CLINICAL RECOVERY AND LIMITED CURE IN CANINE VISCERAL LEISHMANIASIS TREATED WITH AMINOSIDINE (PAROMOMYCIN)


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Abstract. Three groups of three, six, and 12 dogs with parasitologically proven clinical visceral leishmaniasis (Leishmania chagasi infection) were treated with intramuscular aminosidine sulfate at doses of 20 mg/kg/day for 15 days; 80 mg/kg/day for 20 days, and 40 mg/kg/day for 30 days, respectively. Follow-up was by parasitologic examination of bone marrow and skin, serology using the indirect immunofluorescent antibody test, and clinical examination for signs of visceral leishmaniasis or adverse effects of treatment. In animals treated with 20 mg/kg/day, for 15 days, there was dramatic clinical improvement with disappearance of conjunctivitis, increase in appetite, weight gain, and recovery of normal skin condition and a healthy coat, but parasitologic relapse occurred between 50 and 100 days after initiation of treatment. Adverse effects were seen with treatment with 80 mg/kg/day for 20 days; three dogs died during or just after treatment, two showed temporary recovery, and one showed total clinical and parasitologic cure that was maintained for four years. Although adverse effects and relapses were seen in some dogs treated with 40 mg/kg/day for 30 days, three of 12 dogs showed complete parasitologic and clinical cure that was sustained for at least four years. Aminosidine treatment cannot be recommended as an alternative to the humane destruction of dogs for the control of canine visceral leishmaniasis because ineffective treatment may prolong carrier status or encourage development of drug resistance. This drug may be a therapeutic option if there is no danger of a dog acting as a reservoir of infection. Achievement of clinical recovery and limited cure with aminosidine suggests that further trials would be of value, possibly in combination with other anti-leishmanial drugs and with supportive measures to reduce adverse effects.

Canine visceral leishmaniasis (VL) is caused by the protozoan parasite Leishmania infantum in the Old World and by Leishmania chagasi in the New World. These two Leishmania species are very closely related and some authorities consider them to be virtually indistinguishable. Clinically, Old World and New World canine VL give similar presentations, causing signs such as swelling of the popliteal lymph nodes, dermatitis around the eyes and nose, disseminated alopecia, weight loss, edema, and hepatosplenomegaly. The dog is considered to be an important reservoir of infection for human VL, except in endemic areas of the Old World where the disease agent is L. donovani, and not L. infantum/chagasi. Although residual or ultra-low volume insecticide spraying may be useful for attacking the sandfly vectors, there are two principal difficulties obstructing the direct control of canine VL.

First, diagnosis of canine VL is unreliable, due to the lack of a proven, highly sensitive, specific, simple assay for antibodies that can be performed on site during household visits. Serologic screening followed by the elimination of seropositive dogs has been adopted as the primary direct method for control of canine VL in Latin America, and yet its impact on disease transmission is not clear. The alternative approach of parasitologic diagnosis requires intensive effort, and diagnostic DNA probes for field use have yet to be perfected.

Second, there is no effective drug for the treatment of canine VL. Pentavalent antimonials, which are of great value for treatment of the human disease, are used in some countries of the Mediterranean region to treat symptomatic canine VL or asymptomatic dogs that are serologically positive. Clinical signs of canine VL may disappear, although infections usually relapse soon after antimonial treatment is withdrawn. Treatment is often repeated every few months but such repeated use of noncurative doses of antimonials is hazardous: drug-resistant organisms are likely to arise, which may be transferred to humans, and apparently healthy infected dogs may act as a prolonged source of infection to sand flies. Attempts to use antimonials for the treatment of canine VL in the New World have been unsuccessful.

During the last decade there has been renewed interest in the anti-leishmanial activity of the aminoglycoside antibiotic aminosidine (paromomycin). The drug is active in vitro and in vivo against various Leishmania species. Clinical studies have confirmed that it is a very useful alternative to antimonials for the treatment of human VL, although like antimonials it requires parenteral administration. Single-agent aminosidine treatment was found to have limited efficacy in cutaneous leishmaniasis in South America. Aminosidine has been used with great success in clinical trials of combination therapy with antimonials for human VL.

The aims of these experiments were to follow clinical recovery, relapse, and clinical and parasitologic cure in symptomatic canine VL treated with aminosidine.

