SHORT REPORT: EVALUATION OF THE POTENCY AND STABILITY OF A CANDIDATE VACCINE AGAINST AMERICAN CUTANEOUS LEISHMANIASIS

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Abstract. Availability of a safe, immunogenic, and affordable vaccine would represent the best strategy for control of cutaneous leishmaniasis (CL). Stability in field conditions is a essential property for any candidate vaccine. The stability and immunogenicity of three different preparations (thimerosal-preserved, autoclaved, and lyophilized) of a killed *Leishmania amazonensis* vaccine were assessed using fresh products and after 12 months of storage at 4°C. Autoclaving was associated with a time-dependent decrease in the immunogenicity of the vaccine, as measured by the leishmanin skin test and production of interferon-γ. These findings are of importance in the decision of which preparation of candidate killed CL vaccines should move to phase III trials.

The leishmaniases are parasitic diseases caused by protzoa of the genus *Leishmania*. They are considered endemic in 88 countries in four continents, with most of them developing countries. The annual incidence of cutaneous leishmaniasis (CL) is estimated to be 1–1.5 million new cases.1

Control of CL is problematic due to the sylvatic nature of both vectors and reservoirs, making the insecticidal spraying and elimination of reservoirs specially difficult.2 Immunization of the population at risk appears to be the more cost-effective prophylactic measure against CL.1 In Brazil, several studies have been carried out using a preparation of killed promastigotes.4–6 It has been demonstrated that this candidate vaccine is safe and capable of inducing a protective immune response, as measured by the delayed hypersensitivity test result conversion (Montenegro skin test [MST]).5,6 Further characterization of the immune response induced by the candidate vaccine has shown development of cellular immunity with a TH1 profile7,8, an important feature for an effective vaccine against an intracellular pathogen.8

The candidate vaccine is composed of a suspension of killed promastigotes forms in phosphate-buffered saline preserved in thimerosal; therefore, it can undergo proteolytic degradation after storage.10 It is critical to know the effect of proteolysis on immunogenicity and whether different methods used for product stabilization can preserve it. The purpose of this study was to compare the immunogenicity and determine the stability of different preparations of a single-strain, killed candidate vaccine.

In the first phase of study carried out from February to May 1996, healthy adult volunteers living in the rural areas of Catatinga, Brazil, were randomly allocated to receive one of the following recently produced vaccine preparations: 1) autoclaved vaccine, 2) lyophilized vaccine and, 3) non-autoclaved vaccine. Twelve months later, another group of volunteers from the same geographic region received the vaccine preparations that were stored at 4°C.

The candidate vaccine under investigation was produced by bioBrás (Montes Claros, Brazil) under the good manufacturing practice guidelines using promastigote forms of *Leishmania amazonensis* (Leishvacin®, lot 5031016-1V), according to the methods originally described by Mayrink and others.4 All volunteers were MST-negative; each group was composed of 30 individuals. Volunteers and personnel involved in the trial were blinded with regard to which vaccine preparation was used. All volunteers received two doses of vaccine equivalent to 360 μg of nitrogen per dose at 21-day intervals. Adverse reactions were actively monitored for 72 hr after each injection. Forty days after the second dose, blood was collected for *in vitro* assessment of the proliferative response to *L. amazonensis* antigenic stimulus, and interferon-γ (IFN-γ) and interleukin 4 (IL-4) were measured in the supernatants of the mononuclear cell cultures. At this time, MST conversion was also assessed. Levels of IFN-γ were measured by a double-sandwich ELISA technique using B133.1 and B113.5 monoclonal antibodies (kindly provided by Dr. G. Trinchieri, Wistar Institute, Philadelphia, PA). Levels of IL-4 were determined using a commercial ELISA kit (InterTest-4®, Genzyme, Cambridge, MA). This study was approved by the Ethical Review Committee of the Federal University of Minas Gerais. Individual informed consent was obtained from study participants.

The groups were comparable in regards to gender (males comprised 52% of the total) and age (median = 32 years). Recently produced preparations induced similar MST conversion rates and levels of IFN-γ (Table 1), indicating that lyophilization or autoclaving processes did not impair the immunogenicity of fresh products. However, the autoclaved preparation showed a significant decrease in its immunogenicity after 12 months of storage at 4°C. The non-autoclaved and the lyophilized preparations retained their immunogenicity after the storage period. Production of IL-4 was not detected in any of the volunteers. The decreased immunogenicity of the autoclaved product has been also demonstrated in animal models.11

The autoclaving process seems to shorten the shelf-life of this *Leishmania* candidate vaccine. A recently published study carried out in Iran, using autoclaved-killed *L. major* plus bacille Calmette-Guérin, showed an MST conversion rate of 16.5% after one dose of vaccine.12 One of the possible reasons for this low immunogenicity might be the autoclaving process. Conversely, a field trial conducted in Ecuadorian children with two doses of a fresh, three-strain, killed *Leishmania* vaccine showed similar MST conversion rates.
Table 1

<table>
<thead>
<tr>
<th>Vaccine preparation</th>
<th>MST conversion (%)</th>
<th>No. of individuals producing IFN-γ (%)</th>
<th>Mean (SD) levels of IFN-γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoclaved recent</td>
<td>25/28 (89.3)</td>
<td>13/28 (46.4)</td>
<td>145.7 (68.9)</td>
</tr>
<tr>
<td>Non-autoclaved recent</td>
<td>25/28 (89.3)</td>
<td>13/28 (46.4)</td>
<td>131.4 (38.7)</td>
</tr>
<tr>
<td>Lyophilized recent</td>
<td>23/25 (92.0)</td>
<td>14/25 (56.0)</td>
<td>174.5 (71.9)</td>
</tr>
<tr>
<td>Autoclaved 12 months</td>
<td>8/30 (26.7)*</td>
<td>11/29 (37.9)†</td>
<td>62.2 (113.1)*</td>
</tr>
<tr>
<td>Non-autoclaved 12 months</td>
<td>28/30 (93.3)</td>
<td>17/26 (65.4)</td>
<td>171.8 (166.7)</td>
</tr>
<tr>
<td>Lyophilized 12 months</td>
<td>24/30 (80.0)</td>
<td>18/24 (75.0)</td>
<td>293.3 (209.7)</td>
</tr>
</tbody>
</table>

*P < 0.005.
† P = 0.005.

as those reported here. In future field trials of killed *Leishmania* vaccines, either thimerosal-preserved or lyophilized vaccines will be used instead of the autoclaved preparations.

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