ACTIVITY OF PENTAMIDINE-LOADED POLY (D,L-LACTIDE) NANOPARTICLES AGAINST LEISHMANIA INFANTUM IN A MURINE MODEL

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INTRODUCTION

The leishmaniases are parasitic diseases caused by haemoflagellate protozoa which are obligate parasites of the mononuclear phagocyte system of mammalian hosts. Clinical manifestations are multifaceted, ranging from self-healing cutaneous ulceration to progressive and lethal visceral infection. The leishmaniases cause important morbidity and mortality in the Old and New Worlds (Desjeux, 1996). Leishmania/HIV co-infections are regarded as emerging diseases, especially in southern Europe, where 25 to 70 % of adult visceral leishmaniasis (VL) cases are related to HIV infection, and 1.5 to 9 % of AIDS cases suffer from newly acquired or reactivated VL (Gradoni et al., 1995). In the Mediterranean basin, Leishmania infantum usually causes the visceral type of infection, which is fatal if not treated (Rosenthal et al., 1995). Pentavalent antimonials, available as sodium stibogluconate (Pentostam®) or N-methylglucamine antimoniate (Glucantime®) have been the standard first line treatment of VL for over 40 years (Neal, 1987). Pentamidine, a diamidine compound, and amphotericin B, an antifungal agent also active against Leishmania, are the second line treatments after antimony failure or intolerance. Today no other new major antileishmanial drug is available. However, clinical relapses occur regularly in HIV patients and resistances to antimonials are increasingly reported for some species as L. donovani (Modabber et al., 1992). Therefore, alternative therapies using new formulations of existing drugs are of poten-

Summary:

The activity of pentamidine-loaded poly(D,L-lactide) nanoparticles was compared, by determination of median effective doses (ED_{50}), to that of free pentamidine in a murine model of visceral leishmaniasis induced by Leishmania infantum. BALB/c mice were infected intravenously on day 0 with promastigotes and then treated on days 4, 14, 16, and 18. Groups of 5 mice received either 0.57, 1.14 and 2.28 mg/kg of free pentamidine (expressed in pentamidine base) or 0.055, 0.11, 0.22 and 0.44 mg/kg of pentamidine-loaded nanoparticles. In the control group, 12 mice received normal saline. The liver parasite burden was evaluated using the Stauber method 72 h after the last injection and drug levels in livers and spleens were determined. Bound pentamidine was 3.3 times more active than free drug (ED_{50} value = 0.32 mg/kg versus 1.05 mg/kg for free drug). Drug levels showed a weak accumulation in hepatic and splenic tissues following bound pentamidine administration. A lack of acute toxicity was noted in all groups treated by bound pentamidine. Results obtained with this biodegradable carrier may be of particular interest as no new major antileishmanial compound is today available.

KEY WORDS: pentamidine, poly(D,L-lactide) nanoparticles, mouse model, effective doses, leishmania infantum, visceral leishmaniasis.

Résumé: L'efficacité de nanoparticules d'acide poly lactique chargées en pentamidine sur un modèle murin infecté par L. infantum

Les nanoparticules d'acide poly(lactide) chargées en pentamidine ont été comparées, par la détermination des doses efficaces 50 % (ED_{50}), à celle de la pentamidine libre dans un modèle murin de leishmaniose viscérale induite par Leishmania infantum. Douze souris BALB/c ont été infectées par voie intraveineuse avec des formes promastigotes et ont ensuite été traitées à J14, J16 et J18. Des lots de 5 souris ont reçu soit 0.57, 1.14 et 2.28 mg/kg de pentamidine libre (exprimée en pentamidine base) soit 0.055, 0.11, 0.22 et 0.44 mg/kg de pentamidine vectorisée. Douze souris ont reçu du chlorure de sodium isotonique dans le groupe témoin. La charge parasitaire a été évaluée dans le foie par la méthode de Stauber 72 heures après la dernière injection et les concentrations en pentamidine ont été dosées dans les tissus hépatiques et spléniques. La pentamidine vectorisée a été 3.3 fois plus active que la pentamidine libre avec une ED_{50} égale 0.32 mg/kg versus 1.05 mg/kg pour la pentamidine libre. Les dosages dans le foie et la rate ont montré une faible accumulation du principe actif dans ces organes après administration de la pentamidine vectorisée comparativement à la forme libre. Une absence de toxicité aiguë a été notée pour tous les groupes traités par la pentamidine vectorisée.