METHODS

Study area. This study took place in Teresina, the capital of Piaui state in northeastern Brazil, where human and canine VL are endemic. There have been two epidemics of human disease, one in 1993, and a previous outbreak in the early 1980s. The city is located between and adjacent to two rivers, the Parnaiba and the Poty, which influence the vegetation and climate. The climate in Teresina is tropical
with temperatures fluctuating between 22°C and 32°C. There is a rainy season between December and April (approximate cumulative rainfall = 160 mm) and a dry season from May to November (rainfall = 20 mm). Transmission of VL is also thought to be seasonal with changing vector abundance.

A new kennel facility was built at the Department of Parasitology, Federal University of Piauí such that treated dogs could be kept hygienically and separately without exposure to sandfly bites. Doors and windows were screened against sandflies with fine mesh, and the entire building was sprayed monthly with residual pyrethroid insecticide.

**Selection of animal groups.** A total of 21 dogs were treated. All animals had been brought to the Department of Parasitology for clinical, parasitologic, and serologic examination because the owners suspected the presence of canine VL. Dogs were parasitologically proven to be infected with *L. chagasi* before entering the treatment trial and were allocated sequentially into the experimental groups. The owners of the dogs donated the animals to the trial in knowledge that in accordance with local public health recommendations, the only alternative was to have them destroyed.

The first group of dogs consisted of three adult Doberman pinschers. The second group of six adults was composed of two Doberman pinschers, one German shepherd, one tawny boxer, one English cocker spaniel, and one toy terrier. The third group of 12 adults was composed of three Dobermann pinschers, one German shepherd, one fila Brasileiro, two toy terriers, one Irish setter, one short-haired dachshund, one bichon frise, and two of unidentified breed.

**Treatment.** Treatment was with aminosidine sulfate (Gabbromicina®; Farmitalia Carlo Erba, Milan, Italy) dissolved in sterile distilled water and administered by intramuscular injection, in accordance with the manufacturer’s instructions. Group one was given 20 mg/kg of body weight daily for 15 days, group two 80 mg/kg for 20 days, and group three 40 mg/kg for 30 days.

The three groups were not treated simultaneously; results from group 1 were available before groups 2 and 3 were started. No specific control group of untreated animals was set up for this experiment, but during the course of this project several animals of similar clinical and parasitologic status were maintained for experiments on the transmissibility of canine VL to sandflies.23, 24 All such animals showed progressive symptomatic canine VL, none recovered, and towards the terminal stages of the disease they were humanely killed.

**Clinical, parasitologic, and serologic examination.** All animals were examined clinically for signs of depilation and exfoliative dermatitis, external lesions, abnormal claws, and conjunctivitis/keratitis, and compared with the normal weight range for the breed.25 Parasitologic examination was by microscopy of Giemsa-stained impression smears of skin biopsies and sternal bone marrow aspirates. All animals included in the study had amastigotes in the skin and/or bone marrow. Bone marrow samples were obtained by elevation of the head of the dog in a sitting position and sternal puncture, which was rapid and well-tolerated. Serologic examination was by the indirect immunofluorescent antibody test (IFAT) as described previously, based on comparisons of the IFAT, enzyme linked immunosorbent assay (ELISA) and the direct agglutination test.25 Clinical, parasitologic, and serologic examinations were performed prior to treatment, repeated five days after start of treatment and subsequently approximately every 15 days, or less frequently for dogs with clinical recovery and long-term survival. Impression smears of spleen and liver were examined post-mortem.

Possible adverse effects of treatment were followed by monitoring loss of appetite, weight loss, ocular changes, and lack of response to auditory or olfactory signals. True adverse effects were considered to be those occurring within one month of the end of treatment. Nevertheless, we also noted and report here any event that occurred during follow-up at any time since we could not predict long-term effects of the drug.

**RESULTS**

**Treatment with 20 mg/kg/day for 15 days.** The most striking observation from this preliminary group of three dogs was the early and dramatic clinical improvement, with the disappearance of conjunctivitis, increase in appetite, weight gain, and general improvement in skin and coat condition. All three animals were considered to be underweight prior to commencement of treatment, although there was no evidence that they had been denied food and apparent loss of appetite/weight loss was one reason why the owners had asked for the animals to be examined. The only detectable side effect was a temporary widespread loss of hair over five days during the second week of treatment and prior to replacement by a healthy coat. At the end of treatment, three animals were parasitologically negative by examination of both bone marrow and skin. Between 50 days (dogs 1 and 2) and 100 days (dog 3) after initiation of treatment, however, amastigotes were again found, first in the skin and then in the bone marrow (Figure 1). About the same time, or slightly later, symptoms of VL returned, becoming severe with weight loss, disseminated dermatitis, hair loss, and conjunctivitis. Deterioration was progressive and all three animals were killed at day 157 because there was no prospect of recovery, to prevent suffering. The dramatic weight gain, to levels typical for healthy examples of the breed, that accompanied clinical improvement, and the subsequent decrease in body weight associated with relapse are shown in Figure 2.

