Serum concentrations of acute-phase proteins in dogs with leishmaniosi during short-term treatment

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Objective—To evaluate changes in serum concentrations of acute-phase proteins in dogs with leishmaniosi during short-term therapy in accordance with 2 treatment protocols and determine whether concentrations of acute-phase proteins could be used to monitor the initial response of dogs to treatment.

Animals—12 dogs naturally infected with Leishmania infantum.

Procedure—Dogs were allocated into 2 groups. Dogs of group 1 were treated by use of meglumine antimoniate (100 mg/kg, SC, q 24h) administered concurrently with allopurinol (15 mg/kg, PO, q 12h) for 20 days and then with allopurinol alone at the same dosage for the subsequent 30 days. Dogs of group 2 were treated by administration of allopurinol alone (15 mg/kg, PO, q 12h) for 60 days. Blood samples were obtained before and during treatment for measurement of serum concentrations of acute-phase proteins and determination of CBC counts, serum biochemical analyses, and electropherograms.

Results—All dogs evaluated in the study had increased concentrations of C-reactive protein, haptoglobin, and ceruloplasmin at the time of diagnosis of leishmaniosi. Mean concentration of serum amyloid A before treatment was also increased, but some of the dogs had concentrations of serum amyloid A that were within the reference range. Concentrations of C-reactive protein and ceruloplasmin decreased significantly in all dogs at the end of the study period.

Conclusions and Clinical Relevance—Measurement of concentrations of selected acute-phase proteins, such as C-reactive protein or ceruloplasmin, could be used to evaluate the initial response of dogs with leishmaniosi to treatment. (Am J Vet Res 2003;64:1021–1026)

The acute-phase response refers to a nonspecific inflammatory reaction of the host that occurs shortly after any tissue injury. Origin of the response can be attributable to infective, immunologic, neoplastic, traumatic, parasitic, or other causes.1,2 Basically, the acute-phase response includes changes in concentrations of plasma proteins, which are referred to as acute-phase proteins, some of which decrease in concentration (eg, albumin) and others that increase in concentration (eg, haptoglobin, ceruloplasmin, C-reactive protein [CRP], and serum amyloid A [SAA]).3 Changes in concentrations of various acute-phase proteins have been detected in numerous species and pathologic processes, such as mastitis, pneumonia, foot-and-mouth disease, and fatty liver syndrome in cows; pneumonia, meningoencephalitis, and stress in pigs; and leptospirosis, parvovirus infection, ehrlichiosis, and neoplastic conditions in dogs.4

Leishmaniosi in dogs is a slow-developing systemic disease caused by a diphasic protozoan parasite of the Leishmania genus. It is considered a zoonotic disease with wide distribution in South America, Africa, and the Mediterranean area, but sporadic cases have also been reported in other geographic regions.5 In an epidemiologic study,6 it was reported that the disease affects approximately 19% of the canine population in endemic areas.

The drugs most commonly used for the treatment of dogs with leishmaniosi are pentavalent antimony derivatives and allopurinol. Pentavalent antimony compounds, such as meglumine antimoniate and sodium stibogluconate, selectively inhibit leishmania enzymes that are required for glycolytic and fatty acid oxidation. Allopurinol inhibits the growth of leishmania by blocking RNA synthesis. Treatment can induce clinical remission; however, it rarely achieves a parasitologic cure, and relapses are frequent so several cycles of treatment are necessary.7 These circumstances make it difficult to decide when to discontinue treatment; thus, a useful method to monitor the response of dogs treated for leishmaniosi and predict possible relapses of the disease is clearly needed.

In leishmaniosi in humans, parasitologic culture of splenic aspirates has been used to monitor parasite burden, but the sensitivity of such methods is low.8 However, a study9 on the use of acute-phase proteins to determine whether Leishmania parasites are still in the spleen suggested that they might represent useful noninvasive markers for monitoring disease activity, response to therapy, and relapse.10 In addition, measurements of CRP are reportedly of prog nostic value in visceral leishmaniosi.10 Concentrations of acute-phase proteins (haptoglobin, ceruloplasmin, and CRP) reportedly increase in serum of dogs infected with Leishmania infantum, revealing a high sensitivity to detect infected dogs with and without clinical signs of disease, and measurement of these acute-phase proteins has been suggested as a possible tool to evaluate the response to treatment.11

The objective of the study reported here was to evaluate changes in concentrations of acute-phase proteins in dogs during short-term treatment of leishmania-
ious in accordance with 2 widely used therapeutic protocols. This enabled us to analyze the possible use of these proteins for monitoring the initial response to treatment.