MOTS CLÉS: pentamidine, nanoparticules, acide poly lactique, modèle murin, doses efficaces, Leishmania infantum, leishmaniose viscérale.
tial interest. Drug delivery systems, already used in cancer treatment (Astier et al., 1988), may reduce the toxicity and improve the activity of drugs as antileishmanial compounds. This approach is also applicable to diseases involving intracellular pathogenic agents as bacteria, viruses or parasites (Fattal et al., 1989).

The different classes of carriers that have been used to target antileishmanial compounds are red cell ghosts, liposomes and nanoparticles. Pentamidine containing human red cell ghosts gave promising results in vitro but their biological origin and their delicate mode of preparation prevented their further development (Berman et al., 1986). Liposomes loaded with antimicrobial drugs led to an improved anti-leishmanial activity but the commercial production of liposomal Sb5 was abandoned because of toxicity in monkeys (New et al., 1980). Liposomes loaded with amphotericin B (Ambisome®) proved to be a progress in the fight against VL (Torre-Cisneros et al., 1993; Davidson et al., 1996). Results of clinical trials with Ambisome® in Europe suggest that short courses and low doses may reliably cure immunocompetent VL patients, whereas patients co-infected with HIV may require maintenance therapy. However the prohibitive cost of the currently available formulation of liposomal amphotericin B is a major impediment to its use. Other lipid formulations (Amphocil®, Abelcet®) less expensive than Ambisome® are currently being evaluated (Hiemenz et al., 1996). Nanoparticles have been used to target antileishmanial drugs in experimental studies. Amphotericin B was incorporated into poly(D,L-lactide-coglycolide) nanoparticles and showed a good activity in vitro (Venant-Julienne et al., 1995). Primaquine-loaded poly(DL-lactide) nanoparticles (PLA) were more active than free drug in a model associating Leishmania donovani and BALB/c mice (Rodrigues et al., 1994). In our previous studies, pentamidine loaded on polymethacrylate nanoparticles (PM) was 6 fold more active than free drug in a model associating L. infantum and BALB/c mice (Durand et al., 1997). The main problem of these nanoparticles was their low biodegradability. Conversely, lactide polymers and co-polymers are known to be biodegradable (Makino et al., 1985; Lewis et al., 1990). These polymers are slowly hydrolysed in D and L lactic acid. The latter is a metabolite of the Krebs cycle (Bazile et al., 1992). The aim of the present study was to evaluate the activity of pentamidine-bound PLA nanoparticles by determination of median effective doses against Leishmania infantum in a murine model.

**MATERIAL AND METHOD**

Pentamidine isethionate was purchased from Roger Bellon (France). The phospholipid mixture (Lipoid S 75) was supplied by Lipoid GmbH (Ludwigshafen, Germany). Poloxamer (Symperonic PE/F-68®) was purchased from ICI (Clamart, France). Polymer poly(D,L-lactide) (PLA, mol. wt. (GPC) 200,000) was supplied by Boehringer Ingelheim (Ingelheim, Germany). Other reagents were analytical grade.

**PENTAMIDINE-LOADED ON POLY (D,L-LACTIDE) NANOPARTICLES**

Pentamidine base was previously obtained by precipitation of pentamidine isethionate solution in alkaline medium (25 % ammonium hydroxide) at 4 °C. The precipitate was filtered and dried under vacuum. PLA nanoparticles were prepared by adaptation of the method reported by Fessi (Fessi et al., 1987). One milligram of pentamidine base, 125 mg of phospholipids and 125 mg of PLA were dissolved in acetone (25 ml). This solution was mixed to the alkaline aqueous solution (1 ml of 25 % ammonium hydroxide) containing 125 mg of Poloxamer. After 15 minutes of stirring, the acetone and part of the water were evaporated under vacuum. The final volume was 10 ml. The colloidal suspension was monodispersed. The size of nanoparticles was 150 ± 20 nm. This formulation was stable for at least one month at 4 °C. Before use, nanoparticles were diluted in normal saline to obtain a final concentration of 8 µg of pentamidine base per mg of polymer.

