Clinical, serologic, and parasitologic follow-up after long-term allopurinol therapy of dogs naturally infected with Leishmania infantum

Cavaliero, Taja; Arnold, Pierre; Mathis, Alexander; Glaus, Toni M; Hofmann-Lehmann, Regina; Deplazes, Peter

Abstract: Canine leishmaniasis usually is treated with antimony compounds, but frequent relapses, adverse effects, high costs, and development of resistance to long-term antimonial therapy emphasize the importance of searching for alternative antileishmanial drugs. Allopurinol was used at a dosage of 10 mg/kg/day PO to treat 10 dogs naturally infected with Leishmania infantum for a period of 2-24 months. Nine dogs recovered within 2-6 months of chemotherapy, and no relapses were observed during the treatment of up to 20 months. However, 3 of 4 dogs relapsed after treatment was discontinued. These dogs again recovered clinically when therapy was resumed. Parasite-specific immunoglobulin concentrations (IgG2) were high in all dogs before therapy and remained high even in clinically cured dogs during or after therapy. On the other hand, specific IgG1 reactions, which have been shown to be detectable in symptomatic animals, persisted in 7 dogs for long periods after clinical recovery. Three of these dogs relapsed within 2-4 weeks after interrupting therapy. However, 1 dog with no detectable specific IgG1 reaction at the end of therapy did not relapse in the following 4 months. Parasites could be detected in 8 of 9 dogs after clinical improvement by in vitro cultivation or polymerase chain reaction (PCR) testing of lymph node aspirates. In 4 of these dogs, parasites also were detected in blood samples by PCR. Hence, these clinically cured dogs must be regarded as reservoirs of Leishmania and allopurinol cannot be recommended in endemic areas.

DOI: 10.1111/j.1939-1676.1999.tb02190.x

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: http://doi.org/10.5167/uzh-129732

Veröffentlichte Version

Originally published at:
Clinical, Serologic, and Parasitologic Follow-Up after Long-Term Allopurinol Therapy of Dogs Naturally Infected with *Leishmania infantum*

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Canine leishmaniasis usually is treated with antimony compounds, but frequent relapses, adverse effects, high costs, and development of resistance to long-term antimonial therapy emphasize the importance of searching for alternative antileishmanial drugs. Allopurinol was used at a dosage of 10 mg/kg/day PO to treat 10 dogs naturally infected with *Leishmania infantum* for a period of 2–24 months. Nine dogs recovered within 2–6 months of chemotherapy, and no relapses were observed during the treatment of up to 20 months. However, 3 of 4 dogs relapsed after treatment was discontinued. These dogs again recovered clinically when therapy was resumed. Parasite-specific immunoglobulin concentrations (IgG2) were high in all dogs before therapy and remained high even in clinically cured dogs during or after therapy. On the other hand, specific IgG1 reactions, which have been shown to be detectable in symptomatic animals, persisted in 7 dogs for long periods after clinical recovery. Three of these dogs relapsed within 2–4 weeks after interrupting therapy. However, 1 dog with no detectable specific IgG1 reaction at the end of therapy did not relapse in the following 4 months. Parasites could be detected in 8 of 9 dogs after clinical improvement by in vitro cultivation or polymerase chain reaction (PCR) testing of lymph node aspirates. In 4 of these dogs, parasites also were detected in blood samples by PCR. Hence, these clinically cured dogs must be regarded as reservoirs of *Leishmania* and allopurinol cannot be recommended in endemic areas.

**Key words:** Diagnosis; Leishmaniasis; Polymerase chain reaction.

Leishmaniasis is a globally widespread disease. In Mediterranean countries, it affects humans and dogs and is considered to be the most common opportunistic parasitic infection among human immunodeficiency virus-positive persons. In Mediterranean countries, dogs are the main reservoir host for visceral leishmaniasis caused by the protozoan parasite *Leishmania infantum*, which is transmitted between vertebrate hosts by female sandflies of the genus *Phlebotomus*. The incubation period in dogs is variable, ranging from 3 months to several years, and most dogs develop a chronic disease with anemia, hypergammaglobulinemia, generalized lymphadenopathy, skin lesions, epistaxis, and chronic renal failure with eventual death.

