New approach in treatment of cutaneous leishmaniasis in Hamsters

AL-OBOSI,J. A.,S.[1] ; AL- AZAWI,A. W.[2] ; AZIZ, S.B. [3] ; AZIZ,S.Z.[3];
, LATIF,M.B[4]. and Munzir M.E. AHMED[5]
1.Al-Yarmouk college. Pathology Dept. SUDAN. 2. Al-Yarmouk college. Microbiol.and Parasitol. Dept. SUDAN. 3. Al-Yarmouk college. Comm.medicine Dept. SUDAN. 4.College of Vet.Med. Parasit.Dept. Baghdad Univ. 5.Faculty of Medicine, Biochemistry Dept. Al-Gadarif Univ. SUDAN.

ABSTRACT

The current study aimed to treat cutaneous leishmaniasis in hamsters experimentally infected with new drug. Four groups of golden hamsters (T1,T2,T3 and T_4) each of five animals were inoculated intradermally with *Leishmania tropica*, while the fifth group (5 animals) left untreated as control. After appearance of the clinical signs of cutaneous leishmaniasis which were confirmed by presence of leishman (L.d.) bodies in positive blood smear, treatment commenced as follow and bid.: T_1 with thick film acriflavine cream (0.1% acriflavine, cod liver oil and lanolin) for 10 successive days. T_2 with cod liver oil and lanolin, T_3 with lanolin alone, T_4 left untreated as control for the treated groups, while 5th (C) group used as control for the whole experiment.
The result showed that group T_1 completely recovered without any scar formation, group T_2 recovered after 35 days with large scar so as group T_3 and T_4. Animals in group C didn't show any abnormalities along the experiment.

المستخلص

تهدف هذه الدراسة إلى علاج اللشمانيا الجلدية بالهامستر المصاب تجريبيا بنوع جديد من العلاج. اربعة مجاميع كل مجموعة تكونت من 5 خمسة حيوانات ومجموعة خامسة مثلث (T4و T3و T2و T1) من الهامستر الذهبية , حقنت المجاميع الأولى الأربعة بالأدمة بطفيلي اللشمانيا الجلدية الاستوائية , في حين تُركت (C)السيطرة المجموعة الخامسة بدون أي حقن.

بعد ظهور العلامات المرضية للشمانيا الجلدية على الحيوانات المحقونة والتي أُكد فيها الخمج مختبريا وذلك بظهور أجسام لشمن بالمسحات الدموية , بدأنا العلاج كما يلي وبواقع مرتين يوميا. المجموعة الأولىT1 عولجت بطبقة ثخينة من كريم الأكريفلافين (0,1% اكريفلافين و زيت كبد الحوت وزيت صوف الخراف) ولمدة

10 عشرة ايام متتالية , اما المجموعة الثانية T2عولجت بزيت كبد الحوت زائدا زيت صوف الخراف فقط, والمجموعة الثالثة T3عولجت بزيت صوف الخراف فقط ,اما المجموعة الرابعة فقد تُركت بدون علاج, والمجموعة الخامسة هي مجموعة السيطرة (C) .

أظهرت النتائج ان مجموعة حيوانات T1 تماثلت للشفاء التام دون تكون اي ندبة, اما المجموعة الثانية T2 تماثلت للشفاء بعد مرور 35 يوم مع تكون ندبة كبيرة وكذلك كان حال المجموعتين T3و T4. اما مجموعة حيوانات السيطرة فلم تظهر اي تغير طوال فترة التجربة.

Introduction

Cutaneous leishmaniasis (oriental sore) is the most common form of leishmaniasis all over the world causes cutaneous ulcers on exposed parts of the body, leaving life – long scars. [1] It is a skin infection caused by a single-celled parasite, i.e., protozoan called *Leishmania spp*. that is transmitted by female sandfly bites *Phlebotomus spp*..[2] The Leishmania protozoan was first described in 1903 by Leishman and Donovan, working separately.[3] There are about 20 species of Leishmania that may cause cutaneous leishmaniasis.[4] Cutaneous leishmaniasis occurs in China, India, the Near East, the Mediterranean basin, and Africa as far as Nigeria and Angola. In Baghdad the capital of IRAQ they call it Baghdad boil(Habbat Baghdad). It is characterized by single or multiple sharply demarcated, ulcerating, granulomatous, autoinculable skin lesion. Secondary infection is usual and the only systemic symptoms are those due to secondary infection. *Leishmania tropica* or *L. major* may be demonstrated in smears or cultures of curetting from the side or base of the ulcer.[5] The leishmanin skin test is positive. Healing occurs spontaneously in 2 to 18 month

leaving a depressed scar.[1] Healing from cutaneous leishmaniasis give protection against further cutaneous and visceral leishmaniasis.[2] Acriflavine was developed in 1912 by Paul Ehrlich, a German medical researcher and was used during the first World War against sleeping sickness, a protozoan disease caused by *Trypanosoma spp.* [6]

Materials and Methods

Twenty five male golden hamster of the same age were divided into 5 groups each of 5 animals ; kept in specific cages with daily observation; provided with food and water ad libtum. The first four groups (T_1,T_2,T_3,and T_4) were intradermally inoculated in the foot pad with ($10x10^6$ promastigotes) of *L. tropica* ,after disinfected the feet with Tr. iodine. The fifth (C) group left without treatment as control.

