Development of Topical Treatment for Cutaneous Leishmaniasis Caused by *Leishmania major* in Experimental Animals

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Topical treatment, with drug-containing ointments, of cutaneous leishmaniasis caused by *Leishmania major* in BALB/c mice was studied. Twenty chemotherapeutic agents having potential or established antileishmanial activity were formulated in different ointment and cream bases. Only 15% paromomycin sulfate with 12% methylbenzethonium chloride, 12% benzethonium chloride, 12% cetalkonium chloride, or 12% dimethyl sulfoxide, all incorporated in white soft paraffin (United Kingdom patent application no. 2117237A), were completely effective. Topical treatment twice daily for 6 or more days caused total elimination of the parasites and healing of the lesion in all treated mice. All the other antileishmanial compounds, including sodium stibogluconate, pentamidine, amphotericin B, emetine hydrochloride, metronidazole, co-trimoxazole, allopurinol, and rifampin, either showed a slight effect on the parasites or were highly toxic to the animal host at the concentrations tested.

Cutaneous leishmaniasis (CL), an endemic disease in many parts of the world, continues to present serious therapeutic problems. The disease, although usually self-limiting, can cause considerable morbidity and may result in severe disfigurement.

No single chemotherapeutic regimen has proved satisfactory for the treatment of CL (12). Various efforts, including local irradiation, cauternization, cryotherapy, or local infiltration with antileishmanial drugs, such as pentavalent antimonasc, amphotericin B (9), or berberine sulfate (4), are being made to enhance healing of the ulcer. In the case of long-lasting or disfiguring lesions, systemic drug treatment with sodium stibogluconate (19, 20), pentamidine (22), or amphotericin B (6) is advisable despite side effects.

In a previous paper (8) the effect of various chemotherapeutic agents on the development of *Leishmania major* amastigotes in cultivated macrophages in vitro was described. In the present work, compounds which were shown to be effective in vitro (8) and several related compounds were used for topical treatment of CL in vivo in an experimental mouse model.

**MATERIALS AND METHODS**

**Animals.** BALB/c mice, 8 to 12 weeks old, were used for all in vivo studies of *L. major*. 

**Leishmania strain and its maintenance.** *L. major* LRC-L137, the same strain used in an in vitro study (8), was used in the present work. This strain was isolated from an Israeli case of simple CL in 1967. The parasites are kept either as stablates in liquid nitrogen or in BALB/c mice and are used for each experiment after their cultivation on blood agar at 28°C. For culture preparation from infected mice, the leishmanial lesion was cleaned with 70% ethanol, and material was aspirated from the edge of the lesion with a sterile Pasteur pipette and inoculated into a culture tube. The culture was incubated at 25 to 28°C and used within 4 weeks of cultivation. The possibility exists for development of noninfective parasites in cultures incubated for longer periods.

**Effect of drugs on *L. major* development.** The effect of the drug on in vivo development of *L. major* was monitored in both BALB/c and C3H/He mice by following the development of a local dermal lesion caused by the parasite. The mice were inoculated in the base of the tail with $1 \times 10^5$ to $5 \times 10^5$ infective promastigotes. The development of the lesion was followed macroscopically, and the presence of parasites in biopsy material was monitored microscopically in both smears and cultures. Material aspirated with a fine glass pipette through a small incision made at the margin of the lesion with a sterile surgical blade (B.S. 2982; Swann-Morton, Sheffield, United Kingdom) was stained with Giemsa and cultured as previously described. Cultures were considered negative only after 20 days without growth. These techniques were recently recommended by the World Health Organization for detecting CL in both experimental animals and humans (23). Drug efficacy was monitored in both smears and cultures, and the results were expressed as number of mice cleared per number of living mice.

Lesion size was measured in two dimensions (*D* and *d*) at right angles to each other with a caliper gauge, and the lesion size (*S*) was determined by the following formulation: $S = (D \times d)/2$. Each group of mice was tested with a single preparation.

