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Hexadecylphosphocholine: Skin Treatment of *Cutaneous leishmania*

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Human leishmaniasis is a parasitic disease caused by trypnosomatid of the genus leishmania, transmitted by the bite of a sand fly of genus phlebotomus, a characteristic of all leishmanial infections in the intracellular parasitism of macrophages. Various species of leishmania have been identified causing the disease ranging from simple cutaneous to *Visceral leishmaniasis*. Hexadecylphosphocholine (HePC), a novel phospholipids derivative, was tested against leishmania major and leishmania tropica, the causative agent of *Cutaneous leishmaniasis*. Human volunteer were cured completely, when treated topically with an ointment comprising 15 % HePC and 12 % methyl benzethonium chloride in simple ointment base twice daily for 10 d. No parasites were detected in tissue smears or in culture from treated cutaneous lesions.

Key Words: Hexadecylphosphocholine, *Cutaneous leishmaniasis.*

INTRODUCTION

As no vaccine is yet available for any type of Leishmania; chemotherapy is the only mean of controlling the disease. Pentavalent antimonials in use for over a century are still the first line drugs for treating all kind of leishmaniasis. They are marketed in two formulations sodium stibogluconate solution (Pentostam, 100 mg/5 mL). A daily dose of 20 mg/Kg body weight for duration of 20-30 days is recommended[1]. Pentavalent antimonials are toxic, but their rapid excretion by kidneys is a blessing. Although these drugs have a useful therapeutic index, inadequate dosage and discontinuous use of the drug by patients, especially in poor countries, has resulted in the resistance of parasites to the drug. This has caused serious situation in countries like India and Sudan, when rapid transmission of the resistant parasites has given rise to mass unresponsiveness to antimonials drug and thereafter, a very high mortality rate. The signs of resistance are evident here too, as some patients get up to 40 injections (*ca.* 400 mg/injection) but showed no signs of cure. Amphotericin B and Pentamidine have proved effective as second line drugs in place where resistance to antimonials has developed[2].

Pentamidine is more toxic than antimonials drugs and Amphotericine B, as the rate of excretion of drug by the liver and kidneys is very slow. Treatment of leishmaniasis with pentamidine is the only choice in case of unresponsiveness to these two types of drugs. However, dosage of the drugs for very short duration has

resulted in successful treatment of the disease[3]. Many other drugs have been tried for the leishmanicidal activity and at present are in an experimental stages are under going human trials in various countries. Trials of dapsone in oral dosage are is progress in Balochistan and need to be undertaken in other parts of the world too.

Topical therapy of cutaneous leishmaniasis has many advantages and shows great promise. There is renewed interest in the development of such drugs formulation for topical application directly on the cutaneous lesions. Paromomycin, an amino glycoside has attracted much attention and in the form of a topical ointment is in use for the treatment of cutaneous leishmaniasis[4]. However, injectables paromomycin in combination with antimonials drugs for treating all kinds of leishmaniasis has had a better effect than either drug given alone[5].

In Pakistan also, the pentavalent antimonials are the first line drugs for treating all types of leishmaniasis. The high cost of the drug and its application parenterally are the major causes of avoidance of the drug by poors. More serious is the development of resistance to these drugs[6].

There is a need to develop new drugs which in topical form could be used topically for cutaneous lesions. As hexadecylphosphorylcholine (HePC) showed promising results in animal models, we applied the same to some human volunteer. Topical treatment of cutaneous leishmaniasis with an ointment composed of 15 % hexadecylphosphorylcholine (HePC) and 12 % benzethonium chloride (BCl), in a simple ointment base (15/12 HePC ointment) has been developed.

Preliminary clinical study with HePC ointment shows very potent for different species of leishmania. In this work, the therapeutic effect of HePC ointment with two different concentration of BCl 12% and 5% caused by L. Tropica were tried. Treatment was given for 10-15 d.

Treatment: Three preparations were used: (1) Ointment A, 15 % hexadecylphosphorylcholine (HePC) 12 % benzethonium chloride (BCl) in simple ointment base (15/12) hexadecylphosphorylcholine (HePC) ointment, (2) Ointment B, placebo ointment containing simple ointment base only, (3) Ointment C, 15 % hexadecylphosphorylcholine (HePC) + 5 % benzethonium chloride (BCl) in simple ointment base (15/5 HePC ointment).

