East African Medical Journal Vol. 77 No. 9 September 2000
SAFETY AND IMMUNOGENICITY OF AN AUTOCLAVED *LEISHMANIA MAJOR* VACCINE
E.A.G. Khalil, A.M. Elhassan, E.E. Zijlstra, Leishmaniasis Research Group/Sudan, Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan, O.F. Osman, Faculty of Science, University of Khartoum, Khartoum, Sudan, I.A. Eljack, Faculty of Environmental and Public Health, University of Khartoum M.E., Ibrahim, M.M. Mukhtar, Leishmaniasis Research Group/Sudan, Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan, H.W. Ghalib King Saud University, Abha, Kingdom of Saudi Arabia and F, Modabbers, TDR/WHO, Geneva, Switzerland.

Request for reprints to: E.A.G. Khalil, Institute of Endemic Diseases, University of Khartoum, P.O.Box 45235, Khartoum, Sudan.

SAFETY AND IMMUNOGENICITY OF AN AUTOCLAVED *LEISHMANIA MAJOR* VACCINE

E.A.G. KHALIL, A.M. ELHASSAN, E.E. ZIJLSTRA, O.F. OSMAN, I.A. ELJACK, M.E., IBRAHIM, M.M. MUKHTAR, H.W. GHALIB and F. MODABBERS

ABSTRACT

Objective: **To test the safety and immunogenicity of two doses of autoclaved *L.major* (ALM) vaccine mixed with BCG.**
Setting: **Kala-azar endemic area of eastern Sudan.**
Design: **This was a randomised, double blind and BCG controlled phase I/II study.**
Subjects: **Eighty healthy volunteers (forty children and forty adults) with no past history of kala-azar, no reactivity to leishmanin antigen and with a reciprocal direct agglutination test (DAT) titre of <200 were recruited. Informed consents were obtained from volunteers or their guardians in case of children.**
Main outcome measures: **Conversion in the leishmanin skin and the DAT tests.**
Intervention: **Two intra-dermal injections of either ALM+BCG or BCG alone. The injections were three weeks apart.**
Results: **Side effects were minimal and confined to the injection site, with no significant difference between the ALM+BCG and the BCG alone groups. The leishmanin skin conversion was significantly higher in the ALM+BCG group compared to the BCG alone group ($p<0.0005$). Furthermore, the Leishmanin skin test conversion was significantly higher in children than adults ($p<0.0005$). One adult volunteer in the ALM+BCG group converted in both the Leishmanin skin and the DAT tests.**
Conclusion: **We conclude that two doses of ALM+BCG are safe and immunogenic, especially in children.**

INTRODUCTION

Visceral leishmaniasis (kala-azar) is a major health problem in Sudan(1-5). The only measure of control in the country at present is case detection and treatment with antimonial drugs, which are expensive and not always available. Vector and reservoir control measures are difficult to implement due to the elusive nature of the vector and the diversity of possible reservoir hosts. An effective vaccine offers a suitable control measure since it is known that successful treatment of kala-azar leads to the development of a protective cell-mediated response of the Th-1 type(6-8). We had previously reported that individuals that were leishmanin positive as a result of previous exposure to *L.major* infection and who migrated to an area endemic of visceral leishmaniasis appeared to be protected against kala-azar(9). Recently, Anuradha *et al*(10) showed that vaccination of langur monkeys *(Presbytis entellus)* with ALM+BCG protected against *L. donovani* challenge.

A phase I/II trial was previously carried out in healthy volunteers in a kala-azar non-endemic area using one dose of the ALM+BCG vaccine. This study concluded that one dose of the vaccine is safe and immunogenic (unpublished data).

The objective of this study was to determine the safety and immunogenicity of two doses of a vaccine containing autoclaved *Leishmania major* + BCG in healthy children and adult Sudanese volunteers in a kala-azar endemic area in eastern Sudan.

