure to the drug. *Leishmania* promastigotes inoculated into the base of the tail of Balb/c mice caused the development of local lesions several weeks after infection. These lesions were totally cleared of parasites after 20 days topical treatment with an ointment composed of 15% PR and 12% methylbenzethonium chloride (MBCl) in white soft paraffin (P-ointment). Similar results were also obtained with *L. braziliensis* infections. Isoenzyme analysis was found to be the method of choice for parasite strain identification. Excreted factor (EFS) serotyping was only partially effective, and promastigote agglutination gave negative results.

In a clinical study, 53 Belizean patients suffering from cutaneous leishmaniasis (CL) caused by either *L. braziliensis* or *L. mexicana* in Belize were treated topically with the P-ointment. After a treatment period of 14-21 days, 68% of the patients have been healed from the disease, 6% presented a delayed cure, and 26% were considered not responding to this treatment regimen. No toxic effect of the ointment was observed.
INTRODUCTION

During the last decade numerous laboratory and clinical studies have been performed in different parts of the world in order to find out a more efficient and easier way in the topical treatment of CL. The trend was, and still remains to reach a simple, topical preparation, effective, easy to use, with a good compliance rate and accessible to every patient worldwide. Two of us, (J.E-O. & L.W.), are involved more than 10 years in the search for such a drug and numerous related articles have been published on this topic.

CL, an endemic disease afflicting millions of people every year in the Old and in the New World as well, still presents a major therapeutic challenge. The existing treating modalities are either toxic and potentially with side effects (antimonium compounds, ketoconazole), expensive and not easy available (ketoconazole, rifampicin) or not handy and feasible for mass cure, especially in less developed countries (liquid nitrogen, Grenz rays).

Recently, a topical treatment of CL using the P-ointment was developed, treatment that was found to be highly effective against various strains of Leishmania.

CL in Belize is caused by two major parasite species: L. braziliensis, infecting approximately 75% of patients. and L. mexicana causing the remaining 25% of cases. Both species are associated with the development of large, nodulo-ulcerative lesions that heal slowly, leaving ugly scars.

No accurate estimation of the incidence of the disease in Belize is available at the present time. However, according to the
local medical authorities, hundreds of cases are annually reported, especially in the southern and western parts of the country. Many of these patients are soldiers of the Belize Defence Force.

The present study is aimed at determining the efficacy of the P-ointment treatment against Belizean Leishmania strains, both in vitro in culture, in vivo in experimental animals and in humans.

MATERIALS AND METHODS

I. Parasite strains and host animals

Eleven Belizean strains of Leishmania parasites including: 7 isolates of L. mexicana (BEL 44; BEL 46; BEL 47; BEL 58; BEL 64; BEL 66 and BEL 69) and 4 isolates of L. braziliensis (BEL 75; BEL 101; BEL 102 and BEL 103) have been used in this study. All strains have been isolated from patients infected in Belize. They were kept as stabilities in liquid nitrogen and subsequently maintained in vitro at 28°C by twice weekly passage on modified Tobie’s blood agar medium. Infective parasites were maintained in vivo in Balb/c mice. Rabbits were used as a source of normal blood for preparing culture media and for raising specific antisera. Blood, whether normal or immune, was collected by cardiac puncture.

II. Parasite identification

Identification of the leishmanial strains was made using: a) morphological description of the promastigotes which developed in culture at 28°C; b) parasite microagglutination test; c) isoenzyme analysis and d) excreted factor (EFS) serotyping.
a) Morphological characterization

Parasites were cultivated in modified Tobie's medium using RPMI 1640 medium (Beth Haemek, Israel) as overlay. On the fourth day of cultivation, when the parasites were at the logarithmic phase, the cells were examined under phase microscope and their morphology described.

b) Parasite microagglutination test

A microagglutination test using trypsin-treated, formalin-fixed, Coomassie blue-stained Leishmania promastigotes was performed according to the method of Harith et al. Agglutination results were recorded macroscopically and microscopically after 18 hr incubation at room temperature.

c) Isoenzyme analysis

The isoenzyme profiles of the Belizean isolates were compared using the following enzymes: alanine amino-transferase (ALAT); aspartate amino-transferase (ASAT); glucose-6-phosphate dehydrogenase (G6PD); glucose phosphate-isomerase (GPI); malic enzyme (MI); malate dehydrogenase (MDH); mannose phosphate-isomerase (MPI); 6 phosphogluconate dehydrogenase (6PGD); phosphoglucomutase (PGM) and superoxide dismutase (SOD).

d) Excreted factor (EFS) serotyping

1. EFS preparation.

Promastigotes and their EF's were harvested from cultures grown in either blood agar medium or RPMI 1640 medium. For EFS preparation, the culture at the logarithmic phase was centrifuged for 10 min at 1300xg at room temperature. The supernatant was harvested and centrifuged again at 12000xg for 30 min at 4°C, filtered through Milipore filter of 0.45 um pore size,
concentrated 10-fold by freeze drying and stored at -20°C until used.

2. Antisera production in rabbits.

Antisera were raised in male rabbits, 2.5-3 kg, against living promastigotes following the regimen of Adler et al. Six intravenous inoculations (1 x 10^7, 2 x 10^7, 8 x 10^7, 16 x 10^7, 32 x 10^7), were given at weekly intervals, and blood samples were collected ten days after the last injection. The ability of the antibody produced to precipitate leishmanial EFS and to agglutinate Leishmania promastigotes was tested.

3. Immunodiffusion and immunoelectrophoresis.

Immunodiffusion and immunoelectrophoresis were performed in agarose 1.1% in 0.041 M barbiturate buffer pH 8.2 containing 0.02% sodium azide. The preparations were developed for 1-4 days in moist diffusion chambers at 4°C, and photographed after rinsing with saline to remove excess protein, drying and staining with amino black.

III. Effect of paromomycin on leishmanial development in vitro.

The effect of paromomycin sulphate (PR) (Farmitalia, Italy) on the growth of leishmanial promastigotes was tested at 28°C in RPMI 1640 medium supplemented with 20% FCS, 100 units of penicillin and 100 ug streptomycin per ml. The promastigotes were first grown in modified blood agar medium, then transferred to RPMI medium containing various concentrations of PR on day of the experiment. Parasite growth was assessed by daily haemocytometer counts and the growth rates, as compared with the normal untreated controls calculated.
IV. Effect of P-ointment on the leishmanial development in vivo.