**Treatment with 80 mg/kg/day for 20 days.** In view of the relapse of all three dogs in group 1, it was decided to use a higher dose (80 mg/kg) and a slightly prolonged schedule for treatment (20 days) in a second group of animals. A third group (below) was also set up with an intermediate dose level (40 mg/kg) and a more prolonged schedule (30 days).

Dogs in the 80 mg/kg group either had severe VL (symptomatic) or less severe but obvious signs of VL (oligosymptomatic; Table 1) and all had amastigotes in both skin and bone marrow. Response to treatment was not uniform. Two dogs died within five days or one day before treatment ended, and a third dog died four days after treatment had ended. All three of these animals showed adverse effects including appetite loss, weight loss, acute dehydration (sunken eyes), loss of scent perception, and deafness; two also had keratitis. There were no detectable amastigotes in the liver or spleen post-mortem.
A fourth dog survived treatment with dramatic clinical improvement, including restored appetite, weight gain, and disappearance of edema, was apparently entirely healthy apart from incipient blindness that began around day 60, but died suddenly of unknown causes. A single atypical amastigote was found post-mortem in the liver. A fifth dog also recovered dramatically, relapsed and became parasitologically positive seven months after treatment began; deafness and blindness were noted during the terminal phase of the disease. One dog in this group survived and after four years is in excellent general health, parasitologically negative, but with defective vision that arose two years after treatment.

**Treatment with 40 mg/kg/day for 30 days.** One dog lost weight and died before treatment was complete and a second died shortly after treatment; amastigotes were found in both animals post-mortem. Two dogs that did not clear their skin infection during treatment were killed 30 days after treatment and had amastigotes in both the liver and bone marrow post-mortem. Five dogs appeared to have cleared their infections, with weight gain and dramatic clinical improvement, but relapsed between one and four months later with progressive canine VL and parasites in the skin and bone marrow. Three of the 12 dogs have survived for more than four years with no detectable *L. chagasi* infection (Figure 3).

Possible side effects in this group were weight loss in one dog that died during treatment, signs of deafness in four dogs (beginning at between days 25 and 60), and keratitis leading to partial or total blindness in six dogs (beginning at between days 28 and 500). Two of the three dogs that have survived for more than four years and are apparently totally cured of infection have no detectable side effects. The overall results of the trial in terms of clinical status before treatment and outcome of treatment are summarized in Table 1.

**DISCUSSION**

Pentavalent antimonials are tolerated in dogs, although there is a narrow therapeutic window, but are not a suitable treatment for canine VL. Animals with clinical signs usually relapse rapidly after a course of antimonial therapy. Repeated treatment carries the hazard of maintaining a reservoir for the propagation of new infections and, more importantly, of selecting *Leishmania* populations that are less susceptible to treatment, or have become resistant to drugs that are first line therapeutic agents for human VL. Failure of antimonial therapy for canine VL may in part be due to the more rapid elimination of antimonials in dogs as compared with humans, even when administered by the subcutaneous route.26

Aminosidine has become an important drug for treatment of human VL, especially in combination with pentavalent antimonials, with excellent cure rates and a good record of tolerability. Possible types of toxicity are primarily ototoxicity and nephrotoxicity, but the only significant side effect...
recorded in humans treated for VL is minor loss of hearing range, and this occurs rarely. The mode of action of aminosidine is inhibition of protein synthesis by attachment to the small ribosomal subunit. Doses used for treatment of human VL are between 6 mg/kg and 20 mg/kg, once a day for up to 21 days alone or combined with antimony. We have tested the ability of aminosidine to produce clinical improvement and clinical and parasitologic cure in canine VL.