**Topical preparations.** Chemotherapeutic agents with potential or established antileishmanial activity have been incorporated in different ointment and cream formulations and tested for their efficacy when applied topically. The drugs tested were pentamidine isethionate, metronidazole (Flagyl; May and Baker), sodium stibogluconate (Pentostam; Burroughs Wellcome Co.), stibophen (Sandos), emetine hydrochloride, berberine sulfate, allopurinol (Sigma Chemical Co.), co-trimoxazole (Resprim; Ikapharm), amphotericin B (Fungizone; E. R. Squibb & Sons), rifampin (Lepefit), and the aminoglycosides paromomycin (PR) (Humatin; Parke, Davis & Co.), netilmicyn, amikacin, tobramycin, sisomicyn, neomycin, gentamicin, and kanamycin, all as sulfate (Teva Pharmaceutical Industries). In addition, the following were used as pharmaceutical adjuncts for achieving suitable topical formulations: the quaternary ammonium compounds, methylbenzethonium chloride (MBCI), ben-
was
All the water. Treatment was with held for up to 5 months to evaluate the efficacy of the treatment in advanced infection. After the development of the successful PR formulation, subsequent ointments were prepared, analyzed, and supplied by Teva Pharmaceutical Industries, Jerusalem, Israel.

RESULTS

Development of cutaneous leishmaniasis in BALB/c mice. A nodule develops 2 to 3 weeks after the inoculation of $1 \times 10^6$ to $5 \times 10^6$ promastigotes of L. major (LRC-L137) into the base of the tail of a BALB/c mouse. Two weeks later the nodule transforms into an ulcer which increases in size, leading to the death of the animal in a period of 4 to 6 months (Fig. 1). The spleen, liver, bone marrow, and lymph nodes become heavily parasitized as the disease progresses. No spontaneous healing or recovery from infection could be observed with these mice. Increasing the inoculum size from $1 \times 10^6$ to $5 \times 10^6$ affected neither the time required for lesion development nor the variability of the lesion size obtained in each group of mice.

Antileishmanial compounds. In the initial testing for antileishmanial activity of ca. 40 combinations of drugs and ointment or cream bases, none of the preparations proved effective. It was then that an ointment comprising the aminoglycoside PR in white soft paraffin containing the quaternary ammonium compound MBCI was examined. After 10 days of treatment, twice daily, with this combination, the parasites in all the infected animals disappeared from the lesion area, and the open lesion completely healed in a period of 30 days (Fig. 1 and Table 1). In all cases, the topical treatment did not show any effect on the parasites in the internal organs (i.e., spleen, liver, or bone marrow). Generally, the parasites reappeared in the skin at the site of infection and in the surrounding skin area as well as in the nose and footpads 90 to 150 days after the end of treatment.

It was a result of a successful combination of an aminoglycoside with a surface active quaternary ammonium compound that prompted the following series of experiments. In one series of experiments PR was replaced by other aminoglycosides; in a second series, MBCI was replaced by other quaternary ammonium compounds; and in a third series of experiments, the known antileishmanial drugs, which were earlier unsuccessfully tested in the different ointment bases, were tested in the MBCI base (Table 1).

Replacement of MBCI in a PR-containing ointment by other quaternary ammonium compounds, as well as DMSO, did not improve the efficacy of the ointment (Table 1). They all showed a therapeutic effect for at least 80 days after the termination of treatment with the one exception of PR-12% benzalkonium chloride. In this case the lesion healed, but...
some animals died as a result of the toxicity of the benzalkonium chloride. In this group necrosis of the tail was observed at the end of treatment.

Ointments comprising other aminoglycosides, namely, neomycin, kanamycin, gentamicin, and sisomycin, with MBCI were not found to be as effective as the PR preparation. Gentamicin and kanamycin were the only aminoglycosides causing total elimination of the parasites from the lesion at the end of treatment. However, the disease relapsed in the treated mice 10 to 30 days after the end of treatment (Table 1). Furthermore, loss of weight as well as metastasis formation in the tail and legs were apparent in the mice treated with gentamicin combined with MBCI. These phenomena were also observed with amikacin, tobramycin, netilmicin, and sisomycin 27 to 35 days after the termination of treatment.

