

Evaluation of the Anti-Chagas "PF" Vaccine Against two Strains of *Leishmania* sp. in Hamsters (*)

Jorge R. Arias (**)
Humberto Menezes (***)
Rui A. de Freitas (**)

Abstract

Amastigotes of two strains of *Leishmania* sp. were inoculated into golden hamsters (*Mesocricetus auratus*) 8 and 15 days after inoculation with the anti-chagas "PF" vaccine. There was no significant difference in reduction of infection between the "PF" treated and the control (untreated) hamsters after inoculations with either strain of *Leishmania* sp.

INTRODUCTION

The origin and activity of the anti-chagas "PF" vaccine has been thoroughly discussed by Menezes (1969a, b, 1970, 1972a, b, 1974, 1975) and will not be covered here.

This experiment was suggested by Prof. Warwick E. Kerr, and its main purpose was to find whether or not the "PF" vaccine would delay the activity of *Leishmania* sp. in hamsters.

METHODS AND MATERIALS

Eighty golden hamsters (*Mesocricetus auratus*) were randomly placed in 8 groups of 10 each. Later, 2 of these groups of 10 were divided in half, becoming 4 groups of 5 each.

One of the groups of 10 hamsters was maintained untreated to serve as the control (C). The remaining 5 groups of 10 hamsters were inoculated subdermally with the "PF" vaccine. Of these, one group was not treated further and became the "PF" control (PF). Two of the remaining 4 groups of 10 hamsters were inoculated subdermally, in the nose, with amastigotes of each of the 2 strains of *Leishmania* sp. 8 days after the "PF" treatment,

(PF + 8 + LA) and (PF + 8 + LJ) respectively. The remaining 2 groups of 10 hamsters were similarly inoculated with both strains of *Leishmania* sp. 15 days after the "PF" treatment, (PF + 15 + LA) and (PF + 15 + LJ) respectively.

The 4 groups of 5 hamsters each were inoculated with of the strains of *Leishmania* sp. at the same time as those mentioned above, to serve as *Leishmania* controls (to insure infectivity of the tissue triturate which was used as the infective agent). These were (8 + LA), (8 + LJ), (15 + LA) and (15 + LJ) respectively.

The "PF" vaccine used, and method of preparation, was the same as described in Menezes (1975). The vaccine was prepared from the 400th passage of the "PF" *Trypanosoma cruzi* strain maintained in culture media as described by Warren (1960) for 27 days. The final suspension contained about 50% motile trypanosomes and almost 5% were metacyclic forms. All hamsters, whose mean weight was 35 gms, were inoculated subdermally with approximately 3.9×10^5 motile parasites.

The origin of the *Leishmania* sp. Strain J is that of a 31-year-old male patient who manifested over 50 verrucose papilomas varying in diameter from 0.2 to 3.0 cm over his entire body. Inoculation into a hamster's nose produces an initial small verrucose growth at site of inoculation, approximately one to two months after infection. This wart remains for 1 - 2 weeks when it either drops off and typical *Leishmania* manifestations follow, or it gradually becomes a typical lesion.

(*) — This project was partly supported by the CNPq Grant SIP/08-131.

(**) — Department of Medical Sciences, Instituto Nacional de Pesquisas da Amazônia, Manaus.

(***) — Department of Genetics, School of Medicine, Ribeirão Preto, SP.

This parasite develops relatively rapidly in hamsters. Hamsters inoculated with this strain develop histiocytomas in the feet within 3 months after inoculation. For this reason, we believe that the parasite belongs to the *Leishmania mexicana* complex, as defined by Lainson and Shaw (1973).

The origin of the *Leishmania* sp. Strain A is of a 41 year-old female patient with diffuse leishmaniasis. It is being maintained in our laboratory due to its rapid growth and high virulence. Its rapid development in hamsters, as well as its metastization in the hamsters' feet, suggests it also belongs to the *L. mexicana* complex (Lainson and Shaw 1973).

The amastigotes used in this experiment were obtained from tissue triturates of biopsies of leishmania lesions from infected hamster noses and were maserated in a mortar with a pestle with 2 ml of physiological saline. Small quantities of this triturate were inoculated subdermally into the noses of the test hamsters.

A hamster was recorded as "positive" when a definite warty growth or lesion on the nose developed. Persistence of the lesion and future metastization in the hamsters' extremities were also observed.

RESULTS

This experiment was terminated 150 days after inoculation with the respective *Leishmania* sp. No change in results had been noticed during the final 75 days, and only three hamsters which had previously been considered "suspicious" became "positive" after the 7th week post vaccination (Table 1).

As can be seen in Table 1, none of those hamsters which were maintained untreated and served as the controls (C), nor those inoculated with the "PF" vaccine alone (PF), became infected. One hundred percent of all hamsters inoculated with Strain A became "positive"; not only those solely inoculated with Strain A, but also those inoculated with the "PF" vaccine 8 and 15 days previously. Strain J showed itself to be much less virulent, only infecting 62.5% of inoculated hamsters.

It is interesting to observe that with every "PF" treatment there is a slight delay of *Leishmania* infectivity in the hamsters, as compared to the same treatment without the "PF" vaccine. This delay, however, may be due to the fewer number of treatments in the groups without the "PF" treatment.

TABLE 1. — N.º of hamsters which became positive with leishmaniasis after various treatments.

Treatment (1)	Weeks post-treatment											N.º		% Pos.	X N.º Weeks to become pos.
	1	2	3	4	5	6	7	8	9	10	11	Treat.	Pos.		
G	—	—	—	—	—	—	—	—	—	—	—	10	0	0	—
PF	—	—	—	—	—	—	—	—	—	—	—	10	0	0	—
8 + LA	—	—	—	—	1	4	—	—	—	—	—	5	5	100	5.8
PF + 8 + LA	—	—	—	2	2	3	2	—	—	1	—	10	10	100	6.0
15 + LA	—	—	—	—	5	—	—	—	—	—	—	5	5	100	5.0
PF + 15 + LA	—	—	—	—	4	4	2	—	—	—	—	10	10	100	5.8
8 + LJ	—	—	—	1	1	1	—	—	—	—	—	5	3	60	5.0
PF + 8 + LJ	—	—	—	—	5	2	—	1	—	—	1	10	9	90	6.2
15 + LJ	—	—	—	3	—	—	—	—	—	—	—	5	3	60	4.0
PF + 15 + LJ	—	—	—	—	2	2	—	—	—	—	—	10	4	40	5.5

(1) — See text for abbreviations.

CONCLUSIONS

Hamsters were utilized because of their intensive use as a laboratory tool in *Leishmania* studies. The "PF" vaccine as an anti-leishmanial vaccine is far from being adequate. Another vaccine produced from *Leishmania* parasites may suggest an avenue of attack to the high incidence of leishmaniasis in the Central Amazon region.

SUMÁRIO

A vacina antichagásica "PF" foi testada em hamsters contra duas cepas de *Leishmania*. Os animais vacinados com a cepa PF foram desafiadas 8 e 15 dias mais tarde com estas duas cepas de *Leishmania*. Após o término da experiência, i. e. 150 dias após inoculação, notou-se que a vacina "PF" não tinha efeito protetor contra *Leishmania*, em hamsters. Os leves aumentos do tempo de latência nos animais vacinados, sugerem que possivelmente uma vacina seja a arma para controlar a alta incidência desta doença na Amazônia Central.

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