Blood smears were taken from the foot pad before and after inoculation, fixed with absolute methanol for 2 minutes, giemsa stained and examined microscopically under the oil lens. Animals were observed for appearance of specific lesions then treatment commenced as follow bid till the end of the experiment: group T_1 treated topically with thick film of acriflavine cream, group T_2 with cod liver oil and lanolin, group T_3 only with lanolin, while group T_4 left untreated as control for the treated animals.

Statistical Analysis

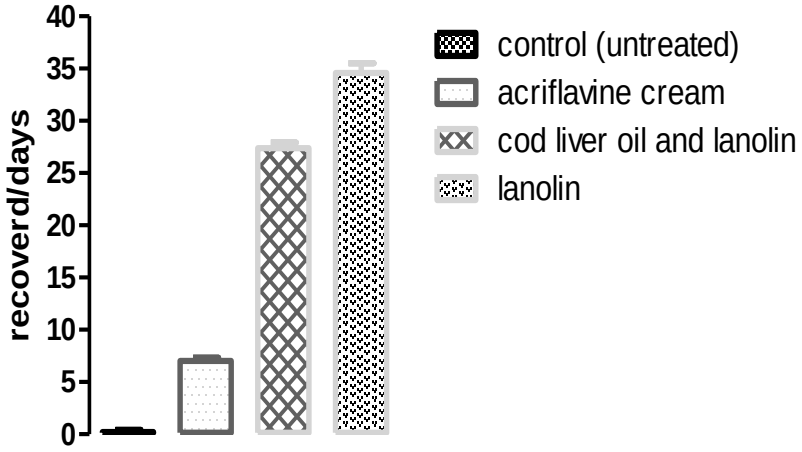
All values reported in the text are mean ± standard error mean. Comparisons between multiple groups or, results form two different treatments were performed with one-way ANOVA followed by t-test for multiple statistical comparisons. A p-

value <0.05 was determined to be significant. All statistical analyses were performed with statistical GraphPad Prism software.

Results

The clinical signs of the infection were evident at 10 days after the inoculation, represented by redness, itching and swelling of the foot pad. Group T_1 which was treated by acriflavine cream showed complete recovery after the 7^{th} day of the beginning of the treatment without leaving scar, whereas after appearance of cutaneous leishmaniasis pictures, group T_2 , T_3 ,and T_4 showed lacerated skin and ulcer formation with exudation till recovery occurred between 28-35 days leaving large obvious scar.

Blood smears taken before inoculation of the parasite were negative for the presence of (L.d.) bodies, while smear taken from T_1, T_2, T_3, and T_4 10 days after inoculation were positive i.e., large number of (L.d.) bodies (amastigote) inside the cytoplasm of many monocytes and lymphocytes.



Treatment of cutaneous leishmaniasis in Hamsters by Acriflavine

Discussion

The isolate used in this experiment was very virulent because it infect all the inoculated animals. On comparing the result of the four group (T_1, T_2, T_3, and T_4) we concluded that acriflavine was highly effective in the treatment of cutaneous leishmaniasis with100% efficacy, and the recovery was due to the effect of acriflavine alone, rather than cod liver oil with lanolin (as in T_2) and lanolin alone (as in T_3), and this was documented by the fact that improvement didn't occur in T_2 and T_3 animals whenever this line of treatment didn't contain acriflavine so they clearly exhibited all signs and phases of the disease, as T_4 animals which was left untreated and recovered spontaneously after a long period leaving scar formation.[1] These results were corresponding to the finding of [5] who noticed that not all patients require treatment and many lesions heal spontaneously.The mode of action of

acriflavine on Leishmania was due to the selective inhibition of kinetoplast DNA synthesis which was caused by a selective localization of acriflavine in the kinetoplast. The loss of kinetoplast DNA leads to a respiratory defect which then leads to a decrease in biosenthetic reactions and eventual cell death.[7] Acriflavine cream is more save in treatment of cutaneous leishmaniasis than pentavalent antimonials which was used in American military study as it caused many serious and reversible side effects.[8]

Conclusion

This study revealed that acriflavine was very effective against cutaneous leishmaniasis and the presence of cod liver oil in the cream which contain vitamin A and vitamin D promote the rapid regeneration of the epithelial cells of the epidermis as a labile cells, and the lanolin act as a good vehicle which held acriflavine deep in the dermis through the

epidermis to kill the parasite, and the dermatitis which occurred due to the infection and the damage of the epidermis was cured with first

intention i.e., without scar formation because the parasite hadn't enough time to damage dermis and epidermis as did in T_2, T_3, and T_4, which healed by second intention, i.e., repair by substitution with granulation tissue due to the extensive damage which occurs in dermis and epidermis by the effect of the parasite and secondary bacterial infections.

Recommendations

1. We recommend to try this treatment (acriflavine + cod liver oil + lanolin) in human cutaneous leishmaniasis.
2. Acriflavin is a very cheap compound and if proved its success in the treatment of human leishmaniasis, we advise to provide the endemic areas with this pharmaceutics formula treatment.

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