The other antileishmanial compounds dispersed in an ointment containing 12% MBCI were only partially or not effective against the disease when applied topically (Table 1). In these mice no healing of the lesion was achieved. In a few cases, parasites were not detected in the lesion at the end of treatment but reappeared shortly afterwards. The usual drugs of choice against the disease, sodium stiboglucone, pentamidine, and amphotericin B combined with 12% MBCI or 12% DMSO, were only slightly effective. Moreover, the trivalent antimonial stibophen and pentamidine isethionate were also toxic to the host animals. When a pentamidine-containing ointment was used, of six mice treated, one died on day 4 and one died on day 8 of treatment and two died on day 2 and one died on day 29 after the end of treatment (total, 83%). When stibophen was used, one of six mice treated died on day 8 of treatment and three mice died during the first 29 days after termination of treatment. Allopurinol ointment (15%) containing 12% MBCI killed all the treated mice in a period of 19 days after the end of treatment. Emetine hydrochloride at 0.5% was not effective and at a concentration higher than 0.5% was found to be lethal to the mice.

A representative successful treatment with an ointment comprising 15% PR and 12% MBCI in white soft paraffin is depicted in Fig. 2.

**Duration of treatment.** The duration of treatment appears to be an important factor in the effectiveness of ointment (Table 2). Treatment for only 2 or 4 days was not sufficient to eliminate all the parasites, although an improvement was noted. However, treatment for 6, 8, or 10 days totally eliminated the parasites, followed by complete healing of the lesion.

**Effect of concentrations.** The effect of different concentrations of PR with various percentages of MBCI on the development of *L. major* in BALB/c mice is given in Table 3. No significant differences between ointments containing 12% MBCI with 5, 10, or 15% PR were observed. Lower concentrations of PR were less effective.

### Table 1. Effect of topical treatment on *L. major* in BALB/c mice

<table>
<thead>
<tr>
<th>Therapeutic agent (% in ointment)</th>
<th>Treatment regimen</th>
<th>No. of mice in group</th>
<th>Mean (±SD) lesion size (mm²)</th>
<th>No. of cured lesions/no. of survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day after inoculation</td>
<td>Pretreatment</td>
<td>30 days after end of treatment</td>
<td>Pretreatment</td>
</tr>
<tr>
<td><strong>PR</strong> (15)</td>
<td>MBCI</td>
<td>PR (15)</td>
<td>60</td>
<td>5</td>
</tr>
<tr>
<td>Amphotericin B (15)</td>
<td>MBCI</td>
<td>35</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>Rifampin (15)</td>
<td>MBCI</td>
<td>35</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>Pentamidine isethionate (15)</td>
<td>MBCI</td>
<td>35</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>Berberineb (15)</td>
<td>MBCI</td>
<td>35</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Sodium stiboleucanate (1.7)</td>
<td>MBCI</td>
<td>35</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Stibophen (15)</td>
<td>MBCI</td>
<td>35</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Metronidazole (15)</td>
<td>MBCI</td>
<td>35</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Co-trimoxazole (15)</td>
<td>MBCI</td>
<td>35</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Allopurinol (15)</td>
<td>MBCI</td>
<td>35</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Emetine hydrochloride (0.5)</td>
<td>MBCI</td>
<td>35</td>
<td>12</td>
<td>9</td>
</tr>
</tbody>
</table>

a The mice were treated topically twice daily, starting 30 to 60 days after injection with 2 × 10⁷ to 5 × 10⁷ promastigotes.

b As chloride.

c As sulfate.

d Four mice died: one on day 4, one on day 8, and two on day 2 after the end of treatment.

† One mouse died on day 29 after the end of treatment.

‡ One mouse died on day 8 of treatment.

§ Five mice died.
Using different concentrations of MBC1 with 10% PR indicated that 1% MBC1 was not sufficient to eliminate the parasites from all the treated mice during the 76 days of experiment. However, with higher concentrations of MBC1 (5%), no parasites were detected 55 days after the end of treatment, and with 12%, no parasites were observed 75 days after termination of treatment. Ointments containing either PR alone or MBC1 alone had only a partial effect on the parasites (Table 3).

Topical treatment of an advanced stage of the disease. Male BALB/c mice, 8 to 12 weeks old, were inoculated in the base of the tail with $5 \times 10^6 L. major$ promastigotes. After 145 days when very large lesions (average lesion size, 223 $\pm$ 61 mm$^2$) had developed, the mice were treated with 15% PR-ointment containing 2% MBC1, twice daily for a period of 10 days.

The results obtained showed that all the five treated mice recovered locally from infection, the lesion healed, and no parasite was detected in the treated lesion for a period of 57 days.