**PENTAMIDINE ISETHIONATE**

Concentrations were expressed in base which represented 57 % of the salt molecular weight. The different dilutions were carried out in 5 % dextrose because of the low stability of pentamidine in normal saline.

**LEISHMANIA STRAIN**

The strain of Leishmania was identified by the Reference Center of OMS (Montpellier) as Leishmania infantum MON 1 (reference MHOM/PT/93/CRE 41). The zymodeme of this strain is usually responsible for visceral leishmaniasis. Leishmania were injected in the hamster by intraperitoneal route to increase its virulence and maintained in NNN-medium at 27 °C for 8 days. Bulk culture of the infectious promastigote forms was initiated and propagated in RPMI 1640 medium (Eurobio, France) supplemented with 0.15 % sodium bicarbonate (BioMérieux, France), 20 % heat-inactivated fetal calf serum (DAP, France), 20 % Schneider-medium (Gibco Ltd, United Kingdom), 1 % L-glutamine (BioMérieux, France) and 0.066 % gentamicine (Pharmacie Centrale des Hôpitaux, Paris, France). The promastigotes reached their infectious metacyclic phase after 8 days, at 27 °C.

**ANIMALS**

Male adult BALB/c mice (5-week old, 20 g ± 2 g) were purchased from IFFA CREDO, (L’Arbresle, France). The infection model used was modified from Neal et al., 1985. The main difference was the use of stationary pro-
mastigotes instead of amastigotes obtained from hamster spleen. Promastigotes were obtained by centrifugation and resuspended in normal saline solution. On day 0, mice were infected by intravenous injection (tail vein) with \(10^7\) infective *Leishmania infantum* promastigotes in a 0.1 ml volume. This procedure induced a heavy *Leishmania* liver burden after 12 days. The mice were randomly assigned into three groups: the control group (normal saline), the group treated with free pentamidine (pentamidine isethionate) and the group treated with pentamidine-loaded poly(D,L-lactide) nanoparticles.

**GROUPS**

In the control group, 12 mice were tested. The different doses were chosen according to our previous studies to obtain the median effective dose (ED<sub>50</sub>) for each regimen (Fusai et al., 1994). In the treated groups, 5 mice received 0.57, 1.14 or 2.28 mg/kg of free pentamidine (expressed in pentamidine base) on D<sub>14</sub>, D<sub>16</sub> and D<sub>18</sub> and 5 mice received 0.055, 0.11, 0.22 or 0.44 mg/kg of pentamidine-loaded nanoparticles (expressed in pentamidine base) in 0.1 ml by tail vein. Twenty-one days after the initial infection, the animals were killed by cervical dislocation. The Guiding Principles for Biomedical Research involving animals were followed during all procedures (CIOMS, 1985). The following parameters were used to assess treatment efficacy:

- Parasite burden: the liver parasite burden was evaluated after Giemsa staining of the smears. The number of amastigotes per 500 hepatocytes was calculated and related to the liver weight (mg), following the Stauber formula (Stauber et al., 1958). The percentage of parasite suppression was calculated as follows:
  
  \[
  \text{Percentage of parasite suppression} = \left[1 - \frac{\text{mean of Stauber count of the treated group}}{\text{mean of Stauber count of control group}}\right] \times 100
  \]

- Median effective doses: the ED<sub>25</sub> and ED<sub>50</sub> (dosages of drug calculated to eliminate 25 % and 50 % of organisms compared to controls) were determined using a Michaelis-Menten model.

- Levels of pentamidine in tissues: weighed aliquots of liver and spleen were immersed into liquid nitrogen. Pentamidine levels in liver and spleen of mice treated with pentamidine isethionate and pentamidine-loaded (D,L-lactide) nanoparticles were determined by high-performance liquid chromatography. Briefly, after thawing, samples were homogenized in 0.5 ml of phosphate buffer saline and pentamidine was extracted by acetonitrile (4 ml) containing hexadimine as an internal standard. The extract was introduced on a solid-phase column (Bond-Elut C<sub>18</sub>, 1 ml) under pressure. Pentamidine was eluted with a 1 ml of a solution composed of 0.5 % heptane-sulfonic acid, 0.02 % tetramethylammonium chloride and 0.1 % phosphoric acid in methanol.

