Efficacy of aminosidine administered alone or in combination with meglumine antimoniate for the treatment of experimental visceral leishmaniasis caused by *Leishmania infantum*

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**Introduction**

Aminosidine is an aminoglycoside antibiotic, with an identical chemical structure to paromomycin and monomycin. Previous experimental studies showed that this drug was effective against *Leishmania major*, *Leishmania tropica* and *Leishmania donovani* with various degree of sensitivity, but limited data on the efficacy of aminosidine against *Leishmania infantum* are available. In human visceral leishmaniasis (VL), several studies reported a favourable outcome after administration of aminosidine alone or combined with pentavalent derivatives of antimony in African and Indian kala-azar. Only four documented cases of *L. infantum* VL were treated with aminosidine, two were cured and a partial parasitological response was seen in the other two, in whom a complete cure was achieved with the addition of sodium stibogluconate or amphotericin B. In southern Europe, therapeutic difficulties remain for the treatment of VL caused by *L. infantum*, which is an important opportunistic infection in AIDS patients. The relapses after initial treatment and the high cost of second-line therapies such as lipid formulations of amphotericin B (AmB) require the assessment of alternative drugs.

**Materials and methods**

**Mice and strain of *L. infantum***

Experiments were conducted with adult BALB/c female mice (Iffa Credo, Lyon, France), at an early or a late stage of infection, following challenge with a strain of *L. infantum* (MHOM/FR/91/LEM2259V) isolated from an AIDS patient. On day 0, each mouse was inoculated iv with $10^7$ promastigotes, i.e. 0.2 mL of inoculum iv per mouse.

**Therapeutic protocols**

Mice were assigned randomly to two groups: (i) the early treatment group, which was treated from day 7 to day 17; (ii) the delayed treatment group, which was treated from day 60 to day 70. Infected and untreated mice were used for control. In each group, the following regimens were evaluated. Meglumine antimoniate (glucantime, Specia, Rhône-Poulenc Rorer, Paris, France) 200 mg/kg body weight was administered daily for 11 days (total dosage, 2200 mg/kg). For each injection, ampoules of meglumine antimoniate (pentavalent antimony 85 mg/mL) were combined with aminosidine sulphate alone or combined with meglumine antimoniate by a sensitive sequential quantification of parasite burdens in the liver and spleen.

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diluted with 5% glucose. Aminosidine sulphate (Gabbromycina, Farmitalia–Carlo Erba, Milan, Italy) 20 or 50 mg/kg was administered daily for 11 days. Vials with 500 mg lyophilized powder of aminosidine sulphate were re-suspended in 4 mL sterile distilled water. Drug solutions were prepared daily, then diluted in 5% glucose before administration and 0.2 mL was injected into each mouse by ip (meglumine antimoniate) or sc (aminosidine) injections.

Evaluation of drug activities

Mice were followed for 4 months after infection. Sequential examinations of parasite burden were performed at the following intervals after infection: days 7, 20, 60 and 120 in the early treatment group, and days 72 and 125 in the delayed treatment group. At each time point, three to five mice from treatment and control groups were killed. Parasite burdens in the liver and spleen homogenates were determined as previously described. 

Parasite burden was expressed as the log_{10} of the number of parasites per gram of tissue and the mean value (± standard error) for parasite burden of three to five mice was calculated for each time point.

Results and discussion

This study was designed to evaluate the efficacy and the tolerance of aminosidine sulphate for the treatment of an experimental VL caused by L. infantum. One of the 30 infected and untreated control mice died before the end of the experiment. All mice treated with meglumine antimoniate 200 mg/kg (n = 24) or treated with aminosidine 20 mg/kg (n = 24) or 50 mg/kg (n = 24) survived. By contrast,

Figure 1. Kinetics of parasite burdens in the liver and spleen of mice infected iv on day 0 with 10^7 promastigotes of L. infantum. Early treatment group received antimicrobial agents from day 7 to day 17 after infection. Mice were treated daily with glucantime 200 mg/kg, aminosidine 20 or 50 mg/kg, and the combination glucantime 200 mg/kg plus aminosidine 20 or 50 mg/kg. Each point represents the mean ± S.E.M. for three to five mice. ○, Control; □, glucantime 200; ▄, aminosidine 20; △, aminosidine 50; ■, glucantime + aminosidine 20; ▲, glucantime + aminosidine 50.

Figure 2. Kinetics of parasite burdens in the liver and spleen of mice infected iv on day 0 with 10^7 promastigotes of L. infantum. Delayed treatment group received antimicrobial agents from day 60 to day 70 after infection. Mice were treated daily with glucantime 200 mg/kg, aminosidine 20 or 50 mg/kg, and the combination glucantime 200 mg/kg plus aminosidine 20 or 50 mg/kg. Each point represents the mean ± S.E.M. for three to five mice. ○, Control; ○, glucantime 200; □, aminosidine 20; △, aminosidine 50; ■, glucantime + aminosidine 20; ▲, glucantime + aminosidine 50.
in both groups of mice treated with the combinations meglumine antimoniate plus aminosidine, six of 24 mice (25%) died during the treatment period.

A n early treatment from day 7 to day 17 (Figure 1) with meglumine antimoniate 200 mg/kg resulted in a marked reduction of the hepatic parasite load compared with untreated mice. In the spleen, the parasite load was only decreased by 1 log_{10} parasites per gram of tissue. Treatment with aminosidine 20 mg/kg never decreased the parasite loads by more than 1 log_{10} in liver and spleen, compared with untreated mice, and did not prove more efficacious than meglumine antimoniate. Treatment with aminosidine 50 mg/kg decreased the parasite loads in liver and spleen by 1 and 0.8 log_{10} respectively, compared with mice treated with meglumine antimoniate. When the drugs were combined, an increased efficacy compared with either drug given alone was observed. No parasites were detected in the liver homogenate subculturings and this negativity persisted for at least 4 weeks after cessation of early therapy. Meanwhile, the combination was inefficient in eradicating parasites, and promastigotes were detectable on day 120 in the liver of all mice, although at a lower level than in the groups treated with aminosidine alone. When treatments were administered alone or combined from day 60 to day 70 post-infection (Figure 2), the mice presented with chronic VL with high and stable parasite loads, and efficacies were lower than in the early treatment group.

Using the same model, we noticed previously that meglumine antimoniate failed to eradicate parasites from liver and spleen of infected mice. In this study, the drug combination aminosidine plus meglumine antimoniate was also unable to eradicate parasites. Experiments conducted by Neal et al. clearly showed variation of the susceptibility of Leishmania species to aminosidine, with activity against L. major, L. tropica and L. donovani, whereas L. infantum was more refractory. The microtitration method that we used for the determination of parasite burdens allowed us to determine the presence of viable parasites in several organs in infected mice, using a long-term follow-up. This method, which proved a sensitive one to evaluate the efficacy of reference therapies against L. infantum, shows the poor efficacy of aminosidine in the treatment of experimental VL caused by L. infantum.

References

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