In an additional experiment, 10- to 12-week-old BALB/c mice were infected with $5 \times 10^6 L. major$ promastigotes. After 105 days when large lesions (average lesion size, 195.7 $\pm$ 50 mm$^2$) and metastases had developed and several mice died as a result of infection, the remaining surviving mice were treated twice daily with 15% PR-ointment containing 1% MBC1 for a period of 10 days. The results obtained showed that of eight mice treated, two died on day 5 of treatment and one died 24 days after termination of treatment. Four of five remaining mice were free of parasites on day 66 after the end of the treatment.

Effect of treatment on adjacent lesions. Male BALB/c mice were inoculated in the base of the tail with $5 \times 10^6 L. major$ promastigotes and in the footpad with $2 \times 10^6$ promastigotes. Twenty-five days after infection, a lesion developed in the base of the tail and a nodule developed in the footpad, both containing parasites. Ninety days after infection, the base of the tail was treated with 15% PR-ointment containing 2% MBC1 twice daily for a period of 10 days, whereas the leg was left untreated.

The results obtained indicated that treatment of the lesion in the base of the tail was followed by the elimination of the parasites only from the treated lesion, whereas the leg remained heavily infected. The parasites in these mice reappeared in the base of the tail at 40 days, and in an additional experiment at 60 days, after the end of treatment.

Follow-up studies in infected C3H/He mice. C3H/He mice are not as susceptible to infection with $L. major$ as are BALB/c mice, and spontaneous recovery occurs 5 to 9 weeks after inoculation of parasites (10). In the present experiment, male, 6-week-old C3H mice were inoculated in the base of the tail with $3.5 \times 10^6 L. major$ promastigotes. At 10 days (group 1) and 30 days (group 2) after infection, the mice were treated with 15% PR-ointment containing 1% MBC1 twice daily for a period of 10 days. The results showed that in both groups of mice nodules or very small lesions developed. Protozoological examination of these mice indicated the presence of viable parasites in 26 of 29 (89.6%) infected mice. After 10 days of treatment, 80% (group 1) and 78.9% (group 2) of mice treated were free of parasites. Total elimination of the parasites from all treated mice was achieved 40 days (group 1) and 20 days (group 2) after the end of treatment. No relapse of the disease in these mice occurred during the 150-day duration of these experiments.

Quantitative determination of ointment applied. To determine the amount of the ointment applied to the lesion, the ointment weight before and after application was determined in 36 BALB/c mice infected with $L. major$ (average lesion size, 25.7 $\pm$ 19.2 mm$^2$). It was found that a 557-mg amount of ointment was used per mouse for 6 days (i.e., 93 mg of ointment per day).

Parenteral treatment with PR. BALB/c mice were inoculated in the base of the tail with $10^6 L. major$ promastigotes. Starting on the day of infection they were injected intraperi-
TABLE 2. Effect of duration of treatment with an ointment containing 15% PR and 12% MBCI on the development of *L. major* in BALB/c mice

<table>
<thead>
<tr>
<th>Duration of treatment (days)</th>
<th>No. of mice in group</th>
<th>Mean (±SD) lesion size (mm²)</th>
<th>Therapeutic response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pretreatment</td>
<td>30 days after end of treatment</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pretreatment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pretreatment</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>22.58 ± 4.9</td>
<td>Nodule</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>35.92 ± 23</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>25.68 ± 19.9</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>20.4 ± 10.1</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>33.7 ± 17.4</td>
<td>0</td>
</tr>
</tbody>
</table>

* Treatment was given twice daily for a period of 10 days, starting 90 days after infection with 4 × 10⁶ promastigotes.

toneally (i.p.) with 1 mg of PR, once daily for a period of 10 days. No parasites were detected in the site of infection 24 days after termination of treatment. However, 6 of 10 mice relapsed on day 35, and the remaining 4 mice relapsed on day 50 after termination of treatment. Administration of the drug i.p. or intraleisonally by the same regimen as above but 25 days after infection indicated the superiority of intraleisional over i.p. treatment. During the 33-day interval after intraleisonal treatment, no parasites were detected in the five mice, whereas with i.p. treatment, parasites were detected in the lesions of three of the five mice. None of these regimens effected cure, for parasites were still present in the spleen at the end of treatment.