The eluate was injected onto a reverse-phase C<sub>18</sub> column (Shandon, Hypersil, 5 μm, 250 mm × 4.6 mm, ID, France). The mobile phase was composed of 700 ml of methanol and 300 ml of a 0.05 M of heptane-sulfonic acid and 0.014 M of diethylamine aqueous solution. The pH of the aqueous solution was adjusted to 3 using phosphoric acid. The flow rate was 0.9 ml/min. Detection of pentamidine was performed by ultraviolet absorption at 280 nm. The limit of detection was 25 ng/g.

**STATISTICAL ANALYSIS**

Results were expressed as mean ± standard error. An one-way analysis of variance or a U-test was performed to compare the influence of the various parameters. A p value lower than 0.05 was considered as statistically significant.

**RESULTS**

**CONTROL EXPERIMENTS**

In mice treated with normal saline solution, there was a mean of 3.5 (range = 3.3 – 3.7, N = 12) *Leishmania infantum* amastigotes per liver cell nucleus at the end of the 21-day period of experimentation. The average liver parasite burden reached was \(13 \times 10^8\) amastigotes (Stauber count) for the control group for \(10^7\) promastigotes inoculated 21 days before as described in table I.

<table>
<thead>
<tr>
<th>Group</th>
<th>Amastigotes counted per 500 hepatocytes</th>
<th>Stauber count 10&lt;sup&gt;6&lt;/sup&gt;</th>
<th>Percentage of suppression %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.745 ± 0.9</td>
<td>12.75 ± 0.65</td>
<td>—</td>
</tr>
<tr>
<td>Pentamidine-loaded nanoparticle dose (mg/kg)</td>
<td></td>
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<tr>
<td>0.055</td>
<td>1.496 ± 0.42</td>
<td>9.63 ± 0.68</td>
<td>24.4 ± 5.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.11</td>
<td>1.302 ± 0.38</td>
<td>8.34 ± 0.33</td>
<td>34.6 ± 2.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.22</td>
<td>1.210 ± 0.49</td>
<td>7.62 ± 0.42</td>
<td>40.2 ± 3.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.44</td>
<td>1.087 ± 0.64</td>
<td>7.53 ± 0.44</td>
<td>58.2 ± 3.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pentamidine isethionate dose (mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.57</td>
<td>1.053 ± 0.24</td>
<td>7.39 ± 0.43</td>
<td>42.1 ± 3.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.14</td>
<td>0.864 ± 0.87</td>
<td>6.22 ± 0.67</td>
<td>51.2 ± 5.3</td>
</tr>
<tr>
<td>2.28</td>
<td>0.785 ± 0.21</td>
<td>5.39 ± 0.26</td>
<td>57.7 ± 2.1</td>
</tr>
</tbody>
</table>

<sup>a</sup>0.055 vs 0.22 and 0.57; 0.11 vs 1.14; 0.44 vs 0.57 = p < 0.05.
<sup>b</sup>0.055 vs 1.14; 0.22 vs 0.44 and 2.28; 0.57 vs 2.28 = p < 0.01.
<sup>c</sup>0.055 vs 0.44 and 2.28; 0.11 vs 0.44 and 2.28 = p < 0.001.

Table I. — Suppression of experimental leishmaniasis in mice treated with pentamidine loaded poly(D,L-lactide) nanoparticles and free pentamidine. The suppression percentage was calculated using Stauber count vs untreated mice. Data are expressed as mean ± standard error for 5 mice per group except control group (n = 12).

U-test was used to compare the different groups.
Fig. 1. — Semilogarithmic plot of suppression of experimental leishmaniasis in mice treated with pentamidine loaded (D,L-lactide) nanoparticles (○: doses ranging from 0.055 to 0.44 mg/kg) or with pentamidine isethionate (●: doses ranging from 0.57 to 2.28 mg/kg). The suppression percentage was calculated using the Stauber count vs untreated mice as described in the experimental section. Suppression was expressed as the percentage of the maximal infestation. Data were fitted to a Michaelis-Menten model (r=0.963) for pentamidine loaded (D,L-lactide) nanoparticles and (r = 0.999) for pentamidine isethionate. Each point represents the mean ± SE for 5 mice per group.