MATERIALS AND METHODS

Volunteers: Two hundred healthy volunteers (100 adults and 100 children) were recruited for the study from two villages in the kala-azar endemic area of eastern Sudan. The inclusion criteria were: age between one and 65 years; absence of past history of kala-azar, cutaneous leishmaniasis or post kala-azar dermal leshmanians (PKDL), absence of chronic systemic or skin diseases, no reactivity to the leishmanin antigen (Razi Institute, Iran), a DAT titre of <200, body temperature ≤37.5°C. Pregnant and lactating ladies were excluded. Volunteers or their guardians were asked to sign informed consent forms written in Arabic, read and explained to them by one of the investigating team. Demographic data and clinical history were recorded in enrolment forms and a physical examination was conducted with particular emphasis to exclude cases of visceral, cutaneous leishmaniasis and PDKL.

Leishmanin skin test: The recruited volunteers were injected intra-dermally with 0.1 ml of leishmanin antigen (Razi Institute, Iran) on the volar aspect of the left arm. As a control 0.1 ml of the

diluent was injected at least 10 cm from the antigen site. The test was read 48 hours later(11). Induration of 5 mm or more and no reaction at the control site was regarded as positive.

Direct agglutination test (DAT): The DAT was performed on capillary blood collected on Whatmann #3 filter paper as described by Harith *et al*(12).

Study group: Forty children (aged ≤15 years) and forty adults who fulfilled the above mentioned criteria were enrolled for the study.

Vaccination and follow up: Volunteers were randomised to receive either 0.1 ml ALM+BCG or 0.1 ml BCG alone (Pasteur Institute, Iran). The BCG concentration was 1/10 of the dose used for routine immunisation. Two intra-dermal injections of ALM+BCG or BCG alone were given to each volunteer. The injections were three weeks apart. Only the on-site monitor knew the group allocation of the volunteers. Volunteers were monitored daily for local reactions (pain, itchiness, erythaema, vesiculation, induration, regional lymph node enlargement) as well as systemic side effects (fever, rash, vomiting) from the vaccination day to day eight post vaccination. Six weeks after vaccination the volunteers were examined clinically with particular reference to evidence of visceral leishmaniasis and were tested by leishmanin and DAT.

RESULTS

The on-site monitor of the Data and Safety Monitoring Board of the Institute of Endemic Diseases, University of Khartoum, broke the code.

Table 1 shows the general characteristics of the study group. Although equal numbers of adult males and females were recruited for the study, most male volunteers tested positive for leishmanin. This is clearly reflected in the low male: female ratio in the adult group.

Table 1

Characteristics of the study group

	No.	M:F ratio	Mean age (years)	Median age (years)
Children (Age<15)	40	19:21	5.8	5.5
Adults (Age>15)	40	9:31	22.0	20.0

Table 2

Total leishmanin skin test conversion in the ALM+BCG and the BCG groups

	Converters	Non-converters	Total
ALM+BCG group	17 (44%)	21 (56%)	38
BCG only group	4 (10%)	36 (90%)	40

P<.0005

Side-effects: The commonest reported side effect was local sharp pain that was experienced by all volunteers. No localised or generalised rash was reported. Complaints of fever were always checked with measurement of axillary temperature. On day three after the first dose of vaccination, two volunteers in each of the ALM+BCG and the BCG group complained of fever. Measurement of axillary temperature showed a slight rise of 37.8°C in one volunteer in each group. Clinical examination revealed no abnormality and thick and thin blood films were negative for malaria parasites. The fever responded to paracetamol treatment. On day three after the second dose one patient from the ALM+BCG group had a temperature of 37.8°C. Clinical examination revealed no abnormality and a blood film was negative for malaria parasite. The rise in temperature responded to a dose of paracetamol treatment. No other major side effects were noted.