The method used to infect mice and score P-ointment efficacy were as described previously. Male Balb/c mice were inoculated intradermally in the base of the tail with $0.5 - 1.0 \times 10^7$ promastigotes. Treatment with P-ointment was started 45 to 80 days after infection. The ointment was applied to the lesions twice daily for a period of 10 to 20 days. Lesion development was inspected macroscopically and the presence of parasites in biopsy material was monitored microscopically, in both smears and cultures. Lesion size was measured in mm by two diameters $(D, d)$ taken at right angles and determined according to the formula: $S(\text{mm}^2) = \frac{D \times d}{2}$. Protozoological examinations were performed prior to treatment, 10 days after the beginning of the treatment and at various time intervals after termination of treatment.

V. Clinical trial.

Fifty-three Belizean patients were included in this study. All the patients were treated for a period of two to three weeks, twice daily with P-ointment. The ointment was prepared by the Department of Pharmacy, Soroka Medical Center in Beer Sheva, Israel.

Treated lesions were left uncovered in most cases. Follow up was done clinically and in the majority of cases also parasitologically by Giemsa's stained smears.

The results obtained were defined as described by El-On et al. in 1986: (1) rapidly effective: no parasites were detected in the treated lesion at the end of treatment, followed by total healing within one month after cessation of treatment; (2) less rapidly effective: the same occur within 8 weeks; (3) ineffective:
parasites still present in the treated lesion and or no healing was achieved after 8 weeks.

RESULTS

I. Strain identification

The results obtained for parasite identification were as follows

a. Morphological characterization

Promastigotes of *L. braziliensis* maintained at 28°C in mid-exponential growth phase were characteristically very small (7.5μ) with very short flagella, the organism usually grew in clumps with almost no movement. *L. mexicana* promastigotes grew as elongated parasites (20μ) with long flagellae. The parasites were highly motile and grew as either free promastigotes or in closely packed rosettes.

b. Microagglutination

The results obtained with the microagglutination test are summarized in Table 1. The highest agglutination titre (1:80) was obtained with *L. mexicana* BEL 69 against the homologous rabbit antiserum. Titers of 1:20 to 1:80 were observed with the other *L. mexicana* isolates. A higher titer (1:320) was obtained with rabbit anti *L. mexicana* antiserum against heterologous *L. major* strain (Table 1).

c. Excreted factors (EFS) serotyping

Schnur, Zuckerman and Greenblatt (1973) showed that leishmanial EFS are precipitated by antibody raised in rabbits against living homologous promastigotes. In the present study, the antiserum raised in rabbits against *L. braziliensis* (BEL 101 and
BEL 103) did not distinguish between \textit{L. mexicana} and \textit{L. braziliensis} strains. All the \textit{L. mexicana} and \textit{L. braziliensis} isolates examined were recognized by these antisera producing 1-3 clear precipitating arcs by immunodiffusion and immunoelectrophoresis. No cross reactivity was obtained between \textit{L. mexicana} and \textit{L. major} when either rabbit anti \textit{L. mexicana} or rabbit anti \textit{L. major} antisera were used.

II. Effect of paromomycin on \textit{L. mexicana} promastigote development \textit{in vitro}

The antileishmanial activity of PR against the various \textit{L. mexicana} isolates is shown in Figs. 1 and 2. PR at 10\mu gml\textsuperscript{-1} inhibited the growth of the parasites by 47\% - 80\%, and this drug at 100 \mu gml\textsuperscript{-1} inhibited the growth by 85\% - 99\% within 4 days of exposure to the drug. BEL-44 was slightly more resistant to PR than the other strains, showing about 85\% growth inhibition rate after 4 days of exposure to PR at 100 \mu gml\textsuperscript{-1}.

III. Effect of topical treatment on \textit{L. mexicana} development in Balb/c mice

Only 6 isolates of \textit{L. mexicana} (BEL 44, BEL 46, BEL 58, BEL 64, BEL 69, BEL 73) and 3 isolates of \textit{L. braziliensis} (BEL 101, BEL 102, BEL 103) were found to be infective to Balb/c mice. The remaining isolates: \textit{L. mexicana} BEL 47 and \textit{L. braziliensis} BEL 75 did not infect the mice even when high doses of 1 x 10\textsuperscript{8} promastigotes were inoculated into young, 4 weeks old mice. Treatment with P-ointment, twice daily for only 10 days was not sufficient to eliminate all the parasites, although an improvement was noted. After 20 days of treatment, parasites were no longer detectable in the area of the lesions in all the infected animals,
and open lesions completely healed within a period of 30 - 40 days (Fig. 3, Table 2). The P-ointment was only slightly less effective against L. major infection in Balb/c mice (Table 2). After 20 days treatment, parasites were still found in the lesions. However, the parasites were undetectable 20 days after termination of treatment.

IV. Clinical study

Results are summarized in Table 3. There were fifty-three Belizean patients, forty-nine males and four females, thirty-two of them soldiers from the Belize Defence Force and the remaining twenty-one civilians in the study group. All of them have been informed about the nature of the disease, the modality of therapy with P-ointment and the alternative current treatments, and a consent was obtained prior to beginning of treatment.

The age range was from 10 to 45 years (mean 26), having a number of lesions between 1 and 8 with a mean of 1.55.

The majority of the patients presented typically, big nodulo-ulcerative lesions with a size range between 0.12 and 50.00 (mean 5.75) square centimeters (Fig. 4). The duration of the lesions in their early or advance infection period varied from 2 to 260 weeks, with a mean of 14.75 weeks.