**DISCUSSION**

*L. major*, the etiological agent of CL in humans, is a parasite of the skin (24). This species is not found in the peripheral blood, and it rarely metastasizes to other sites or invades the reticuloendothelial cells of the viscera. The introduction of the parasite into the skin is followed by the development of a local lesion with a depressed ulcerated center, which persists for a period of 6 to 18 months. The disease is generally terminated after such a period, leaving a scar in the lesion area. Chemotherapy depends, for the most part, upon a small group of compounds (6, 19, 20, 22), with each having its own efficacy and attendant toxicity. Very few studies have dealt with problems addressed to topical treatment in any form of leishmaniasis, although this procedure is commonly used in the treatment of topical bacterial, viral, and fungal infections. The present study has been an attempt to develop topical treatment for CL.

Although *L. major* produces visceral infection in addition to the local lesion in the base of the tail of the BALB/c mice (7, 14), it seems a suitable model for the present study. The fact that a spontaneous healing cannot be achieved in these mice indicates that the local clearance of the parasites obtained is due to topical treatment only.

To develop a suitable semisolid antileishmanial preparation, it was decided to test different ointment and cream bases in combination with the various potential or established antileishmanial drugs. White soft paraffin (petrolatum) was selected as a typical oleaginous ointment base in view of its widespread use for many pharmaceutical ointments (see reference 23). The incorporation of surface-active agents, with the ability of forming oil-in-water or water-in-oil emulsions with water, has often been used for increasing drug penetration (11). Hence, an ointment base comprising white soft paraffin and the water-in-oil emulsifying agent sorbitan sesquioleate (Arlacel) (an absorption ointment base) and several ointment bases with white soft paraffin and different oil-in-water emulsifying waxes (emulsifying ointment bases) were prepared. These emulsifying waxes, often referred to as complex emulsifying agents, are generally compounded from cetostearyl alcohol and a second emulsifier such as cetrimide, ceteomacrogol, or sodium lauryl sulfate (2). Because of the ionic nature of cetrimide (cationic) and sodium

**TABLE 3. Effect of different combinations of PR with MBCI in white soft paraffin on *L. major* development in BALB/c mice**

<table>
<thead>
<tr>
<th>Ointment composition (%)</th>
<th>Treatment regimen</th>
<th>No. of mice in group</th>
<th>Mean (±SD) lesion size (mm²)</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>No. of cured lesions/no. of survivors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pretreatment</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>PR  MBCI</td>
<td>Day started after inoculation</td>
<td>Days of treatment</td>
<td></td>
<td>Days after end of treatment</td>
</tr>
<tr>
<td>0</td>
<td>12</td>
<td>70 10 8</td>
<td>32.7 ± 18.6 8.4 ± 2.5</td>
<td>0/8</td>
</tr>
<tr>
<td>1</td>
<td>12</td>
<td>70 10 5</td>
<td>27.6 ± 17.9 3.6 ± 0.5</td>
<td>0/5</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>70 10 5</td>
<td>23.2 ± 11.5 0</td>
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<tr>
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<td>12</td>
<td>70 10 5</td>
<td>15.68 ± 6.1 0</td>
<td>0/5</td>
</tr>
<tr>
<td>15</td>
<td>12</td>
<td>70 10 5</td>
<td>11.52 ± 6.2 0</td>
<td>0/5</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>70 10 10</td>
<td>21.8 ± 13.0 3.5 ± 0.8</td>
<td>0/10</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>70 10 5</td>
<td>27.4 ± 12.1 0</td>
<td>0/5</td>
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<tr>
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<td>17.5 ± 8.2 0</td>
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<tr>
<td>10</td>
<td>12</td>
<td>70 10 5</td>
<td>29.7 ± 16.4 0</td>
<td>0/5</td>
</tr>
</tbody>
</table>

* Mice were infected with 5 × 10⁶ promastigotes.
lauryl sulfate (anionic), incompatibilities with specific drugs limited their use. The incorporation of a single surfactant into white soft paraffin was also tested as a means of increasing drug efficacy. The specific choice of cetrimide and methylbenzenethionium chloride was prompted by their known antimicrobial properties (23), hence their dual role as pharmaceutical adjuncts and potential anti­leishmanial agents.