Materials and Methods

Animals—Twelve dogs naturally infected with *L. infantum* were used for the study. The diagnosis of leishmanioses was established on the basis of clinical signs, identification of the parasite in smears of aspirates obtained from the lymph nodes or bone marrow, and results of an ELISA for detection of antibodies against *L. infantum* (we used the 1:100 dilution as a minimum value for a positive result on the serologic test). All dogs had negative results for antibody titers against *Ehrlichia canis* and *Babesia canis* (positive results were considered with a titer of 1:20 or higher for *E canis* and 1:80 or higher for *B canis*).

Results of physical examination revealed that the most common manifestations of the disease were weight loss, varying degrees of generalized lymphadenopathy, and skin lesions. Alterations found most commonly in hemograms and serum biochemical analyses were mild nonregenerative anemia (PCV, 31 to 37%), hyperproteinemia, and hypoalbuminemia. Dogs with renal insufficiency (urea concentration > 3.82 mmol/L or creatinine concentration > 106 μmol/L), hepatic damage, or hepatic insufficiency (activity of alanine aminotransferase > 83.2 U/L or aspartate aminotransferase > 50 U/L or fasting bile acid concentration > 8 μmol/L) were not included in the study.

Experimental design—Dogs were randomly allocated into 2 groups. Group 1 included 6 dogs treated by use of meglumine antimoniate (100 mg/kg, SC, q 24 h) administered concurrently with allopurinol (15 mg/kg, PO, q 12 h for 20 days and then allopurinol alone administered at the same dosage for the subsequent 30 days. Blood samples were obtained for measurement of acute-phase proteins and other determinations on the day before (day 0) and days 10, 20, 35, and 50 after initiation of treatment.

Group 2 included 6 dogs treated by use of allopurinol alone (15 mg/kg, PO, q 12 h for 60 days). Blood samples were obtained from this group on days 0, 10, 30, and 60 after initiation of treatment.

Clinical and routine laboratory examinations—Clinical and laboratory assessment for each dog included physical examination, CBC counts performed by use of an automated blood counter, blood smear examination, serum biochemical analyses performed by use of an automated chemistry analyzer, evaluation of electrophorograms, and measurement of concentrations of acute-phase proteins before treatment and during the monitoring period. Electrophoretic assays were performed on a multicapillary instrument designed for automation of serum protein electrophoresis and validated for analysis of serum proteins in canine samples. Electrophorograms were divided into 7 fractions in accordance with the guidelines of Martinez-Subiela et al. Serum total protein concentrations were estimated by use of a refractometer.

Measurement of concentrations of acute-phase proteins—Concentrations of CRP were estimated by use of a solid-phase sandwich immunoassay specific for canine CRP, validated in our laboratory. Final absorbance of samples was measured by use of a microtiter plate reader at 450 nm.

Concentrations of SAA were determined by use of a solid-phase sandwich ELISA. The ELISA was designed for use in determining concentrations of SAA in various animal species, and it was validated in our laboratory for canine serum samples. Final absorbance of samples was measured by use of a microtiter plate reader at 450 nm.

Statistical analysis—An ANOVA was used to evaluate whether mean concentrations of acute-phase proteins at the various time points during treatment for each group were significantly different from the mean group value obtained before treatment. Repeated-measures ANOVA was used to evaluate whether concentrations of acute-phase proteins in each dog during treatment were significantly different from concentrations before treatment.

Differences were considered significant at a value of *P* ≤ 0.05. All analyses were conducted by use of a statistical program.

Results

Clinical and routine laboratory examinations—Improvement in the health of all dogs was observed after commencement of treatment. Dogs gained weight, and the major clinical signs began to disappear approximately 20 days after initiation of treatment. All serum biochemical variables, except for total protein, albumin, and globulin concentrations, remained within reference ranges established by our clinical pathology laboratory until the end of the study. An increase in PCV was observed in dogs with anemia that returned to values within the laboratory reference range (37 to 55%) approximately 30 days after initiation of treatment. Total protein concentrations of all dogs decreased gradually during the study period. The most evident changes, although not significantly different, observed in the electrophoretic fractions during the course of treatment were an increase in albumin concentrations and a decrease in α2 and γ fractions (Table 1). By the end of the study, all dogs were in good general health.

