Oxidative stress in dogs with coccidiosis

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SUMMARY

The aim of this study was to determine the occurrence of an oxidative stress situation in dogs with coccidiosis. For that, the coccidiosis diagnosis was confirmed in 10 dogs presenting compatible clinical symptoms (bloody diarrhoea, weight loss and dehydration) by detection of oocysts in stool samples whereas 10 apparently clinically healthy dogs without oocyst in stool served as negative controls. Two coccidian species were identified after stool culture: Isospora canis in 7 dogs and I. ohioensis in 3 dogs. Plasma MDA concentrations were determined as a marker for lipid peroxidation and plasma glutathione peroxidase (GSHPx) and catalase (CAT) activities coupled to plasma glutathione (GSH) and serum vitamins C, E, A and β-carotene concentrations were measured for evaluating the antioxidant efficiency. Marked increases of plasma MDA concentrations compared to healthy dogs were evidenced in infected dogs (P < 0.05) whereas all antioxidant compounds except for GSHPx and β-carotene were significantly depressed in the infected group. In addition, plasma MDA concentrations as well as the variations of the CAT activities and of the concentrations of the vitamins A and C were significantly associated with the severity of clinical signs. Furthermore, in the infected group, a positive correlation was found between the vitamin A concentrations and the CAT activities (P < 0.01) while vitamin C concentrations were negatively associated with CAT activities and with vitamin A and β-carotene concentrations (P < 0.05). These results demonstrate the involvement of the oxidative stress as an aggravating factor during coccidiosis in dogs.

Keywords: Dog, oxidative stress, antioxidant status, coccidiosis, Isospora canis, Isospora ohioensis.

Introduction

Coccidiosis in the dog is an enteric disease caused by protozoa predominantly in the genus Isospora and can result in a serious or even fatal colitis [7, 12] and enteritis [7, 9]. Many coccidian species infect the intestinal tract of dogs. Some Isospora spp of dogs can facultatively infect other mammals and produce in various organs an encysted form that is infective for the dog [7]. It is common to observe intestinal parasites in canines of all ages, but the prevalence of infection is usually high in puppies, because young dogs have not yet acquired immunity to parasites [3, 36]. In kittens, it is seen primarily during weaning stress. The clinical signs in severe cases are diarrhoea (haemorrhagic enteritis), weight loss, and dehydration. Usually, coccidiosis is associated with other infectious agents, stress or immunosuppression [7].

Many potentially toxic reactive oxygen species (ROS) are generated through normal oxidative metabolism, and ROS in low concentrations is necessary for some physiological processes [15, 18, 22]. Oxidative stress may be defined as an alteration in the steady-state balance between oxidant and antioxidant agents in the cells; when the ROS accumulated into cells, several physiological processes may be disturbed [13, 27, 29]. Oxidative stress is a secondary aggravating factor in most diseases. The oxidative defence mechanisms against ROS, although activated, might be not enough efficient and clinical symptoms of illness may occur [17]. The imbalance between increased production of radicals and availability of antioxidant molecules may result in increased oxidative stress [4, 31]. Excessive formation of free radicals and comitmit damage at cellular and tissue levels are controlled by antioxidant defence systems [19, 21, 41, 42] which act in synergy [18]. Some antioxidant enzymes such as glutathione peroxidase (GSHPx), catalase (CAT) and the reduced glutathione (GSH) may have important functions in alleviating the toxic effects of ROS [21, 32, 34, 42]. Non enzymatic, chain breaking anti-oxidant vitamins such as vitamin C, vitamin E, vitamin A and β-carotene, has also eliminate oxidants constantly produced in the organism under normal circumstances [18, 28, 34].
The aim of this study was to determine the occurrence of an oxidative stress in dogs with coccidiosis by assessing lipid peroxidation intensity throughout measurement of plasma Malondialdehyde (MDA) concentrations in one hand and by evaluating the involvement of enzymatic antioxidant systems (GSHPx and CAT) and of non-enzymatic chain-breaking antioxidants (GSH, vitamins E, C, A and β-carotenes) in the other hand.