Table 3

Leishmanin skin test conversion in the ALM+BCG and BCG only group in children and adults

	Leishmanin test	
	ALM+BCG groups Converters/non converters	BCG group Converters/non-converters
Children	11/9 (55%)	2/18 (10%)
Adults	6/12 (33.3%)	2/18 (10%)

P<0.0005

Immunogenicity: Forty two days after the second dose of vaccination, follow up could be achieved for 78 volunteers (98.7%). Leishmanin skin test conversion was detected in 17 volunteers (44%) in the ALM+BCG group compared to only four (10%) in the group that received BCG only (p<0.0005). When the result was further stratified by age it was clear that children (<15 years) had a higher Leishmanin skin conversion of 55% compared to 33.3% in adults in the ALM+BCG group (p<0.0005). The Leishmanin conversion in the BCG group was similar in children and adults (10%), Tables 2 and 3. One adult in the ALM+BCG group converted in both the leishmanin and the DAT.

DISCUSSION

Visceral leishmaniasis is a serious health problem in many areas of eastern and southern Sudan. Treatment is expensive and not always available. Vector control measures are expensive and difficult to implement. It is clear that an effective vaccine is the ideal solution to this problem in Sudan. Extensive studies on vaccines for cutaneous leishmaniasis have been carried out(13-19), but studies on vaccination against human visceral leishmaniasis are limited and were on experimental animal models(20,21).

This pilot trial is the first step to test for vaccines against human visceral leishmaniasis and it was carried out to test the safety and immunogenicity of ALM+BCG vaccine in an endemic area of visceral leishmaniasis. It is clear that the vaccine is safe and acceptably immunogenic. It is also evident that the rate of leishmanin skin test

conversion is higher in children than in adults. This will be an important finding if the vaccine proved to be protective in Phase III trials, because visceral leishmaniasis is mainly a disease of children in Sudan.

BCG coverage is very low in the area where the study was carried out so it was decided to use BCG instead of a placebo. BCG also leaves a scar that made it difficult for the investigators to tell who received what and thus reducing investigator bias. We conclude that ALM+BCG vaccine is safe and immunogenic when used in healthy volunteers. A field trial is underway to determine the efficacy of the vaccine against visceral leishmaniasis in the Sudan.

ACKNOWLEDGEMENTS

This investigation received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. The support of MSF-Holland/Khartoum is highly appreciated.