In endemic areas, veterinarians should inform dog owners that infected asymptomatic, nontreated and treated dogs may be a source of infection for sandflies even after successful clinical therapy. Therefore, from an epidemiologic point of view, infected dogs should be euthanized because elimination of infection is not achieved by chemotherapy. The most commonly used drugs are pentavalent antimonial compounds recently has been reported. However, despite clinical cure, relapses have been observed, and parasite clearance in clinically cured dogs treated solely with allopurinol has not been investigated.

The aim of this study was to test the efficacy of long-term allopurinol treatment in dogs with naturally acquired leishmaniasis with respect to clinical cure and parasite elimination, as assessed by serology, in vitro cultivation of promastigote stages of the organism, and polymerase chain reaction (PCR).

**Materials and Methods**

**Dogs**

Ten dogs, 6 females and 4 males, of different breeds and ranging in age from 1 to 10 years were included in this study. Six of these dogs acquired infection in their country of origin (Spain and Italy) and 4 dogs by visiting countries where leishmaniasis is endemic (France, Greece, and Italy).

**Specific Antibody Detection**

The specific detection of immunoglobulins (IgG1 and IgG2) against an extract of soluble *L. infantum* promastigote antigen was performed by enzyme-linked immunosorbent assay (ELISA).

**Parasitologic Diagnosis**

Parasitologic diagnosis was made every 1 or 2 months by in vitro cultivation of lymph node aspirates and detection of promastigote stag-
es or by PCR of lymph node aspirates or heparinized blood. In cases proven positive by in vitro cultivation, only blood samples were tested by PCR. If in vitro cultivation was either negative or could not be evaluated because of heavy bacterial contamination, samples also were tested for the presence of parasites by PCR.

**Chemotherapy**

Dogs were treated PO with allopurinol (Allopur, GEA, Frederiksborg, Denmark) at a dosage of 10 mg/kg/day. The period of treatment varied between 2 to 24 months. After remission of clinical signs, we intended to stop chemotherapy in order to see whether relapses would occur. Being aware that the dogs could still be infected with the parasite despite their healthy appearance, the owners of dogs 1, 2, 3, 7, and 8 did not agree to interruption of therapy. Treatment was discontinued from months 20 to 21 in dog 4 and from months 5 to 6 in dog 5. Dog 6 had been treated before with meglumine antimoniate but relapsed and therefore was treated with allopurinol. The owner of dog 6 interrupted therapy after 14 months for 2 weeks. Therapy was stopped after 4 months in dog 10.

**Follow-Up Examinations**

Dogs were presented for follow-up investigations every 1–2 months at the beginning of the study, but at longer intervals later with clinical improvement. Details of the treatment course are summarized in Tables 1, 2.

Clinicopathologic data included CBC, serum biochemistry, and urinalysis. In addition, globulins were analyzed for their γ and β fractions. The dogs’ serologic responses to therapy were monitored by measuring specific IgG (gamma specific), IgG1, and IgG2 by ELISA, and the presence of parasites was assessed by periodic in vitro cultivation and PCR of lymph node aspirates and blood.

**Results**

**Clinical Response**

Clinical follow-up during allopurinol therapy is depicted in Tables 1, 2. At the beginning of the study, all dogs had clinical signs associated with leishmaniasis, but with different severity. Four dogs had localized skin lesions on the head, ears, and nose and 4 dogs had generalized skin lesions. Lymphadenopathy was observed in 6 dogs. Two dogs had recurrent episodes of epistaxis, and 1 dog had acute nephritis accompanied by generalized lymphadenopathy. Nine of the 10 dogs recovered clinically after treatment for 2–6 months and no relapses occurred during long-term treatment. Dogs 4, 5, and 6 relapsed after interruption of their therapy. The relapses were characterized by the occurrence of skin lesions and enlargement of lymph nodes. Dogs 4 and 6 recovered again when therapy was resumed. No further information is available about dog 5. Therapy of dog 10 was stopped after 4 months and the animal re-

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**Table 1. Clinical course of leishmaniasis in 6 naturally infected dogs after treatment with allopurinol.**

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Months*</th>
<th>Clinical Symptoms</th>
<th>Parasite Detectionb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>Generalized alopecia, skin ulcers</td>
<td>LN/in vitro+, B/PCR+</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>Lymphadenopathy, skin lesions on head and ears</td>
<td>B/PCR−</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>Epistaxis, skin lesions on head and ears</td>
<td>LN/in vitro+, B/PCR+</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>Epistaxis, lymphadenopathy</td>
<td>LN/in vitro+, B/PCR+</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>Skin lesions on ears and nose</td>
<td>LN/in vitro+, B/PCR+</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>Renal failure, lymphadenopathy</td>
<td>LN/in vitro+, B/PCR+</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>Anemia</td>
<td>B/PCR+</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>Healthy</td>
<td>B/PCR+</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>Healthy</td>
<td>B/PCR+</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>Healthy</td>
<td>B/PCR+</td>
</tr>
</tbody>
</table>

LN, lymph node; B, blood; PCR, polymerase chain reaction.