Materials and Methods

ANIMALS AND SAMPLES

The study was performed on 10 dogs with coccidiosis and 10 clinically healthy dogs. In both groups, animals were 2-5 months old. Blood samples were taken by venipuncture into heparinised and non-heparinised vacutainer sterile tubes. The non-heparinised blood samples were allowed clotting at room temperature for 2 hours before centrifugation whereas heparinised blood samples were directly centrifuged (1 000 g, at room temperature for 10 minutes) and supernatants were carefully harvested and stored at -20°C until analysis. Stool samples were directly collected from the rectum of all dogs for flotation and culture.

ANALYTICAL PROCEDURES

The stool samples were centrifuged with saturated NaCl solution for flotation and were examined under the light microscope [38]. After flotation examination of stools, the detected oocysts were sporulated in 2.5% potassium dichromate at 22°C and the species identification was performed.

The lipid peroxidation intensity in plasma was proportional to the concentration of thiobarbituric acid reactive species [35]. The amount of produced MDA was used as an index of lipid peroxidation. Briefly, one volume of the test sample and two volume of stock reagent (15% w/v trichloroacetic acid in 0.25 N HCl and 0.375%, w/v thiobarbituric acid in 0.25 N HCl) were mixed in a centrifuge tube. The solution was heated for 15 minutes in boiling water. After cooling, the precipitate was removed by centrifugation at 500g for 10 minutes and then the supernatant absorbance was measured at 532 nm against a blank containing all the components except for the test sample with a spectrophotometer [35]. The lipid peroxidation level was expressed as μmol/L.

The protein concentration was measured by the method of LOWRY [28].

The plasma GSHPx activity was determined according to the method of LAWRENCE [25]. Plasma (0.1 mL) was added to 0.8 mL of the reaction mixture containing 50 mM potassium phosphate buffer pH 7.0, 1 mM EDTA, 1 mM sodium azide (NaN3), 0.2 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH), 1 U/mL oxidized glutathione (GSSG)-reductase, 1 mM GSH and 0.25 mM H2O2 and incubated for 5 minutes at 25°C before the reaction initiation by addition of peroxide solution (1 mL). The absorbance at 340 nm was recorded for 5 minutes with a spectrophotometer and blank value (plasma was replaced with distilled water) was subtracted from each observed value. The plasma GSHPx activity was calculated from the slope of the NADPH disappearance curve (micromoles of NADPH oxidized per minute) and expressed as U/g of proteins with the proteinemia determination in parallel.

The plasma GSH concentrations were measured spectrophotometrically using the Ellman’s reagent [37] and results were expressed as mmol/L.

The plasma CAT activity was measured as previously described by GOTH [16]. Briefly, 0.2 mL of plasma samples was incubated in 1.0 mL substrate (65 μmol hydrogen peroxide per mL in 50 mM phosphate buffer, pH 7.0) at 37°C for 60 seconds. The enzymatic reaction was terminated with 1.0 mL of 32.4 mM ammonium molybdate solution. Absorbance was measured at 405 nm with a spectrophotometer (Shimadzu UV-1208 UV-VIS, Japan) against a blank containing all the components except for the enzyme. The catalase activity was expressed as kU/L.

The serum vitamin A and β-carotene concentrations were spectrophotometrically determined according to the method described by SUZIKI and CATOH [39], those of the vitamin C were colorimetrically determined using the phosphotungstic acid method described by KYAW [23] and those of the vitamin E were determined spectrophotometrically according to the MARTINEK’s method [30].

STATISTICAL ANALYSIS

The statistical analysis was performed using SPSS Ms package program (Windows Release 10.0). T tests for independent samples were used for evaluating data between groups. Pearson correlations were calculated between the different biochemical parameters. Results were expressed as means ± standard deviations, and P < 0.05 was taken as the level of significance.

Results

There was no history of coccidian-related symptoms in the controls at the time of sampling and this situation was confirmed throughout the faecal examinations. In the other dog group (n = 10), the coccidiosis diagnosis was made on the basis of oocyst detection by microscopic examination of stool samples. After stool culture, 2 coccidian species were identified, namely Isospora canis and Isospora ohioensis, which were characterized by a sporulation time of 7-10 days and 6-7 days respectively and by an oocyst size of 36.3-31.1 μm and 23.6-19.2 μm respectively. Seventy percent (7/10) of dogs were infected with Isospora canis and 30% (3/10) of them with Isospora ohioensis. Bloody diarrhoea, weight loss, dehydration and fatigue were observed in infected dogs throughout clinical examination. Furthermore, the clinical symptoms were more severe in I. canis infected dogs.