Four weeks after termination of treatment, rapid clearing was obtained in 36 (67.92%) patients. Less rapid clearing was demonstrated after eighth weeks in 3 (5.66%) patients and no effect was considered in further 11 (20.75%) after 12 weeks and 3 (5.66%) patients after 16 or more weeks (Table 3, Fig. 5) Age, sex, size of the lesions and duration since infection, as well as the presence of crusts and superimposed bacterial contamination did
not influence the response to treatment. Generally the clinical appearance of the lesion during and at the end of treatment was worse than at the start. This effect was observed also in previous studies and is caused by the development of contact dermatitis. Various degrees of inflammation, depending on the lesion size and site and the host response, were associated with this treatment. A burning sensation at the site of treatment sometimes occurred during the first ointment applications. None of the patients was dropped from the study due to side effects. Discoloration of the treated area developed in most of the patients.

DISCUSSION

Belize, known as British Honduras till becoming an independent nation in 1981, borders on Mexico to the North, Guatemala to the West and South and on the Caribbean sea to the East (Fig. 6). With a surface of about 8900 square miles, including numerous small islands (cays) and forests occupying some 65% of the area, the population is estimated at 160 000.

There are no statistic figures regarding epidemiology of the disease in Belize but the majority of the cases occur in the western part of the country, especially in the Mountain Pine Ridge area, Cayo and Toledo districts, and only sporadic cases on the eastern shores and cays. Our treated patients were mainly from these areas.

Most of the registered patients are soldiers of the Belize Defence Force but practitioners all over the country are reporting the presence of the disease along the civilian population as well.
Tourists and British soldiers stationed in Belize are also not immune to CL.

According to people with some experience with CL in this part of the world like Drs. David S. Jolliffe from the Ministry of Defence in London and David Evans from the London School of Hygiene and Tropical Medicine, about 75% of the cases of CL in Belize are provoked by \textit{L. braziliensis}, and only the remaining 25% by \textit{L. mexicana}.

Until recently, the drug of choice for the treatment of CL in Belize was stibophen (pentasodium antimony-biscatechol-3:5-disulphonate) given intramuscularly. Side effects like nausea, headache, and arthralgia were usually reported. Local treatment using a forest vine ("tie-tie") juice applied to the lesions is also used by bush doctors. Presently, the pentavalent antimonial compound Glucantime (antimony-N-methyl-glutamine) is given intramuscularly, generally with mild side effects.

In the present study, it has been found that the Belizean \textit{L. braziliensis} strains barely grow at 28°C in modified Tobie's blood agar medium containing RPMI 1640 as overlay. Inactivation of the rabbit blood added to the solid phase of the medium did not improve the growth. In all cases the parasites grew slowly with low motility. However, fresh rabbit blood of up to 2 weeks old improved development of the parasites. Unlike the \textit{L. braziliensis} strains, all the \textit{L. mexicana} isolates grew easily in modified Tobie's blood agar medium. Also, after short adaptation, these strains as well as those of the \textit{L. braziliensis} BEL 101, BEL 102 and BEL 103 grew in RPMI 1640 liquid medium supplemented with 20% heat inactivated FCS. According to Evans et al. (1984),
reducing the incubating temperature to 23°C, improved the
*L. braziliensis* growth. These results were confirmed in our study.
Identification of the leishmanial strains is generally made by
either EF serotyping, isoenzyme analysis and DNA hybridization.
In this study, an attempt was made to use simple techniques and
simple facilities for parasite identification. Three techniques
were used to identify the Belizean isolates: microagglutination
test, EF-serotyping and isoenzyme analysis. The microagglutination
test that was recently found to be highly indicative in visceral
leishmaniasis, and in CL, and the EF serotyping proved to be
only partially effective in identifying the Belizean leishmanial
isolates. However, the isoenzyme analysis was shown to be a highly
reliable and reproducible method for distinguishing leishmanial
species. The Belizean *L. mexicana* strains were found to be very
susceptible to PR treatment, showing 85% inhibition of
promastigote growth within 4 days of exposure to PR at 100 ug/ml
Topical treatment of Belizean *L. mexicana* lesions in mice was also
highly effective. Although 10 days treatment was not sufficient to
eliminate all the parasites from the lesions, total elimination
was achieved at the end of 20 days treatment. The results obtained
with the Belizean strains were even better than those obtained
with the *L. major* strain. In our previous study, six days of
treatment were sufficient to cure the CL lesions due to *L. major*
in Balb/c mice, while in the present study, even 20 days treatment
did not totally eliminate *L. major* parasites. An intensive study
made recently *in vitro* in cultivated infected macrophages and
*in vivo* in infected Balb/c mice, revealed that the PR batch
received was not as effective as the original batch received
several years ago. We have recently learned that each new batch of PR should be examined against Leishmania both in vitro in tissue culture and in vivo in experimental animals prior to its application in a clinical study. PR is considered effective only if: a) PR at 10 ug/ml totally eliminates the intracellular amastigotes from infected macrophages within 4 days of exposure to the drug, and b) 15/12 P-ointment applied twice daily to the CL lesion caused by L. major in Balb/c mice, cured the lesion after 6 days treatment. Indeed, with a new batch of PR recently received, 6-10 days treatment with P-ointment was sufficient to eliminate all the L. mexicana and L. braziliensis parasites from the CL lesions.

Topical treatment of CL patients with P-ointment was found to be highly effective in 68% of the patients and effective in an additional 6%, after two to three weeks of drug application. Although the lesions caused by American leishmaniasis are generally big, ulcerated and exudating, only a few limited side effects like stinging and burning sensation were reported.

In one case nodular satellites appeared in the vicinity of the initial lesion during the treatment period, probably due to the dissemination of the disease, satellites that cleared together with the healing of the "mother" lesion. We would like to emphasize that nodular satellite appearance was also observed in untreated patients suffering from CL caused by L. major in Israel.

It is well accepted that topical treatment is not feasible in CL caused by L. braziliensis. However, in Belize, no dissemination to the mucocutaneous junctions has been reported, and it is for this reason that no Leishmania typing was performed. Nevertheless,
we do not recommend the use of topical treatment in areas with known mucocutaneous leishmaniasis, and serotyping is imperative in such areas prior to treatment.

Topical treatment with a cream or an ointment is a very simple procedure, easy to apply, not time consuming and doesn’t request hospitalization or thight medical supervision. Such a modality is of particular value especially in less developed countries where medical facilities and transportation are not always available.