Treatment was started and dose adjusted as two time per day. Ointment was applied twice daily for 12 d.

Clinical examination: An overall clinical assessment of patients including hemoglobin estimation, total leucocytes count and liver function tests were performed before and after termination of treatment. Samples were taken for the culture from the lesion before and at the end of the treatment and at each visit. For the follow up examination, several visits for up to 6 months were made. At the end of the therapy the lesions in all patients were found parasitologicaly negative. The lesion disappeared from 10 d to upto 30 d in all the cases.

(1) **Parasitological and clinical cure:** No parasite have found in the treated lesion, (2) **Delayed cure:** leishmania parasite were still present but disappeared

within next 5 days, (3) **Parasitological cure:** No parasite was detected in the treated lesion at the end of the treatment. (4) **Failure:** Neither parasitological nor clinical cure was achieved with in the 30 days after termination of the treatment.

EXPERIMENTAL

76 Patients with cutaneous leishmania participated in the therapeutic trial. The patients were of different age group between ages of 15 months and 56 years. Each patient signed a statement of inforibed consent. Six patients were dropped because of some side effect (feeling burning sensation). Duration of the lesion ranged form 3 weeks to 3 months. Lesions were mainly on the exposed part of the body like face, arms and feet. Forty eight patients had a single lesion while 22 patients had multiple lesions (max, 3). Demonstration of leishmania from skin lesions by the slit smears technique was necessary for inclusion of a single lesion. The sample was inoculated in biphasic medium containing NNN and overlay of 199 and 10 % heat inactivated foetal calf serum (HIFCS) and pen strip as an antibiotics. The entire samples were kept for cultivation of Amastigotes in incubator at 22 ºC for 3 to 4 d. Confirmed cases were included in the study. Forty four patients were divided into two groups, that were treated with either 15/12 HePC ointment (44 patients) or 15/5 HePC ointment (14 patient). HePC were gifted by Asta Pharma, Italy for the preparation of ointment base. All the chemicals or reagents used were of AnalaR grade. Clinical trials were carried out in Combined Military Hospital (CMH), Sandmen Hospital and OPDs of private clinic under the supervision of skin specialist. Ointment base have been prepared in the Department of Pharmacy University of Balochistan, Quetta, Pakistan.

RESULTS AND DISCUSSION

Topical treatment with 15:12 HePC ointment: The effect of 15/12 hexadecylphosphorylcholine (HePC) ointment on patient with CL is given in Table-1. One patient showed no response while 9 patients showed delayed cure. This may be due to the patient not regular during follow up. The cure rate for those patient treatment with 15/12 hexadecylphosphorylcholine (HePC) ointment was (99 %) (43 of 44 patients). Clinical improvement of the treated lesion was demonstrated up to 6 weeks after treatment, although no parasites were demonstrated in these treatment lesions at the end of the treatment except one patient. In the cured patient parasites were totally eliminated from the lesion by the end of the treatment and the lesion were clinically healed.

Topical treatment with 15/5 hexadecylphosphorylcholine (HePC) ointment is shown in Table-2. Ten out of 14 patients were cured of the parasites after 10 days of treatment where as one had showed delayed cure and took 5 days more to eliminate completely leishmania parasites.

Placebo treatment: The placebo group was treated for 12 days of treatment, no response appeared except the lesion become clean and soft.