The oxidant and antioxidant status of dogs with coccidiosis and of healthy dogs were presented in Table I. The plasma MDA concentrations were markedly increased in naturally infected dogs compared to controls (P < 0.05).
Moreover, dogs infected with *I. canis* tended to exhibit higher plasma MDA concentrations than dogs infected with *I. ohioensis* and this marker correlated with the severity of clinical signs.

By contrast, the plasma GSH concentrations (*P < 0.05*) and the serum concentrations of the vitamins E (*P < 0.05*), C (*P < 0.05*) and A (*P < 0.001*) were significantly depressed in the infected group compared to the control group. In addition, the plasma CAT activities were also greatly decreased in dogs with coccidiosis (*P < 0.001*). However, the plasma GSHPx activities and the serum β-carotene concentrations did not significantly differ in this group from control values, although the enzyme activity and the pro-vitamin A concentrations appeared to be lower in the infected group. In addition, the decrease of the different antioxidant systems (especially vitamin A and C, and CAT activity) appeared to be related to the severity of clinical signs.

Whereas any significant correlation was obtained in healthy dogs, a positive correlation was determined between CAT and vitamin A (*r = 0.770*, *P < 0.05*), and the vitamin C concentrations were negatively correlated with CAT activities (*r = -0.645*, *P < 0.05*), with the vitamin A concentrations (*r = -0.638*, *P < 0.05*) and with the β-carotene concentrations (*r = -0.737*, *P < 0.05*) in infected dogs (Table II).

### Discussion

Intestinal parasites are among the most common pathogenic agents encountered by veterinarians dedicated to companion animals and they constitute one of the main causes of pathologies of the intestinal tract in dogs [2]. The presence of these intestinal parasites is common in dogs of all ages, but the prevalence of infection is usually high in puppies, mainly due to the fact that certain modes of transmission are exclusive to the newly whelped or neonates, and also because the acquired immunity to parasites is not complete in young dogs [3, 36]. Isosporosis is a protozoan infection of dogs all over the world. Once the animals have overcome the infection they can develop immunity in varying degrees [40]. Infections

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Injured dogs</th>
<th>Healthy dogs</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (µmol/L)</td>
<td>1.27 ± 0.37</td>
<td>0.93 ± 0.09</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>GSHPx (U/g protein)</td>
<td>12.04± 0.23</td>
<td>12.34± 0.45</td>
<td>NS</td>
</tr>
<tr>
<td>GSH (µmol/L)</td>
<td>0.115 ± 0.008</td>
<td>0.128 ± 0.01</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>CAT (kU/L)</td>
<td>26.48 ± 9.04</td>
<td>53.05 ± 15.52</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Vitamin E (mg/L)</td>
<td>0.16 ± 0.03</td>
<td>0.22 ± 0.07</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Vitamin C (mg/L)</td>
<td>6.65 ± 1.22</td>
<td>8.48 ± 1.47</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Vitamin A (mg/L)</td>
<td>3.26 ± 0.72</td>
<td>5.46 ± 0.86</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>β-carotene (µg/L)</td>
<td>97.85 ± 15.78</td>
<td>106.88 ± 24.39</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table I : Oxidant and antioxidant status in dogs with coccidiosis (n = 10) and in healthy dogs (n = 10). Results are expressed as mean ± standard deviation.