The cure rate achieved in this study with the Belizean strains of CL indicates that the P-ointment should be taken into consideration for the treatment of American leishmaniasis.
REFERENCES


TABLE 1

Direct agglutination of Leishmania promastigotes by rabbit anti L. mexicana (BEL 69) antiserum

<table>
<thead>
<tr>
<th>Leishmania strain</th>
<th>10</th>
<th>20</th>
<th>40</th>
<th>80</th>
<th>160</th>
<th>320</th>
<th>640</th>
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<tr>
<td>L. mexicana BEL 44</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>L. mexicana BEL 46</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>L. mexicana BEL 58</td>
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<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L. mexicana BEL 64</td>
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<td>+</td>
<td>+</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>L. mexicana BEL 69</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>-</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L. major LRC-L137</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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Table 2

THE EFFECT OF A TOPICAL TREATMENT WITH PR OINTMENT ON L. MAJOR AND L. MEXICANA IN BALB/C MICE. THE MICE WERE TREATED TWICE DAILY FOR 20 DAYS.

<table>
<thead>
<tr>
<th>Site</th>
<th>No. of mice in group</th>
<th>Day started after innoc.</th>
<th>Days of treat.</th>
<th>Mean lesion size (mm²) ±SD pretreat.</th>
<th>30 days after end of treat.</th>
<th>Therapeutic response</th>
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<td></td>
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<td></td>
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<td>13/M 137</td>
<td>10</td>
<td>45</td>
<td>20</td>
<td>0.65±0.37</td>
<td>0.1±0.15</td>
<td>0/10</td>
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<td>13/M 44</td>
<td>5</td>
<td>80</td>
<td>20</td>
<td>0.08±0.01</td>
<td>0</td>
<td>0/5</td>
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<tr>
<td>13/M 46</td>
<td>5</td>
<td>70</td>
<td>20</td>
<td>0.38±0.25</td>
<td>0</td>
<td>0/5</td>
</tr>
<tr>
<td>13/M 58</td>
<td>5</td>
<td>70</td>
<td>20</td>
<td>0.64±0.3</td>
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<tr>
<td>13/M 64</td>
<td>5</td>
<td>85</td>
<td>20</td>
<td>0.63±0.4</td>
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<td>0/5</td>
</tr>
<tr>
<td>13/M 69</td>
<td>5</td>
<td>85</td>
<td>20</td>
<td>0.24±0.01</td>
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<td>0/5</td>
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Table 3. The effect of topical treatment with P-ointment on cutaneous leishmaniasis.

<table>
<thead>
<tr>
<th>Effect of treatment</th>
<th>Weeks to heal from end of treatment</th>
<th>No.</th>
<th>%</th>
<th>Average±SD</th>
<th>Range</th>
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<tr>
<td>Rapidly effective</td>
<td>4</td>
<td>36</td>
<td>67.92</td>
<td>26.12±8.08</td>
<td>10-45</td>
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<tr>
<td>Less effective</td>
<td>8</td>
<td>3</td>
<td>5.66</td>
<td>21.66±3.05</td>
<td>19-25</td>
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<tr>
<td>Ineffective</td>
<td>12</td>
<td>11</td>
<td>20.75</td>
<td>24.81±3.78</td>
<td>22-31</td>
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<tr>
<td>Ineffective</td>
<td>&gt;16</td>
<td>3</td>
<td>5.66</td>
<td>23.00±0.99</td>
<td>22-24</td>
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</table>

<table>
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<tr>
<th>No. of lesions</th>
<th>Description of lesions</th>
<th>Average±SD</th>
<th>Range</th>
<th>Duration in weeks</th>
<th>Average±SD</th>
<th>Range</th>
<th>Size (cm²)</th>
<th>Average±SD</th>
<th>Range</th>
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<tr>
<td>1.43±0.82</td>
<td>1-4</td>
<td>14.94±39.67</td>
<td>2-240</td>
<td>4.28±7.89</td>
<td>0.12-50.00</td>
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<tr>
<td>1.00±0.00</td>
<td>1</td>
<td>5.33±2.30</td>
<td>4-8</td>
<td>3.25±1.50</td>
<td>2.00-4.00</td>
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<td>2.45±2.29</td>
<td>1-8</td>
<td>20.38±41.28</td>
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<td>4.40±6.09</td>
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<td>2.00±1.00</td>
<td>1-3</td>
<td>6.66±2.30</td>
<td>4-8</td>
<td>1.42±1.62</td>
<td>0.30-2.00</td>
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</table>
EGENDS TO FIGURES

Figure 1.
The effect of paromomycin sulphate on the growth of various *L. mexica* solates, either untreated (*) or treated with the drug at 10 ug/ml ( ), or 1 ug/ml, in culture at 28°C.

Figure 2.
The effect of paromomycin sulphate on the growth of *L. mexicana* isolates culture at 28°C.

Figure 3.
The effect of topical treatment with PR ointment on *L. mexicana* BEL 64 in Balb mice.
The mice were inoculated in the base of the tail with 1x10 promastigote treatment was applied twice daily for 20 days, starting 85 days after parasit inoculation (A). The numbers indicate days after the end of treatment.

Figure 4.
Typical cutaneous leishmaniasis lesions in Belizean patients.

Figure 5.
A patient with cutaneous leishmaniasis before and after treatment with ointment. The lesion was treated twice daily for 20 days. A. before treatment B. and C. are 4 and 10 weeks after termination of treatment respectively.

Figure 6.
A map of Belize districts.
Figure 1.

The effect of paromomycin sulphate on the growth of various *L. mexicana* isolates, either untreated (*) or treated with the drug at 10μg/ml (o), or 100μg/ml (x) in culture at 28°C.
The effect of paromomycin sulphate on the growth of *L. mexicana* isolates in culture at 28°C.
Figure 3.

The effect of topical treatment with PR ointment on *L. mexicana* BEL 64 in Balb/C mice. The mice were inoculated in the base of the tail with \(1 \times 10^7\) promastigotes. Treatment was applied twice daily for 20 days, starting 85 days after parasites inoculation (A). The numbers indicate days after the end of treatment.
Figure 4.

Typical cutaneous leishmaniasis lesions in Belizean patients.
Figure 5.

A patient with cutaneous leishmaniasis before and after treatment with P ointment. The lesion was treated twice daily for 20 days. A. before treatment B. and C. are 4 and 10 weeks after termination of treatment respectively
Figure 6.