Concentrations of acute-phase proteins—Changes in the concentrations of acute-phase proteins were observed for each treatment protocol (Tables 2 and 3).

Mean serum concentration of CRP before treatment was 27.23 and 28.54 mg/L for groups 1 (meglumine and allopurinol) and 2 (allopurinol only), respectively. Mean serum concentration of CRP in dogs of group 1 decreased from days 0 to 35, followed by a slight increase on day 50 to reach a concentration of 7.6 mg/L. Concentration of CRP in dogs of group 2 decreased gradually from days 0 to 60, when the concent
Thereafter, the concentration of SAA decreased slowly, reaching a concentration of 6.29 mg/L on day 10. Commencement of treatment resulted in a rapid decrease of 35.29 and 51.1 mg/L for groups 1 and 2, respectively.

ANOVA of data for each dog in a group was conducted; all dogs had significant decreases beginning on day 30. Mean serum concentration of ceruloplasmin decreased significantly beginning on days 2 on day 60 (Tables 1 and 2). Mean ceruloplasmin concentration decreased during the study period; however, an ANOVA of mean values revealed significant decreases in concentrations beginning on day 10.

Mean serum concentration of haptoglobin before treatment was 0.55 and 0.32 g/L in groups 1 and 2, respectively. For group 1, mean haptoglobin concentration increased after commencement of treatment and reached a maximum on day 10; it then decreased gradually to a value of 3.18 g/L on day 50 (Table 1). Mean haptoglobin concentration decreased during the course of treatment for group 2 (Table 2). The ANOVA for the 2 entire groups did not reveal significant decreases during the study period; however, an ANOVA of data for each dog revealed that 4 dogs in group 2 had significant decreases in the concentration of SAA beginning on day 10.

None of the dogs had all values for acute-phase proteins within the reference range at the end of the study period.

**Discussion**

The possible use of acute-phase proteins as biomarkers for monitoring treatment of dogs with leishmaniosis was evaluated by the use of 2 treatment protocols (meglumine antimoniate plus allopurinol and allopurinol alone). Pentavalent antimony derivatives (meglumine antimoniate) have been the treatment of choice for many years; however, the drugs are expensive, potentially nephrotoxic, and can result in the development of resistant organisms in the event of long-term treatment. Allopurinol has many advantages, such as the fact it is relatively nontoxic to the host, efficacious for improving clinical status of patients, has a low cost, and can be administered orally; however, it only acts as a leishmaniosstatic drug. Allopurinol, alone or in combination with meglumine antimoniate, is used routinely in practice.

The prognosis of dogs with leishmaniosis is guarded, because leishmania parasites are extremely resistant to treatment. Anti-leishmanial treatments often achieve temporary clinical improvement in dogs; however, they are not associated with elimination of the parasites, and relapses are common. Various methods, such as sequential parasitologic culture of bone marrow or skin, cytologic examination of bone marrow smears, and determination of serologic titers against leishmania organisms, have been used to assess the clinical course of the disease and monitor dogs for relapses.

**Table 2**—Mean serum concentrations of acute-phase proteins in 6 dogs with leishmaniosis that were treated by administration of meglumine antimoniate plus allopurinol

<table>
<thead>
<tr>
<th>Acute-phase protein</th>
<th>Day 0</th>
<th>Day 10</th>
<th>Day 20</th>
<th>Day 35</th>
<th>Day 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>27.23 ± 9.29</td>
<td>15.19 ± 8.09</td>
<td>8.79 ± 4.80</td>
<td>5.02 ± 4.00</td>
<td>7.60 ± 6.95</td>
</tr>
<tr>
<td>Haptoglobin (g/L)</td>
<td>4.80 ± 1.88</td>
<td>8.77 ± 4.81</td>
<td>6.95 ± 2.96</td>
<td>4.60 ± 2.34</td>
<td>3.18 ± 1.97</td>
</tr>
<tr>
<td>Ceruloplasmin (g/L)</td>
<td>0.95 ± 0.17</td>
<td>0.23 ± 0.16</td>
<td>0.16 ± 0.13</td>
<td>0.11 ± 0.07</td>
<td>0.11 ± 0.06</td>
</tr>
<tr>
<td>Serum amyloid A (mg/L)</td>
<td>35.29 ± 41.44</td>
<td>6.29 ± 6.29</td>
<td>4.57 ± 5.16</td>
<td>3.17 ± 5.85</td>
<td>2.09 ± 1.20</td>
</tr>
</tbody>
</table>

Values reported are mean ± SD. **Within a row, values with different superscript letters differ significantly (P < 0.05).** Day 0 = Day of initiation of treatment.