* After start of therapy.

b By in vitro cultivation and/or PCR.
Table 2. Clinical course of leishmaniasis in 4 dogs before and after interruption of treatment with allopurinol.

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Months&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Clinical Symptoms</th>
<th>Parasite Detection&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>4&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0</td>
<td>Hyperkeratosis, lymphadenopathy</td>
<td>LN/in vitro+, B/PCR+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Improvement of skin lesions, smaller lymph nodes</td>
<td>LN/in vitro+, B/PCR-</td>
</tr>
<tr>
<td>5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Healthy</td>
<td></td>
<td>B/PCR-</td>
</tr>
<tr>
<td>7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Healthy</td>
<td></td>
<td>B/PCR-</td>
</tr>
<tr>
<td>11&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Relapsed</td>
<td></td>
<td>LN/in vitro+, B/PCR+</td>
</tr>
<tr>
<td>24&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Healthy</td>
<td></td>
<td>LN/in vitro+, B/PCR-</td>
</tr>
<tr>
<td>5&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0</td>
<td>Lymphadenopathy, hyperkeratosis, skin ulcers</td>
<td>LN/in vitro+, B/PCR-</td>
</tr>
<tr>
<td>1&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Improvement of skin lesions</td>
<td></td>
<td>Not done</td>
</tr>
<tr>
<td>3&lt;sup&gt;h&lt;/sup&gt;</td>
<td>No skin lesions, lymphadenopathy</td>
<td></td>
<td>LN/in vitro-, LN/PCR-, B/PCR-</td>
</tr>
<tr>
<td>4&lt;sup&gt;i&lt;/sup&gt;</td>
<td>Healthy</td>
<td></td>
<td>LN/in vitro+, B/PCR+</td>
</tr>
<tr>
<td>6&lt;sup&gt;j&lt;/sup&gt;</td>
<td>Relapsed</td>
<td></td>
<td>Not done</td>
</tr>
<tr>
<td>10&lt;sup&gt;k&lt;/sup&gt;</td>
<td>Skin lesions on head, lymphadenopathy</td>
<td></td>
<td>LN/in vitro+</td>
</tr>
<tr>
<td>2&lt;sup&gt;l&lt;/sup&gt;</td>
<td>Few skin lesions and lymphadenopathy</td>
<td></td>
<td>LN/in vitro-, LN/PCR+, B/PCR+</td>
</tr>
<tr>
<td>3&lt;sup&gt;m&lt;/sup&gt;</td>
<td>Healthy</td>
<td></td>
<td>B/PCR-</td>
</tr>
<tr>
<td>5&lt;sup&gt;n&lt;/sup&gt;</td>
<td>Healthy</td>
<td></td>
<td>LN/in vitro+, B/PCR-</td>
</tr>
<tr>
<td>7&lt;sup&gt;o&lt;/sup&gt;</td>
<td>Healthy</td>
<td></td>
<td>B/PCR-</td>
</tr>
<tr>
<td>9&lt;sup&gt;p&lt;/sup&gt;</td>
<td>Healthy</td>
<td></td>
<td>B/PCR-</td>
</tr>
</tbody>
</table>

LN, lymph node; B, blood; PCR, polymerase chain reaction.

<sup>a</sup> After start of therapy.
<sup>b</sup> By in vitro cultivation and/or PCR.
<sup>c</sup> Therapy discontinued between months 20 and 21.
<sup>d</sup> Therapy discontinued between months 5 and 6.
<sup>e</sup> Therapy interrupted during month 14 for 2 weeks.
<sup>f</sup> Therapy discontinued after 4 months.

mained healthy for 5 months until the end of the study. Dog 9 was euthanized 2 months after beginning therapy because of severe renal failure. Dog 7 had no more characteristic signs of leishmaniasis after 8 months of treatment but suffered from diarrhea and vomiting. Dogs 1, 2, and 9 had concomitant infections with *Ehrlichia canis* and dog 6 had concomitant infection with *Dirofilaria repens*.