A map of Belize districts.
TOPOCAL TREATMENT OF CUTANEOUS LEISHMANIASIS IN BELIZE: IN VITRO AND IN VIVO STUDIES WITH LEISHMANIA MEXICANA

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*Department of Microbiology and Immunology, Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva 84105, Israel
‡Belize City Hospital, Belize, Belize
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Abstract—El-On, J., Cawich, F., Evans, D. A. and Weinrauch, L. 1993. Topical treatment of cutaneous leishmaniasis in Belize in vitro and in vivo studies with Leishmania mexicana. International Journal for Parasitology 23: 121-127. Strains of Leishmania mexicana isolated from Belizean patients were found to be highly susceptible to paromomycin sulphate (PR) treatment. This drug at 100 μg ml⁻¹ destroyed 85-99.5% of in vitro cultivated Leishmania promastigotes within 4 days of exposure to the drug. Leishmania promastigotes inoculated into the base of the tail of Balb/ c mice caused the development of local lesions several weeks after infection. These lesions were totally cleared of parasites after 20 days of topical treatment with PR ointment, comprised of 15% paromomycin sulphate and 12% methylbenzethonium chloride in soft paraffin. Similar results were also obtained with L. braziliensis infections. Isoenzyme analysis was found to be the method of choice for parasite strain identification. Excreted factor serotyping was only partially effective and promastigote agglutination gave negative results.

INDEX KEY WORDS: Leishmania mexicana; identification; paromomycin; topical treatment.

INTRODUCTION

Cutaneous leishmaniasis (CL) in Belize is caused by two major parasite species: Leishmania braziliensis infecting approximately 75% of patients and L. mexicana causing the remaining 25% of cases (Lainson & Strangways-Dixon, 1963; Evans, Lanham, Baldwin & Peters, 1984). Both species are associated with the development of large nodulo-ulcerative lesions that heal slowly leaving ugly scars.

No accurate estimation of the incidences of the disease in Belize is available at the present time. However, according to the local medical authorities, hundreds of cases are annually reported, mainly in the southern and western parts of the country. Many of these patients are soldiers of the Belizean Defence Forces.

Recently, a topical treatment of CL using an ointment comprised of 15% paromomycin sulphate (PR) and 12% methylbenzethonium chloride (MBCI) (PR ointment) was developed. This treatment was found to be highly effective against CL caused by various strains of Leishmania (see El-On, Jacobs & Weinrauch, 1987). The present study is aimed at determining the efficacy of this treatment against Belizean Leishmania strains, both in vitro in culture and in vivo in experimental animals, before its application to humans.

MATERIALS AND METHODS

Parasite strains and host animals. Eleven Belizean strains of Leishmania parasites including seven isolates of L. mexicana (BEL 44, BEL 46, BEL 47, BEL 58, BEL 64, BEL 66 and BEL 69) and four isolates of L. braziliensis (BEL 75, BEL 101, BEL 102 and BEL 103) have been used in this study. All strains were isolated by one of us (D.A.E.) from patients infected in Belize. They were kept as stablates in liquid nitrogen and subsequently maintained in vitro at 28°C by twice weekly passage in modified Tohe's blood agar medium. Infective parasites were maintained in vivo in Balb/c mice. Rabbits were used as a source of normal blood for preparing culture media and for raising specific antisera. Blood, whether normal or immune, was collected by cardiac puncture.

Parasite identification. Identification of the leishmanial strains was made using: (a) morphological description of the promastigotes which developed in culture at 28°C; (b) a parasite microagglutination test; (c) isoenzyme analysis and (d) excreted factor (EFS) serotyping.

Morphological characterization. Parasites were cultivated
TABLE 1—DIRECT AGGLUTINATION OF Leishmania promastigotes by rabbit anti-L. mexicana (BEL 69) antisemum

<table>
<thead>
<tr>
<th>Leishmania strain</th>
<th>Agglutination titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. mexicana BEL 44</td>
<td></td>
</tr>
<tr>
<td>L. mexicana BEL 46</td>
<td></td>
</tr>
<tr>
<td>L. mexicana BEL 58</td>
<td></td>
</tr>
<tr>
<td>L. mexicana BEL 64</td>
<td></td>
</tr>
<tr>
<td>L. mexicana BEL 69</td>
<td></td>
</tr>
<tr>
<td>L. mexican BEL 73</td>
<td></td>
</tr>
<tr>
<td>L. major LRC-L137</td>
<td></td>
</tr>
</tbody>
</table>

in modified Toeb's medium using RPMI 1640 medium (Beth Haemek, Israel) as overlay. On the fourth day of cultivation, when the parasites were at the logarithmic phase, the cells were examined under the phase microscope and their morphology described (Evans et al., 1984). Parasite microagglutination test A microagglutination test using tryptan-treated, formalin-fixed, Coomassie blue-stained Leishmania promastigotes was performed according to the method of Haith, Laerman, Mintier-Goodblood, Kaper & Kolk (1987). Agglutination results were recorded macroscopically and microscopically after 18 h incubation at room temperature.

Isoenzyme analysis. The isoenzyme profiles of the Belzien isolates were compared using the following enzymes: alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), glucose-6-phosphate dehydrogenase (G6PDH), glucose phosphate isomerase (GPI), malate dehydrogenase (MDH), mannose phosphate isomerase (MPI), 6-phosphogluconate dehydrogenase (6-PGDH), phosphoglucomutase (PGM) and superoxide dismutase (SOD) (Evans et al., 1984).

EFS preparation Promastigotes and their EFS were harvested from cultures grown in either blood agar medium or RPMI 1640 medium. For EFS preparation, the culture at the logarithmic phase was centrifuged for 10 min at 1300 g at room temperature. The supernatant fluid was harvested and centrifuged again at 12,000 g for 30 min at 4°C, filtered through a Milipore filter of 0.45 μm pore size, concentrated 10-fold by freeze drying and stored at -20°C until use.