The addition of water to either the absorption or emulsifying ointment base forms a semisolid emulsion or cream. Very often the presence of water can enhance drug abs­orption (18).

Another ointment base that has gained popularity for many commercial preparations is polyethylene glycol ointment base, hence its use in the present investigation.

The incorporation of the aprotic agent DMSO into phar­maceutical ointments for increasing drug penetration has been reported (reviewed in reference 11). It was used in the present study in a number of bases.

It has been found that of the 10 antileishmanial compounds used for topical treatment, only 1, PR, with various quater­nary ammonium salts (as well as DMSO), was highly effective in both healing the lesion and eliminating the parasites. With these ointments, total elimination of the parasites was achieved after ca. 10 days of treatment of both early and advanced infections. Furthermore, this duration of treat­ment was sufficient to clear the lesion of both the leishmanial parasites and the secondary infection, mainly bacterial, accompanying this disease; hence, the healing was achieved only 1 month after termination of treatment. In addition, the presence of parasites in the first 2 weeks of infection and their reappearance several months after termination of treatment, as shown by protozoological examination several days before lesion development, indicate the superiority of proto­zoological examinations over measurement of the lesion size as the criterion for the determination of efficacy of the treatment.

In the treated BALB/c mice reappearance of the parasites at the site of infection as well as in other sites on the skin was noted a considerable time after the end of treatment. In addition, treatment of a leishmanial lesion caused the elimi­nation of the parasites only from the treated lesion. Almost no effect was observed on other untreated lesions of the same animal. These findings suggest a limited penetration of the drug and may support the idea that the relapse of the disease at the site of inoculation is caused by unaffected parasites migrating from the internal organs. This hypothesis is supported by further study with C3H/He mice. In these mice, in which no "post kala-azar dermal leishmanial" phenomenon is known to develop (10), no relapse of the disease was observed after successful topical treatment. However, this model was not considered suitable for this study in view of the very short duration of the infection and hence the existence of difficulties in differentiating treated infection from spontaneous healing.

Comparison of all our data indicates that the effect ob­tained in vivo reflects that obtained in vitro (8). Among 11 drugs examined in vitro against L. major in C3H mice macrophages, only 2, berberine (10 μg/mL, \(1.3 \times 10^{-5} \) M) and PR (10 μg/mL, \(1.3 \times 10^{-5} \) M), caused total elimination of the parasites in a period of 3 to 4 days after drug administration. However, the therapeutic index of PR is 20 times higher than that of berberine. In the present work it was found that the most effective drug in vitro gave the highest cure rate, whereas all the others were only partially effective.

PR is an antibiotic substance or substances produced by the growth of Streptomyces rimosus subsp. paromomycin. Similar to other aminoglycosides, PR has a short plasma half-life. In normal subjects the plasma half-life is 2.47 h after a single 500-mg intramuscular injection of the drug (16). PR given parenterally was found to be highly effective against L. major in experimental animals (15) and humans (1, 21). However, PR has been shown to give rise to nephrotoxic and oto­toxic effects when given in high dosage by the parenteral route (5, 17). The use of PR for topical treatment may reduce toxicity and allow its use at higher concentrations. In the present work, the results obtained with the ointment were better than those obtained by injection (i.p. or intraleishmanially). Intraleishional injection of PR (1 mg/day) for a period of 10 days killed all the parasites locally, whereas the same dose given i.p. for the same period of time killed the parasites in only two of five mice treated. Neither treatment was suffi­cient to kill the parasites in the internal organs. In contrast, the utilization of an ointment containing 1% PR (93 mg of the ointment was applied daily; i.e., 0.93 mg of PR) caused the elimination of the parasites from the lesion in all the treated mice. In 80% of these animals, the parasites reappeared 36 days after the end of treatment, whereas in the remaining 20%, they reappeared after 55 days (Table 3). It can be concluded that the utilization of the drug in the form of ointment is as effective as the drug injected intraleishmanially and superior to the same dose of drug given i.p.

The results obtained in this study demonstrate that PR in an ointment base containing specific quaternary ammonium compounds or DMSO is capable of killing the parasites when applied directly to the lesion infected with L. major. The favorable results have prompted both an investigation into the efficacy of this preparation against other Leishmania strains and extensive clinical trials.

ACKNOWLEDGMENTS

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LITERATURE CITED