Three groups of animals were treated. In the first, 20 mg/kg for 15 days produced dramatic clinical improvement but all three animals eventually relapsed with progressive canine VL. There were no side effects, with all signs being consistent with the disease progression, except that the unhealthy coat was shed prior to replacement with fresh growth of healthy hair. Clinical improvement was associated with apparent clearance of infection, such that it was not detectable by skin biopsy or bone marrow aspiration, but it returned to both sites during the relapses.

A four-fold increase in dose and extended treatment produced severe side effects that included loss of appetite, weight loss, evidence of nephrotoxicity, and neurotoxicity affecting hearing, vision, and sense of smell. Only one dog in this group was cured, but it developed impaired vision almost two years after treatment.

An intermediate dose of 40 mg/kg for 30 days was more successful in that three (25%) of 12 dogs survived for more than four years with no evidence of L. chagasi infection. Nevertheless, two animals died during or just after treatment and others either did not clear their infection or relapsed at variable intervals. Possible late side effects were still seen, principally loss of hearing and vision, but since these did not arise until long periods after treatment had ended, the pathogenesis of such delayed toxic effects is not clear, and it is doubtful that they are attributable to the preceding aminosidine therapy. True side effects were considered to be those occurring within one month of the end of treatment (Figure 3).

The clinical condition of dogs prior to treatment is shown in Table 1. It might be expected that dogs with the most severe VL, termed here symptomatic, would be most likely to suffer side effects and die during or just after treatment, possibly in part due to the damage caused by antigen release and deposition of complexes in the kidneys. Similarly, animals not in the terminal phase of the disease (oligosymptomatic) should be best able to withstand treatment and have the best chances of clinical cure. The data in Table 1 are not particularly informative on the first of these predictions but the second trend is clearly apparent: all clinically cured dogs were initially considered to be oligosymptomatic.

As far as we are aware, clinical and parasitologic cure of canine VL with proven skin and bone marrow infections and overt clinical signs of disease has rarely been achieved. Although aminosidine did produce a proportion of such cures, its use clearly has no value at present as a routine control measure against canine VL in endemic areas such as Teresina: it is not a satisfactory alternative to the humane destruction of dogs. For highly prized, irreplaceable pedigree stock, it could be used to save selected animals, assuming that rigorous measures were taken to prevent transmission to sand flies from treatment failures. Ineffective treatment carries the risk of generating organisms resistant to aminosidine, for which three resistance mechanisms have now been proposed. In Leishmania, resistance to aminosidine apparently depends on increased production of ribosomal RNA but not on enzymatic inactivation of the drug or base substitutions in the small ribosomal subunit.

In future studies, it would be of value to investigate the response of relapse infections to a second course of aminosidine treatment, at an early stage in the relapse, when relatively few organisms were present. Isolation of Leishmania pretreatment and during relapses would enable in vitro assays to determine if recrudescent organisms were less susceptible (resistant) to drug action.

Achievement of clinical recovery and cure suggest that further trials with aminosidine may be worthwhile, for example, with slow-release formulations or drug combinations. Combined aminosidine and antimony therapy is said to enhance the persistence of high serum concentrations of antimony, which may allow reduction of therapeutic doses and increased intervals between administrations, but concomitant elevation of levels in skin, presumably crucial to cure of canine VL, has not been investigated. Kidney lesions in canine VL due to glomerular and tubular damage from deposition of immunoglobulins are well known and are likely to be precipitated by antigen release during aminosidine treatment. Supportive therapy might be used to reduce the side effects of aminosidine in dogs.

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<table>
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<th>Dose</th>
<th>No. of dogs</th>
<th>Clinical condition*</th>
<th>Adverse effects†</th>
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<th>Relapsed and died</th>
<th>Cure§ (%)</th>
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* Clinical condition before treatment. Symp = symptomatic; Oligo = oligosymptomatic.
† Adverse effects occurring within one month of the end of treatment (later clinical events were also recorded, see text and Figure 3).
‡ Died within one month of treatment.
§ Clinical and parasitologic cure.
FIGURE 3. Aminosidine, 40 mg/kg/day for 30 days: dogs 2 and 6 both died in good clinical condition but were parasitologically positive, D = died; dogs 10 and 11 both parasitologically negative at 800 days of follow-up. S.A. = still alive. Late occurring blindness and/or deafness in dogs 2, 6, and 10 were not necessarily attributable to preceding aminosidine therapy (see text). +VE = positive; PM. = post-mortem.

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