Antisera production in rabbits. Antisera were raised in male rabbits of 2.5-3.5 kg against living promastigotes following the regimen of Adler, Foner & Montlio (1966). Six intravenous inoculations (1 × 10⁶, 2 × 10⁶, 8 × 10⁶, 16 × 10⁶, 32 × 10⁶) were given at weekly intervals, and blood samples were collected 10 days after the last injection. The ability of the antibody produced to precipitate leishmanial EFS and to agglutinate Leishmania promastigotes was tested.

Immunodiffusion and immunoneutralisation. Immunodiffusion and immunoelectrophoresis were performed in 1.5% agarose in 0.041 M-barbiturate buffer pH 8.2 containing 0.02% sodium azide. The preparations were developed for 1-4 days in moist diffusion chambers at 4°C, and photographed after rinsing with saline to remove excess protein, drying and staining with amino black.

The effect of paromomycin on leishmanial development in vitro. The effect of paromomycin sulphate (PR) (Farmitalia, Italy) on the growth of Leishmania promastigotes was tested at 28°C in RPMI 1640 medium supplemented with 20% FCS, 100 units penicillin and 100 μg streptomycin per ml. The promastigotes were first grown in modified blood agar medium, then transferred to RPMI medium containing various concentrations of PR on day 1 of the experiment. Parasite growth was assessed by daily haemocytometer counts and the growth rates, as compared with the normal untreated controls, calculated.

Effect of PR ointment on the leishmanial development in vivo. The methods used to infect mice and score PR ointment efficacy were as described previously (El-On, Jacobs, Witztum & Greenblatt, 1984). Male Balb/c mice were inoculated intradermally with the same material in the base of the tail with 0.5-1 x 10⁷ promastigotes. Treatment with PR ointment was started 45-80 days after infection. The ointment was applied to the lesions twice daily for a period of 10-20 days. Lesion development was inspected macroscopically and the presence of parasites in biopsy material was monitored microscopically, in both smear and cultures. Lesion size was measured in millimetres by two diameters (D) taken at right angles and determined according to the formula S mm² = D x D/2.

Protozoological examinations were performed prior to treatment, 10 days after the beginning of treatment, at the end of the treatment and at various times after termination of treatment.

RESULTS

Strain identification

The results obtained for parasite identification were as follows.

Morphological characterization. Promastigotes of L. braziliensis maintained at 28°C in mid-exponential growth phase were characteristically very small (7.5 μm) with very short flagella, the organisms usually growing in clumps with almost no movement. L. mexicana promastigotes grew as elongated parasites (20 μm) with long flagella. The parasites were highly motile and grew as either free promastigotes or in closely packed rosettes.

Microagglutination. The results obtained with the microagglutination test are summarized in Table 1. The highest agglutination titre (1:80) was obtained with L. mexicana BEL 69 against the homologous rabbit antisemum. Titres of 1:20-1:80 were observed with the other L. mexicana isolates. A higher titre (1:320) was obtained with rabbit anti-L. mexicana against the heterologous L. major strain (Table 1).

Extended factor (EFS) serotyping. Schurr, Zuckerman & Greenblatt (1972) showed that leishmanial EFS are precipitated by antibody raised in rabbits against living homologous promastigotes. In the present study, the antisem raised in rabbits against L. mexicana BEL 69 and against L. brasilienesis (BEL 101, BEL 103) did not distinguish between L. mexicana and...
Treatment of Belizian cutaneous leishmaniasis

Fig. 1. The effect of paromomycin sulphate on the growth of various \textit{L. mexicana} isolates, either untreated or treated with the drug at 10 or 100 \(\mu\)g ml\(^{-1}\) in culture at 28°C.

\textit{L. braziliensis} strains. All of the \textit{L. mexicana} and \textit{L. braziliensis} isolates examined were recognized by these antisera producing one–three clear precipitating arcs by immunodiffusion and immunoelectrophoresis. No cross-reactivity was obtained between \textit{L. mexicana} and \textit{L. major} when either rabbit anti-\textit{L. mexicana} or rabbit anti-\textit{L. major} antisera were used.

Effect of paromomycin on \textit{L. mexicana} promastigote development in vitro

The anti-leishmanial activity of PR against the
### Table 2: The effect of topical treatment with PR ointment on *L. major* and *L. mexicana* in BALB/c mice

<table>
<thead>
<tr>
<th>Parasite</th>
<th>No. of mice in group</th>
<th>Day started after inoculation</th>
<th>Mean lesion size (mm²) ± s.d.</th>
<th>Therapeutic response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pretreatment</td>
<td>30 days after end of treatment</td>
<td>Number of cured lesions</td>
</tr>
<tr>
<td><em>L. major</em> RC-L137</td>
<td>10</td>
<td>45</td>
<td>0.65±0.37</td>
<td>0.1±1.5</td>
</tr>
<tr>
<td><em>L. mexicana</em> BEL 44</td>
<td>5</td>
<td>80</td>
<td>0.08±0.01</td>
<td>0</td>
</tr>
<tr>
<td><em>L. mexicana</em> BEL 46</td>
<td>5</td>
<td>70</td>
<td>0.38±0.25</td>
<td>0</td>
</tr>
<tr>
<td><em>L. mexicana</em> BEL 58</td>
<td>5</td>
<td>70</td>
<td>0.64±0.30</td>
<td>0.2±3.0</td>
</tr>
<tr>
<td><em>L. mexicana</em> BEL 64</td>
<td>5</td>
<td>85</td>
<td>0.63±0.40</td>
<td>0</td>
</tr>
<tr>
<td><em>L. mexicana</em> BEL 69</td>
<td>5</td>
<td>85</td>
<td>0.24±0.01</td>
<td>0</td>
</tr>
</tbody>
</table>

The mice were treated twice daily for 20 days.
Treatment of Belizian cutaneous leishmaniasis

<table>
<thead>
<tr>
<th>DAYS</th>
<th>TREATED</th>
<th>UNTREATED</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>35</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>80</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
</tbody>
</table>

**Fig. 3.** The effect of topical treatment with PR ointment on *L. mexicana* BEL 64 in Balb/c mice. The mice were inoculated in the base of the tail with $1 \times 10^6$ promastigotes. Treatment was applied twice daily for 20 days starting 85 days after parasite inoculation (A). The numbers indicate days after the end of treatment.