**Table 3**—Mean serum concentrations of acute-phase proteins in 6 dogs with leishmaniosis that were treated by administration of allopurinol alone

<table>
<thead>
<tr>
<th>Acute-phase protein</th>
<th>Day 0</th>
<th>Day 10</th>
<th>Day 20</th>
<th>Day 30</th>
<th>Day 60</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>28.54 ± 10.24</td>
<td>23.28 ± 22.27</td>
<td>14.65 ± 13.28</td>
<td>4.27 ± 3.44</td>
<td></td>
</tr>
<tr>
<td>Haptoglobin (g/L)</td>
<td>7.63 ± 2.51</td>
<td>5.95 ± 1.90</td>
<td>4.90 ± 1.46</td>
<td>4.75 ± 2.25</td>
<td></td>
</tr>
<tr>
<td>Ceruloplasmin (g/L)</td>
<td>0.32 ± 0.09</td>
<td>0.29 ± 0.09</td>
<td>0.18 ± 0.09</td>
<td>0.12 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Serum amyloid A (mg/L)</td>
<td>51.10 ± 70.90</td>
<td>22.86 ± 43.03</td>
<td>6.94 ± 4.13</td>
<td>5.64 ± 5.66</td>
<td></td>
</tr>
</tbody>
</table>

Values reported are mean ± SD. See Table 2 for key.
However, all of these methods have serious limitations. Cytologic examination of bone marrow smears has poor sensitivity to detect the parasite, and repeated collection of bone marrow specimens is a difficult and invasive procedure.11 Serologic tests are not useful in monitoring the progress of treated animals or determining whether an animal is cured.12 Thus, there is not a criterion-referenced standard for evaluating the effectiveness of therapy. Treatments are typically considered successful when clinical signs have disappeared, results of hematologic and serum biochemical analyses are within reference ranges, and an electropherogram is normal. In the study reported here, remission of clinical signs, return of hematologic results to within reference ranges, and a normal electropherogram were detected. These findings revealed that there was a good therapeutic response that confirmed previous reports12,13 on the efficacy of both treatment protocols.

Dogs evaluated in the study reported here had increased concentrations of CRP, haptoglobin, and ceruloplasmin at the time of diagnosis, which was in agreement with results of another report.14 Mean concentration of SAA before treatment was increased, but some dogs had concentrations of SAA within the reference range. These results were in concordance with another study20 in which investigators determined that concentrations of SAA were less sensitive for use in detecting animals infected by Leishmania infantum, compared with results for other acute-phase proteins. Substantial differences in the pattern of distinct acute-phase proteins during treatment of dogs with leishmaniosis were found in this study. Moreover, there were differences among dogs in the pattern for each protein after treatment. Mean serum concentrations of CRP and ceruloplasmin decreased significantly at the end of the study period. However, the exact time at which these acute-phase proteins initially decreased during short-term treatment cannot be definitively stated, because each dog had significant decreases in concentrations of these acute-phase proteins at different times after initiation of treatment.

Mean serum concentration of haptoglobin did not decrease significantly during the study. Moreover, the concentration of this acute-phase protein increased from days 0 to 10 in group 1. Increases in serum concentrations of haptoglobin after administration of various drugs, such as corticosteroids or anthelmintics, have been described elsewhere21,22 suggesting that this protein may increase during the active phase of hepatic functions, such as detoxication of drugs. Although further studies would be necessary, the lack of a significant decrease in concentrations of haptoglobin in group 1 during treatment could have been attributable to possible stimulation of the synthesis of this acute-phase protein as a result of meglumine antimoniate administered to dogs with leishmaniosis. Moreover, additional studies would be desirable to evaluate the effects of meglumine antimoniate on other acute-phase proteins, because although it was not observed in the dogs of the study reported here, it has been indicated that injection of this drug may cause local reactions, muscular pain and fibrosis, and peripheral nerve paralysis, processes that could initiate an acute-phase reaction in dogs subjected to anti-leishmanial treatments. It could be postulated that allopurinol does not substantially stimulate haptoglobin synthesis, because most dogs in group 2 had significant decreases in the concentration of this acute-phase protein during the experimental period.