Microcytic normochromic anemia was observed in dogs 5, 7, and 10 before therapy (data not shown). Despite clinical improvement, anemia persisted in dogs 5 and 7 even after 6 and 8 months of treatment, respectively. The hematologic values in dog 10 were normal 4 months after beginning therapy.

Changes were observed in the concentrations of albumin, β and γ globulins, and total protein performed each 2 months (data not shown). All dogs had high concentrations of globulins at the beginning of treatment. These concentrations decreased to normal (1.12–1.87 g/dL) in dogs 2, 8, and 10 but remained high in dogs 1, 3, 4, 5, 6, 7, and 9 during therapy. After interruption of therapy, globulin concentrations tended to increase in dogs 4, 5, and 6.

At the beginning of treatment, albumin concentrations were low in all dogs except dogs 4 and 6, which had normal concentrations (3.22–4.21 g/dL). The concentration of albumin increased during treatment to normal in dogs 1, 2, 3, 8, and 10 and remained below normal in dogs 5, 7, and 9. After interruption of therapy, the concentration of albumin decreased in dogs 5 and 6.

Hyperproteinemia was observed in all dogs at the beginning of therapy. High total protein concentrations correlated with the increase in γ and β globulin concentrations during treatment (data not shown).

No changes were observed in serum electrolyte concentrations.

**Parasite-Specific Antibody Reactions**

Parasite-specific (IgG1, IgG2, and IgG gamma specific) antibody reactions during therapy were performed every 2 months (data not shown). The pretreatment concentrations of IgG2 and IgG gamma parasite-specific antibodies were high in all dogs. The concentrations of IgG2 in dogs 1, 2, 3, 4, 5, 6, 7, and 9 remained high or decreased only slightly toward the end of therapy. The IgG2 response in dogs 8 and 10 differed from that of the other dogs by progressively decreasing until the end of therapy, but remaining well above the lower limit of detection of the assay. The IgG gamma specific concentrations remained high and correlated with the results of the IgG2 ELISA. At the beginning of therapy anti-leishmania IgG1 concentrations were above
the lower limit of detection of the assay in all dogs except dog 8. During therapy, IgG1 concentrations generally decreased but persisted near the lower limit of detection of the assay in 7 dogs (dogs 1, 2, 3, 4, 5, 6, and 7) for long periods despite clinical cure, excluding dog 4, which showed values below the lower limit of detection of the assay.

**Parasite Detection**

Leishmania infection was confirmed in all dogs before or during chemotherapy by means of in vitro cultivation of lymph node aspirates or PCR (Tables 1, 2). Before therapy, *Leishmania* promastigote stages were detected by in vitro cultivation of lymph node aspirates in 9 dogs. After clinical cure, 9 (60%) of a total of 15 aspirations from 7 dogs were positive for *L. infantum* by in vitro cultivation. In 1 of the 6 negative samples, the presence of the parasite was confirmed by PCR of blood and lymph node aspirate. In 12 (57%) of 21 blood samples from dogs with clinical symptoms, before, during, and after interruption of treatment, circulating *Leishmania* were detected by PCR, whereas only 5 (19%) of 26 blood samples were positive in treated asymptomatic dogs.

**Discussion**

Nine of 10 dogs responded well to the allopurinol therapy at a dosage of 10 mg/kg/day, resulting in improvement or clinical cure after 2–6 months. No apparent adverse effects were associated with treatment as assessed by clinical, hematologic, and blood chemistry testing. Allopurinol has been used in dogs at dosages of 15–30 mg/kg/day for 1–10 months with clinical cure.20,23 Treatment with allopurinol at 11 mg/kg/day for 4 months resulted in clinical cure, but the dog relapsed 1 month later and was treated again with 15 mg/kg/day allopurinol for another 9 months. The dog still was clinically healthy 19 months after allopurinol was discontinued.24 However, parasitologic status of the cured dogs was not thoroughly investigated in these studies. In our study, relapses were observed in 3 of 4 dogs after long-term therapy with allopurinol at 10 mg/kg/day was discontinued for 2 weeks to 1 month. Based on recently published results and the results of this study, we conclude that a dosage of 10 mg/kg/day does not effectively eliminate the parasite, even after long-term administration.