Various *L. mexicana* isolates is shown in Figs. 1 and 2. PR at 10 µg ml$^{-1}$ inhibited the growth of the parasites by 47–80% and this drug at 100 µg ml$^{-1}$ inhibited the growth by 85–99.5% within 4 days of exposure to the drug. BEL 44 was slightly more resistant to PR than the other strains showing about 85% growth inhibition rate after 4 days of exposure to PR at 100 µg ml$^{-1}$.

_Effect of topical treatment on L. mexicana development in Balb/c mice_

Only six isolates of *L. mexicana* (BEL 44, BEL 46, BEL 58, BEL 64, BEL 69 and BEL 73) and three isolates of *L. braziliensis* (BEL 101, BEL 102 and BEL 103) were found to be infective to Balb/c mice. The remaining isolates *L. mexicana* BEL 47 and *L. braziliensis* BEL 75 did not infect the mice even when high doses of $1 \times 10^6$ promastigotes were inoculated into young, 4-week-old mice.

All of the infective *L. mexicana* isolates were found to be highly virulent to Balb/c mice. Four–five weeks after the inoculation of 0.5–1 $\times 10^6$ promastigotes into the base of the tail of Balb/c mice, a big nodule developed. One week later, the nodule transformed...
into an ulcer which increased in size up to 34.7 ± 8.8 mm² and 134.6 ± 28.7 mm² in diameter (average of 27 mice) in a period of 9 and 13 weeks after infection, respectively. Fifty percent of the mice died during the fifth month after infection.

Treatment with PR ointment twice daily for only 10 days was not sufficient to eliminate all of the parasites, although an improvement was noted. After 20 days of treatment, parasites were no longer detectable in the area of the lesions in all of the infected animals, and open lesions completely healed within a period of 30–40 days (Fig. 3, Table 2). PR ointment was only slightly less effective against L. major in Balb/c mice (Table 2). After 20 days treatment, parasites were still found to be present in the lesions. However, the parasites were undetectable 20 days after termination of treatment.

**DISCUSSION**

Identification of the leishmanial strains is generally carried out by either EF-serotyping, isoenzyme analysis or DNA hybridization (Chang & Bray, 1985). In this study, an attempt was made to use simple techniques and simple facilities for parasite identification. Three techniques were used to identify the Belizian isolates: the microagglutination test, EF-serotyping and isoenzyme analysis. The microagglutination test that was recently found to be highly indicative in visceral leishmaniasis (Harth, Kolk, Leeuwenburg, Mungai, Huguen, Jelsma & Kagar, 1988) and CL (Mengistu, Kießling & Akuffo, 1990), and the EF-serotyping proved to be only partially effective in identifying the Belizian leishmanial isolates. However, the isoenzyme analysis was shown to be a highly reliable and reproducible method for distinguishing leishmanial species.

The Belizian L. mexicana strains were found to be highly susceptible to PR treatment showing 85% inhibition of promastigote growth within 4 days of exposure to PR at 100 μg ml⁻¹. Topical treatment of Belizian L. mexicana lesions in mice was also highly effective. Although 10 days treatment was not sufficient to eliminate all of the parasites from the lesions, total elimination was achieved at the end of 20 days treatment. The results obtained with the Belizian strains were even better than those obtained with the L. major strain. In our previous study (El-On et al., 1984) 6 days of treatment were sufficient to cure the CL (L. major) lesions in Balb/c mice, while in the present study, even 20 days treatment did not totally eliminate L. major parasites. An intensive study made recently in vitro on cultivated infected macrophages and in vivo in infected Balb/c mice revealed that the PR batch received was not as effective as the original batch received several years ago. We have recently learned that each new batch of PR should be examined against Leishmania both in vitro in tissue culture and in vivo in experimental animals prior to its application to clinical study. PR is considered effective only if (a) PR at 10 μg ml⁻¹ totally eliminates the intracellular amastigotes from infected macrophages within 4 days of exposure to the drug, and (b) >15-20% PR ointment applied twice daily to the CL lesion caused by L. major in Balb/c mice cured the lesion after 6 days treatment. Indeed, with a new batch of PR recently received, 6–10 days treatment with PR ointment was sufficient to eliminate all of the L. mexicana and L. braziliensis parasites from the CL lesions.

Based on the results obtained in this study, a clinical trial with PR ointment has recently been started in Belize. Preliminary results indicate a high efficacy of PR ointment against both L. mexicana and L. braziliensis. Final results of the clinical study will be published elsewhere.

**ACKNOWLEDGEMENTS**

This study was supported by the AID/CDR Grand No. DPE-5544-G-SS-047-00. Paromomycin sulphate was kindly supplied by Parke Davis Warner Lambert, Italy and Farmitalia, Carlo Erba, Italy.

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Mengistu G., Kießling R. & Akuffo H. 1990. The value of a direct agglutination test in the diagnosis of cutaneous and
Treatment of Belizian cutaneous leishmaniasis


Topical treatment of New World cutaneous leishmaniasis in Belize: A clinical study

Louis Weinrauch, MD, a, *Filiberto Cawich, MD, b Peter Craig, MD, c St. John Xavier Sosa, MD, d and Joseph El-On, PhD e Jerusalem and Beer-Sheva, Israel, and Belize City, Belize

Background: Many studies have been performed during the past decade to find an effective topical therapy for cutaneous leishmaniasis (CL). Such a treatment has been tested in vitro and in vivo in experimental animals and in patients with Old World CL. 1-10

This article summarizes the results achieved in Belize in the treatment of New World CL caused by Leishmania braziliensis or Leishmania mexicana with an ointment containing 15% paromomycin sulfate (PR) and 12% methylbenzethonium chloride (MBCl) in white soft paraffin (P-ointment). The results confirm the cure rate obtained in Israel with the same preparation in Old World CL caused by Leishmania major, Leishmania tropica, or Leishmania aethiopica. 3, 4, 7

During the past decade many studies have been performed to find an efficient and simple topical treatment for cutaneous leishmaniasis (CL). Such a treatment has been tested in vitro and in vivo in experimental animals and in patients with Old World CL.1-10

This article summarizes the results achieved in Belize in the treatment of New World CL caused by Leishmania braziliensis or Leishmania mexicana with an ointment containing 15% paromomycin sulfate (PR) and 12% methylbenzethonium chloride (MBCl) in white soft paraffin (P-ointment). The results confirm the cure rate obtained in Israel with the same preparation in Old World CL caused by Leishmania major, Leishmania tropica, or Leishmania aethiopica.3, 4, 7

MATERIAL AND METHODS

After informed consent had been obtained, 53 patients with confirmed CL were included in this study. All were treated for 2 to 3 weeks, twice daily with P-ointment. Patients self-applied the ointment; treated lesions were left uncovered in most cases. Ten to 15 gm of P-ointment were required on average.