The concentration of SAA decreased during the course of both treatments but not significantly. An explanation could be that although the initial mean value was high, only 3 dogs in group 1 and 4 dogs in group 2 had values above the reference range. Based on this data, 2 subgroups of dogs should be considered: 1 in which SAA concentrations were high before treatment and a significant decrease was observed during treatment and the other in which SAA concentrations were within the reference range before therapy and did not change significantly during treatment.

To our knowledge, we are not aware of other reports of changes in the concentrations of acute-phase proteins in dogs with leishmaniosis during treatment. Decreases in concentrations of CRP and SAA have been reported9,10 in humans after treatment for visceral leishmaniosis, indicating that both of these acute-phase proteins have the potential to be used as markers for the response to treatment. However, in dogs with leishmaniosis reported here, it would have been better to determine serum concentrations of CRP and ceruloplasmin than SAA or haptoglobin concentrations to monitor the treatment response.

Ideally, an untreated control group should have been used in the experimental procedure. However, it would not have been ethical to have a group of untreated patients. Although we are not aware of data on the time course for serum concentrations of acute-phase proteins in untreated dogs with leishmaniosis, there is information to indicate that concentrations of these acute-phase proteins will remain high. A relationship between Leishmania parasites and high concentrations of acute-phase proteins has been reported16,18 in humans with leishmaniosis, and persistence of these increased concentrations after treatment is considered an indicator of possible development of resistance to the drug administered. A similar finding has been reported in dogs with other parasitic diseases, such as Trypanosoma brucei infections.23 Although a significant decrease was found in this short-term study, none of the dogs had serum concentrations of CRP and ceruloplasmin that were within reference ranges established by our laboratory26 at the end of treatment. Additional studies would be needed to assess the return of these acute-phase proteins to concentrations within their respective range, which could be used as an indicator for the decision to discontinue treatment in dogs with leishmaniosis.

It must be pointed out that concentrations of the acute-phase proteins should be interpreted with caution because of the low specificity of these tests, since other pathologic processes attributable to surgery, corticosteroid treatments, or infectious and parasitic diseases could increase and maintain increased concentrations of acute-phase proteins and impede a decrease of these proteins in response to anti-leishmanial treatment. It should also be stated that although most dogs with leishmaniosis had high concentrations of acute-phase
proteins, sensitivity did not reach 100%. Sensitivity for CRP and ceruloplasmin, respectively, was 93 and 90% for detection of dogs with clinical signs of leishmaniosis and 82 and 78% for detection of dogs with leishmaniosis but without clinical signs of the disease. Thus, some dogs with leishmaniosis may have concentrations within the reference range; therefore, acute-phase proteins would not be useful in monitoring the response to treatment in these dogs.

Changes in the various protein fractions were also evaluated in this study. It has been proposed that evaluation of the electrophoretic pattern is a useful technique for monitoring the progress of the disease, contrary to cytologic examination of bone marrow smears or use of serologic tests. Results obtained in the study reported here are in concordance with results of other reports in which investigators documented decreases in concentrations of total protein and γ-globulins and an increase in albumin concentration after administration of various treatments for dogs with leishmaniosis. However, although the changes observed in the various fractions were significant in some dogs (data not shown), they were of lower magnitude than those observed in the acute-phase protein concentrations. On the basis on these results, CRP and ceruloplasmin could have greater potential as biomarkers for monitoring the response to treatment, compared with the use of analysis of electrophoretograms. Moreover, the quantification of the concentrations of these selected acute-phase proteins is more accurate than the determination of various protein fractions in an electrophoretogram, because manual differentiation of the electrophoretic fractions can increase the inaccuracy of the tests and imprecision between laboratories. Additionally, Leishmania parasites do not always cause modifications in the patterns of serum proteins, and in some cases, abnormal electropherograms have been found after successful treatment of leishmaniosis.

Measurement of concentrations of selected acute-phase proteins, such as CRP or ceruloplasmin, can be used to aid in the evaluation of the response to treatment in dogs with leishmaniosis. However, follow-up monitoring of treatment should not be based on the results for acute-phase proteins alone; it should also be correlated with other clinical and laboratory variables, because other pathologic conditions or the administration of some drugs could increase concentrations of acute-phase proteins and mask the response to treatment for a specific dog. Additional long-term studies that involve the use of more dogs would be desirable to test the ability of acute-phase proteins to implement cessation.

References