Table II. — Activity of pentamidine (bound or free) in a Leishmania infantum infected mice. The effective doses (ED) were calculated using a Michaelis-Menten model and were expressed in mg/kg.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ED_{25}</th>
<th>ED_{50}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free pentamidine (F)</td>
<td>0.20±0.06</td>
<td>1.05±0.102*</td>
</tr>
<tr>
<td>Pentamidine-loaded nanoparticles (B)</td>
<td>0.070±0.009</td>
<td>0.32±0.07</td>
</tr>
<tr>
<td>Ratio ED (F/B)</td>
<td>2.9</td>
<td>3.3</td>
</tr>
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</table>

*ED_{50} (F) vs ED_{50} (B) = p < 0.001.

The maximal percentage of parasite suppression was obtained with 0.44 mg/kg of pentamidine-loaded nanoparticles (58.2 ± 3.5 %) (Table I). This result was not significantly different from the one observed with 2.28 mg/kg of pentamidine isethionate (57.7 ± 2.1 %). The relationship between the percentage of parasite suppression and the dose is represented in figure 1. The Michaelis-Menten model was used to fit the data obtained from groups treated with pentamidine-loaded nanoparticles (r = 0.963) or with isethionate pentamidine (r = 0.999). The maximal effect (E_{max}) calculated with pentamidine-loaded nanoparticles was not significantly different from that calculated with pentamidine isethionate (70.4 % vs 65.8 %).

EVALUATION OF THE EFFECTIVE DOSES
Pentamidine-loaded nanoparticles were 3.3 fold more active than free drug: ED_{50}=1.05 mg/kg (pentamidine isethionate) vs 0.32 mg/kg (pentamidine loaded nanoparticles). The ED_{50} values were not determined as a plateau was reached for 70% of parasite suppression. The other values of the effective doses are presented in table II.

PENTAMIDINE LEVELS
Administration of pentamidine-loaded nanoparticles (0.44 mg/kg) led to similar drug levels, in liver and spleen, to that reached with 2.28 mg/kg of isethionate pentamidine (Table III). The maximal concentrations obtained in liver and in spleen were 1.4 μg/g and 0.56 μg/g, respectively. For all dose tested, drugs levels were higher in liver than in spleen. The drug levels in the liver were 2 fold higher than those in the spleen. The relationship between dose and concentration was linear for pentamidine-loaded nanoparticles (Fig. 2) and for pentamidine isethionate.

Table III. — Pentamidine levels in liver and spleen. Mice were killed 3 days after cessation of treatment. Each mouse received 3 doses of free pentamidine or pentamidine loaded on PLA on days 14, 16 and 18.

|Mice were killed 3 days after cessation of treatment. Each mouse received 3 doses of free pentamidine or pentamidine loaded on PLA on days 14, 16 and 18. The values are expressed as means±standard errors for 5 mice per group.|

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Liver (μg/g)</th>
<th>Spleen (μg/g)</th>
<th>Liver (μg/g)</th>
<th>Spleen (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.055</td>
<td>—</td>
<td>—</td>
<td>0.30±0.040</td>
<td>0.20±0.042</td>
</tr>
<tr>
<td>0.11</td>
<td>—</td>
<td>—</td>
<td>0.42±0.060</td>
<td>0.25±0.042</td>
</tr>
<tr>
<td>0.22</td>
<td>—</td>
<td>—</td>
<td>0.73±0.055</td>
<td>0.32±0.065</td>
</tr>
<tr>
<td>0.44</td>
<td>—</td>
<td>—</td>
<td>1.29±0.078</td>
<td>0.56±0.036</td>
</tr>
<tr>
<td>0.57</td>
<td>0.48±0.043</td>
<td>0.24±0.031</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1.14</td>
<td>0.82±0.065</td>
<td>0.32±0.046</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2.28</td>
<td>1.40±0.072</td>
<td>0.54±0.110</td>
<td>—</td>
<td>—</td>
</tr>
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</table>