TABLE-1
EFFECT OF HePC OINTMENT WITH 15/12 ON CUTANEOUS LEISHMANIASI LESION WERE TREATED TWICE DAILY FOR 10 d

Patients	Age	Sex	Number and character of lesion	Site	Duration (d)	Response	Clinical examination
A2	30	Male	1 large wet	Face	10	Rapid healing	No parasite have found
B3	15	Female	Multiple wet	Face and arm	15	Rapid healing	No parasite have found
C4	56	Male	1 small dry	For head	10	Rapid healing	No parasite have found
D3	41	Male	2 small dry	Hand	12	Delayed healing	L. Parasite still present but disappear during next 2 days
E6	32	Male	1 small wet	Elbow	12	Rapid healing	No parasite have found
F1	15	Female	1 small wet	Legs	10	Non healing	Failure
G8	12	Female	1 small dry	Face	10	Rapid healing	L. Parasite still present but disappear during next 5 days
H3	46	Male	3 small dry	Face and arm	15	Delayed healing	L. Parasite still present but disappear during next 5 days
I3	39	Male	3 large wet	Cheek	10	Not follow up	——
J2	26	Male	1 large wet	Arm	10	Not follow up	——
K2	22	Female	2 small wet	Face and hand	10	Delayed healing	No parasite have found
L3	18	Female	1 small dry	Hand	15	Delayed healing	L. Parasite still present but disappear during next 5 days
M4	11	Female	1 small dry	Hand	15	Delayed healing	L. Parasite still present but disappear during next 5 days

TABLE-2
EFFECT OF HePC OINTMENT WITH 15/5 ON CUTANEOUS LEISHMANIASIS LESION WERE TREATED TWICE DAILY FOR 10 d

Patients	Age	Sex	Number and character of lesion	Site	Duration (d)	Response	Clinical examination
A2	20	Female	1 Large wet	Face	10	Rapid healing	No parasite have found
B3	32	Male	2 Large wet	Face	12	Rapid healing	No parasite have found
C4	15	Male	1 Large wet	Hand	10	Rapid healing	No parasite have found
D1	12	Female	2 Large wet	Elbow	15	Delayed healing	Parasite still present but disappear during 5 days
E3	24	Male	1 small dry	Forehead	12	Rapid healing	No parasite have found
F1	18	Male	1 small dry	hand	10	Rapid healing	No parasite have found

TABLE-3
EFFECT OF PLACEBO GROUP ON CUTANEOUS LEISHMANIASIS

Patients	Age	Sex	Number and character of lesion	Site	Duration (d)	Response	Clinical examination
A3	2	Male	1 large wet	Face	12	No response	Parasite were present
B1	6	Female	1 small wet	Face	12	No response	Parasite were present
C2	30	Female	2 large dry	Arm and leg	12	No response	Parasite were present
D4	22	Male	1 large wet	Hand	12	No response	Parasite were present
E2	56	Male	1 large wet	Face	12	No response	Parasite were present

Clinical and laboratory examination: Routine laboratory examination indicated no adverse effects. Various degree of inflammation, a burning sensation and local pain depending on the size and penetration of the host response were associated with this treatment. Five patients were dropped from the study because of not taken interest due to severe pain, inflammation and edema that developed with in 3 to 7 d of treatment with 15/12 hexadecyl-phosphorylcholine (HePC) ointment.

This was performed to determine the *in vivo* efficiency of different concentrations of HePC ointment. It is found that even a concentration as low as 6 % is quite effective against parasites and after the treatment of almost 10 day, no parasites were seen, from the lesions as compared with placebo. Some of the patients showed delayed cure despite the fact that the lesions were cured parasitologicaly without any clinical improvement. These results are almost similar to our previous study on animal models of where 3/6 hexadecylphosphorylcholine (HePC) ointment showed a cure of 87 to 99 %. It is also found that 15/5 hexadecylphosphorylcholine (HePC) ointment was almost as effective as 15/12 (HePC) ointment against *Cutaneous leishmania*, no antibiotic orally or injectable treatment was required for the elimination of the parasites after (HePC) ointment. Generally (except in few cases local inflammation, burning sensation and pain), no side effects were recorded with the (HePC) ointment containing 12 % concentration of benzethonium chloride (BCl) are required to establish to optimal concentration.

In the present study, the cure rate with the ointment was found to be almost 100 % higher then the cure rate in the placebo treated group for short period of treatment with the 15/12 or 15/5 hexadecylphosphorylcholine (HePC) ointment. Although the numbers of patients selected are very small, yet it indicates the efficacy of the ointment. The study needs to be done on large number of subjects and volunteers have to be prepared for placebo test as well.

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