REFERENCES

1. Satti, M.H. Early phases of an outbreak of kala-azar in the southern Fung. *Sudan Med. J.* 1958; **1**:98-111.
2. Perea, W.A., Moren, A., Ancelle, T. and Sondorp, E. Epidemic visceral leishmaniasis in southern Sudan. *Lancet*, 1989; **2**:l222-1223.
3. De Beer, P., Harith A.E., Grootheest van M. and Winkler A. Outbreak of kala-azar in Sudan. *Lancet.* 1990; **335**:224.
4. Zijlstra, E.E., Siddig, M.A., Elhassan, A.M., Eltoum, I., Satti, M., Ghalib, H.W., Sondorp, E.E. and Winkler A. Kala-azar in displaced people from southern Sudan: epidemiological, clinical and therapeutic findings. *Trans. roy. Soc. trop. Med. Hyg.* 1991; **85**:365-369.
5. EL Hassan, A.M., Hashim, F.A., Siddig, M.A., Ghalib, H.W. and Zijlstra, E.E. Kala-azar in Western Upper Nile in southern Sudan and its spread to a nomadic tribe from the north. *Trans. roy. Soc. trop. Med. Hyg.* 1993; **87**:387-395.
6. Badaro R., Jones T.C., Carvalho, E.M.; Sampaio, D., Reed S.G., Barval A., Teixeira R. and Johnson W.D. New perspective on subclinical forms of visceral leishmaniasis. *J. Infect. Dis.* 1986; **154**:1003-1011.
7. Sacks D.L., Lal S.L, Shrivastava S.N., Blackwell J. and Neva F.A. An analysis of T-cell responses in Indian kala-azar. *J. Immun.* 1987; **138**:908-913.
8. Kurthzals J.A. L., Hey A.S., Theander T.G., Odera E., Christensen C.B.V., Githure J.I., Koech D.K., Schaef K.U., Handman E. and Kharazmi A. Cellular and humoral immune reactivity of a population of the Baringo district of Kenya to Leishmania promastigotes Lipophosphoglycan. *Amer. J. trop. Med. Hyg.* 1992; **46**:480-488.
9. Zijlstra E.E., El Hassan A..M., Ismail A. and Ghalib H.W. Endemic Kalaazar in eastern Sudan: a longitudinal study on the incidence of clinical and subclinical infection and post-kala-azar dermal leishmaniasis. *Amer. J. trop. Med. Hyg.* 1995; **52**:299-305.
10. Anuradha D., Sharma P., Srivastava J.K., Misra A., Naik S. and Katiyar J.C. Vaccination of langur monkeys (Presbytis entellus) against leishmania donovani with autoclaved L.major plus BCG. *Parasitology.* 1998; **116**:219-221.
11. Sokal J.E. Measurement of delayed skin test responses. *N. Engl. J. Med.* 1975; **293**:501-502.
12. Harith, A.E., Kolk, A..H..J., Kager, P.A., Leeunburg, J., Muigai, R., Kiugu, S. and Laarman, J.J. A simple and economical direct agglutination test for sero-diagnosis and sero-epidemiological studies of visceral leishmaniasis. *Trans. roy. Soc. trop. Med. Hyg.* 1986; **80**:583-587.
13. Mayrink W., da Costa C.A., Magalhaes P.A., Melo M.N., Dias M., Lima A.O., Michalick M.S. and William P. A field trial of a vaccine against American dermal leishmaniasis. *Trans. roy. Soc. trop. Med. Hyg.* 1979; **73**:385-387.
14. Green M.S., Kark J.D., Witztum E., Greenblat C.L. and Spira D.T. Frozen *L.tropica:* the effect of dose, route for administration and storage on the evolution of clinical lesion. Two field trials in the Israel defense forces. *Trans. roy. Soc. trop. Med. Hyg.* 1983; **77**:152-159.
15. Antune-s C.M.F., Mayrink W., Magalhaes P.A., da Cosa C.A., Melo M.N., Dias M., Michalick M.S., William P., Lima A.O. and Vieira J.B. Controlled field trials of a vaccine against New World cutaneous leishmaniasis. *Intern. J. Epidem.* 1986; **15**:572-580.
16. Modabber F. Experience with vaccines against cutaneous leishmaniasis of man and mice. *Parasitology.* 1989; **98**:S49-S60.
17. Mohebali M., Javadian E.H., Hashemi-Fesharki R., Mohammad zadeh M., Nadim A., Tahvildar-Bidruni G.H. and Mesdaghim A. A Trial of non-living crude vaccine against zoonotic cutaneous leishmaniasis. *Med. J. Islamic Repub. Iran.* 1995; **8**:211-215.
18. Bahar K., Dowlati Y., Shidani B., Alimohammaddian M.H., Khamesipour A., Ehsasi S., Hashemi-Feshari R., Ale-Agha S. and Modabber F. Comparative safety and immunogenicity trial of two killed Leishmania major vaccines with or without BCG in human volunteers. *In: Clinics in Dermatology (Eds.),* Dowlati Y. and Modabber F.; Elsevier Science Inc., New York, 1996, 14, 489-495.
19. Sharifi I., Fekri A. R., Aflatonian M.R., Khamesipour A., Nadim A., Mousavi M.R.A., Momeni A.Z., Dowlati Y., Godal T., Zicker F., Smith P. and Modabber F. Double-blind randomised controlled vaccine trial of a single dose of killed *Leishmania major* plus BCG against anthroponotic cutaneous leishmaniasis in Bam, Iran. *Lancet.* 1998; **351**:1540-3.
20. Jarecki-Black J.C., Hallman K.L., James E. R. and Glassman A.B. Resistance against *Leishmania donovani* induced with an aluminium hydroxide vaccine. *Ann. Clin. Lab. Sci.* 1988; **18**:72-77.
21. Rachamim N. and Jaffe C.L. Pure protein from *Leishmania donovani* protects mice against both cutaneous and visceral leishmaniasis. *J. Immunology,* 1993; **150**:2322-2331.