Despite the recovery and clinical cure of our patients, parasites were detected in 8 of 9 dogs by means of in vitro cultivation or PCR of lymph node aspirates or blood samples. Because blood samples were washed free of plasma and subsequently depleted of erythrocytes, only intracellular *Leishmania* stages within mononuclear cells were concentrated in samples used for PCR. Therefore, it is very unlikely that free DNA or DNA from killed parasites was amplified. The finding that clinically cured dogs that had been treated daily with allopurinol still had circulating *Leishmania* parasites in the blood as detected by PCR indicates that such animals remain potential reservoirs of parasites.

From a diagnostic point of view, the sensitivity of the in vitro cultivation of lymph node aspirates was lower in clinically cured dogs. After therapy and remission of lymphadenopathy, popliteal lymph nodes were very small, and it was difficult to obtain biopsy material. Therefore, errors in collecting material as well as microbial contamination of samples occur quite often and limit the value of in vitro cultivation. PCR has proven to be a good alternative for parasite detection in contaminated samples.

Compared to earlier studies of imported dogs with leishmaniasis studied at the animal hospital in Zurich,9,12,25 mild clinical signs were found in the dogs of this study, except for dog 9. This probably is due to the fact that veterinarians in Switzerland are becoming more familiar with the clinical signs of leishmaniasis, resulting in earlier diagnosis. Furthermore, dogs coming from endemic areas usually are serologically tested with a sensitive ELISA for leishmaniasis.

The hypoalbuminemia and hyperglobulinemia typical of leishmaniasis were also observed in our study. Albumin concentrations tended to increase whereas globulin concentrations tended to decrease during treatment in all dogs that responded to therapy. Even after clinical cure, albumin concentrations did not reach normal values in 2 dogs and globulins were still high in 6 dogs. In the 3 dogs that relapsed after interruption of therapy, globulin concentrations tended to increase again, and in 2 dogs albumin concentrations tended to decrease. These results indicate that the immunopathologic process persisted in most of the dogs although the animals were clinically healthy before therapy was discontinued. This fact may explain the short time between interruption of therapy and relapses, and serum globulin concentrations should be assessed before discontinuing therapy.

No correlation between the relatively high values of IgG2 or IgG gamma specific ELISA at the time of diagnosis and during and after treatment, and the clinical condition of the dogs could be established. High IgG2 ELISA values persist several years after successful treatment with meglumine antimoniate in asymptomatic infected dogs.25 The IgG gamma specific ELISA used in this study detected asymptomatic infections in a seroepidemiologic study in Cyprus (Deplazes, unpublished data). Therefore, IgG2 or IgG gamma specific ELISA as well as dot ELISA22 or direct agglutination test27 do correlate with infection, but have no prognostic value with respect to clinical cure of the dogs.

Anti-leishmania IgG1 concentrations were not as high at the beginning of treatment as described by other authors.25 This result could have been due to early diagnosis of the disease in the dogs of this study. In 7 dogs, IgG1 antibody results remained slightly positive (values near the lower limit of detection of the assay) despite clinical cure. After interrupting therapy in 3 of these dogs, all relapsed within 1 month. However, dog 4 with no detectable specific IgG1 reaction at the end of therapy did not relapse in the following 4 months. Persistent IgG1 antibodies were detected in chronically ill dogs and a decrease of these antibodies to background concentration was observed in recovered dogs after treatment with meglumine antimoniate.25 Therefore, the IgG1 ELISA seems to be of prognostic value and useful to determine the appropriate time to discontinue therapy, which might reduce the likelihood of eventual relapses.

Allopurinol is efficient for the clinical treatment of canine leishmaniasis although further investigations are need-
ed to determine optimal dosage and duration of treatment. The drug is easy to administer, is inexpensive, and there are few reports of adverse effects. Dogs that relapsed after interruption of therapy with allopurinol were successfully retreated with the same drug. Despite clinical cure, dogs may have circulating parasites during or after allopurinol treatment, and these animals may remain active reservoirs of the disease. Clinically cured dogs treated with allopurinol at a dosage of 10 mg/kg/day must be regarded as potential parasite reservoirs. Consequently, allopurinol cannot be recommended for the treatment at this dosage, especially in endemic areas.

References