Fig. 2. — Intratissular concentrations of pentamidine in liver and spleen in Leishmania infantum infected mice, 3 days after the last injection. Mice were treated on days 14, 16 and 18 with pentamidine-loaded (D,L-lactide) nanoparticles (doses ranging from 0.054 to 0.44 mg/kg). Each point represents the mean ± standard error (n = 5).
DISCUSSION

Results showed that bound pentamidine was more potent than free drug against Leishmania infantum in our BALB/c mice model. The ratio of median effective doses (ED$_{50}$) for free and bound drug was 3.3. The low ED$_{50}$ value observed for bound pentamidine (0.32 mg/kg) demonstrated the ability of PLA nanoparticles to induce a higher activity and a lower toxicity of pentamidine for the treatment of VL. It was not possible to obtain the ED$_{50}$ for free and bound pentamidine as the dose response curves reached a plateau at 70% of parasite suppression.

Red cell ghosts containing pentamidine tested in a L. donovani Syrian hamster model led to very similar results with a reported ED$_{50}$ of 0.38 mg/kg (Berman et al., 1986). Immunoglobulin ghost red cells had a better efficacy, nevertheless the use of such natural materials had limited their further development. The enhancement of pentamidine activity observed with PLA nanoparticles was lower than that obtained with polymeric nanocapsules evaluated on the same in vitro model (ED$_{50}$ ratio = 3.3 versus 6) (Durand et al., in press). Conversely, the enhancement of activity of primaquine, an antimalarial compound which also has antileishmanial activity, by targeting it on PLA nanoparticles against L. donovani in a BALB/c mice model, was identical to that observed with pentamidine-bound PLA nanoparticles. However, authors reported a relatively high ED$_{50}$ value (6.6 mg/kg) for bound primaquine (Rodrigues et al., 1994). These findings may be due to a low intrinsic activity of primaquine against L. donovani.

In our model, the parasite load increased to 13 x 10$^8$ amastigotes per liver after 12 days and remained at that level for at least 5 weeks allowing time to test for drug suppression of parasites. The different doses were chosen according to our previous studies to obtain the ED$_{50}$ for each regimen. The unloaded nanoparticles were not tested in this work as their lack of activity has already been reported in a model associating L. donovani and BALB/c mice (Rodrigues et al., 1994). Determination of drug levels 3 days after the last injection showed a weak drug accumulation in hepatic and splenic tissues following PLA bound pentamidine administration compared to free drug. Pentamidine carried on human red cell ghosts or liposomes concentrated more markedly than pentamidine carried on PLA in the liver and in the spleen in rodents (Berman et al., 1986; Debs et al., 1987). Pentamidine levels were lower in the spleen than in the liver as it was previously described by authors (Debs et al., 1987). A drug accumulation in hepatic and splenic tissues following the use of drug carriers was already reported for other antileishmanial compounds (Gangneux et al., 1996).

The drug accumulation in tissues and the drug targeting mainly in the mononuclear phagocytes led to an increase in drug delivery to intracellular leishmania and explained probably most of the enhancement of activity observed.

The lower activity observed with pentamidine-loaded PLA nanoparticles compared to pentamidine-loaded PM nanoparticles may be due to dissimilar kinetics of drug release. Actually, mechanisms of pentamidine binding to PM and PLA carriers are different. Pentamidine was bound by ionic interaction to PM (Paul et al., in press) whereas it was bound mainly by adsorption to PLA (data not shown). Pentamidine release from PLA may be easy and therefore, may occur in close contact to leishmania. In contrast, pentamidine release from PM, which requires a pH value near 5 (found in the parasitophorous vacuole 48 h after the leishmania phagocytosis (Antoine et al., 1990), may be more finely controlled. The major advantage of PLA compared to PM nanoparticles is their biodegradability. PLA nanoparticles are biocompatible and were approved by the Food and Drug Administration for human administration (Makino et al., 1985). PLA were well tolerated in mice at the maximal dose tested of 1,000 mg/kg and no sign of acute toxicity was observed (Rodrigues et al., 1995). Results obtained with L. infantum are of particular interest because it is the most frequently Leishmania species isolated from HIV patients in mediterranean countries. Bound-pentamidine PLA nanoparticles require now further investigations particularly in immunodepressed mice models in order to evaluate more accurately their interest for HIV-Leishmania co-infected patients.

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