The results were evaluated as described by El-On et al. 4: (1) rapidly effective: no parasites detected in the treated lesion at the end of treatment, followed by total healing within 1 month after cessation of treatment; (2) less rapidly effective: the same occurring within 8 weeks; (3) ineffective: parasites still present in the treated lesion and/or no healing after 8 weeks.

RESULTS

The patients' ages ranged from 10 to 45 years (mean 26 years); the number of lesions ranged from one to eight (mean 1.55). The majority of the patients had large noduloulcerative lesions (Fig. 1). The duration of the lesions varied from 2 to 260 weeks (mean 14.75 weeks).

Four weeks after termination of treatment, rapid clearing was obtained in 36 patients (68%) (Fig. 2). Less rapid clearing was observed after 8 weeks in three patients (6%) and no response occurred in 14 (26%) after 12 weeks or more. Age or sex of the patient, size and number of the lesions, the duration of the infection as well as the presence of crusts and superimposed bacterial infection did not influence the response to treatment. Generally, the clinical
appearance of the lesion during and at the end of
treatment was worse than at the start. This has been
observed in previous studies in both responders and
nonresponders and is caused by the development of
contact dermatitis. Various degrees of inflamma-
tion, depending on the lesion size and site and the
host response, were associated with this treatment.
A burning sensation at the site of treatment some-
times occurred during the first applications.
However, none of the patients was dropped from the
study because of side effects. A combination of hy-
popigmentation and hyperpigmentation was
observed in most healed lesions.

DISCUSSION

Leishmania species were not identified in the
study population because the technology was not
readily available. Published reports from this region
indicate that L. braziliensis is the most frequently
identified organism, with L. mexicana documented
in approximately 25% of cases.10-13

Until recently, the drug of choice for the treat-
ment of CL in Belize was intramuscular stibophen
(pentasodium antimony-biscatechol-3:5-disulpho-
nate heptahydrate).13 Side effects such as nausea,
headache, and arthralgia usually occur. Pain at the
injection site is another drawback. Death from dys-
rhythmia has been reported after intravenous large
doses. Local treatment with a forest vine ("tie-tie")
juice is also used by bush doctors.11 Presently, the
pentavalent antimonial compound, meglumine an-
timonate (Glucantime), is given intramuscularly,
generally with mild side effects.

In our study, topical treatment with P-ointment
was found to be highly effective in 68% of the
patients and effective in an additional 6%, after 2 to
3 weeks of drug application.

In one case nodular satellites appeared in the vi-
cinity of the initial lesion during treatment. Prob-
ably this was caused by dissemination of the disease.
However, the satellites cleared with healing of the
"mother" lesion.

The cure rate achieved in this study is as good as
any current parenteral therapy. In a recent study
performed in Guatemala,14 cure rates of 57% and
89% were obtained in L. mexicana-infected patients
treated with sodium stibogluconate (Pentostam) or
detoconazole, respectively. Cure rates of 96% and
30%, respectively, were observed in patients infected
with L. braziliensis and similarly treated. These re-
sults are of particular value because both countries,
Guatemala and Belize, are supposed to have identi-
cal Leishmania parasites.

It is well accepted that topical treatment is not
feasible for CL caused by L. braziliensis. However,
in Belize, no dissemination to mucocutaneous junc-
tions has been reported, and, for this reason, no
Leishmania typing was performed. Nevertheless, we
do not recommend the use of topical treatment in areas with known mucocutaneous leishmaniasis; serotyping is necessary in these areas before treatment.

Paromomycin sulfate was supplied by Farmitalia Carlo Erba, Italy, and Warner-Lambert, Italy.

REFERENCES

NEW SECTION IN THE JOURNAL

Readers will notice a new heading in the Table of Contents of the October 1993 issue of the Journal. Entitled “Pearls of Wisdom,” this section will consist of four features that include articles of interest to the dermatologist.

“Pearls” are not universally accepted diagnostic or patient management techniques that can be found on quick perusal of standard textbooks of dermatology. On the contrary, “pearls” are special, personalized hints that experienced clinicians have found useful in enhancing the well-being of their patients.

The first feature, “Clinical Pearl,” was originally conceived by Dr. Boni Elkefsi as a mycology page, but was broadened by the Assistant Editors to include all categories of clinical dermatology. “Clinical Pearl” will feature those special approaches that practitioners have found especially useful in diagnosis and treatment of skin diseases.

The second feature, “Surgical Pearl,” will be directed by Dr. Stuart Salasche, who currently chairs a symposium on this topic at the annual meeting of the American Academy of Dermatology. As its title implies, “Surgical Pearl” will focus on the surgical management of skin disease.

The third feature, “Abstracts from the Literature,” will help keep readers up to date on articles of interest from journals not readily accessible to the practitioner. These abstracts, which will be prepared by the Assistant Editors, will come from articles that have appeared in nondermatologic journals in the United States and both dermatologic and nondermatologic journals abroad.

Finally, the fourth feature, written by Dr. Jeffrey Bernhard, is entitled “Lotaderma.” Each month, this section will ask (and we hope answer) a difficult question designed to provoke thought or to challenge the reader’s knowledge of dermatologic areas. Curious? Confused? Wait until next month!

The Journal has introduced these features to fulfill its mission to meet the needs of the practicing dermatologist. We would like to make “Pearls of Wisdom” a monthly section and welcome and solicit your input to the “Clinical Pearl” and “Surgical Pearl” features. Please consult the “Information for Authors” pages in the October 1993 issue of the Journal for additional information.—Bruce H. Thiers, MD, Associate Editor