<table>
<thead>
<tr>
<th>GSHPx</th>
<th>GSH</th>
<th>CAT</th>
<th>Vit. E</th>
<th>Vit. C</th>
<th>Vit. A</th>
<th>β-Car.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA</td>
<td><em>r</em> = -0.489, NS</td>
<td><em>r</em> = -0.415, NS</td>
<td><em>r</em> = -0.135, NS</td>
<td><em>r</em> = -0.077, NS</td>
<td><em>r</em> = -0.279, NS</td>
<td><em>r</em> = -0.113, NS</td>
</tr>
<tr>
<td>GSHPx</td>
<td><em>r</em> = -0.081, NS</td>
<td><em>r</em> = 0.208, NS</td>
<td><em>r</em> = 0.292, NS</td>
<td><em>r</em> = 0.287, NS</td>
<td><em>r</em> = 0.257, NS</td>
<td><em>r</em> = -0.141, NS</td>
</tr>
<tr>
<td>GSH</td>
<td><em>r</em> = -0.042, NS</td>
<td><em>r</em> = 0.421, NS</td>
<td><em>r</em> = 0.265, NS</td>
<td><em>r</em> = -0.117, NS</td>
<td><em>r</em> = -0.468, NS</td>
<td></td>
</tr>
<tr>
<td>CAT</td>
<td><em>r</em> = -0.192, NS</td>
<td><em>r</em> = -0.645*, <em>r</em> = 0.770**, <em>r</em> = 0.304 NS, <em>r</em> = 0.085, NS</td>
<td><em>r</em> = 0.146, NS</td>
<td><em>r</em> = 0.011, NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vit. E</td>
<td><em>r</em> = -0.638*</td>
<td><em>r</em> = 0.737*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vit. A</td>
<td><em>r</em> = -0.423, NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Diseased dogs

| MDA | *r* = -0.143, NS | *r* = -0.047, NS | *r* = 0.439, NS | *r* = 0.321, NS | *r* = -0.513, NS | *r* = 0.518, NS | *r* = -0.137, NS |
| GSHPx | *r* = -0.075, NS | *r* = 0.218, NS | *r* = 0.564, NS | *r* = 0.508, NS | *r* = -0.007, NS | *r* = -0.075, NS |
| GSH | *r* = -0.236, NS | *r* = 0.263, NS | *r* = 0.518, NS | *r* = 0.148, NS | *r* = -0.536, NS |
| CAT | *r* = -0.016, NS | *r* = -0.202, NS | *r* = 0.399, NS | *r* = 0.476, NS |
| Vit. E | *r* = 0.065, NS | *r* = 0.450, NS | *r* = -0.557, NS |
| Vit. C | *r* = 0.358, NS | *r* = -0.283, NS |
| Vit. A | *r* = -0.345, NS |

Control dogs

| MDA | *r* = -0.047, NS | *r* = 0.439, NS | *r* = 0.321, NS | *r* = -0.513, NS | *r* = 0.518, NS | *r* = -0.137, NS |
| GSHPx | *r* = -0.075, NS | *r* = 0.218, NS | *r* = 0.564, NS | *r* = 0.508, NS | *r* = -0.007, NS | *r* = -0.075, NS |
| GSH | *r* = -0.236, NS | *r* = 0.263, NS | *r* = 0.518, NS | *r* = 0.148, NS | *r* = -0.536, NS |
| CAT | *r* = -0.016, NS | *r* = -0.202, NS | *r* = 0.399, NS | *r* = 0.476, NS |
| Vit. E | *r* = 0.065, NS | *r* = 0.450, NS | *r* = -0.557, NS |
| Vit. C | *r* = 0.358, NS | *r* = -0.283, NS |
| Vit. A | *r* = -0.345, NS |

Vit.: Vitamin; β-Car: β-carotene ; *: P < 0.05 ; **: P < 0.01, NS: not significant.

Table II : Correlations between the oxidative stress marker (MDA) and antioxidant systems (GSHPx and CAT enzymes, GSH, Vitamins A, C, E and β-carotene) in dogs with coccidiosis (n = 10) and in healthy controls (n = 10).
with *I. ohioensis* without symptoms of diarrhoea have been described, but serious clinical illness of infected puppies may usually occur. On the other hand, *I. canis* infections were always accompanied with symptoms [10]. In this study, bloody diarrhoea, weight loss, dehydration and fatigue were determined on all sick dogs. After the stool culture, *Isospora canis* and *Isospora ohioensis* were identified allowing the diagnosis of coccidiosis.

The determination of lipid peroxidation intensity by measurement of plasma MDA concentrations is among the most widely used methods for determination of the oxidative stress. The increase of malondialdehyde (MDA) concentrations in plasma is a marker of lipid peroxidation [18, 32]. Lipid peroxidation is a non-enzymatic chain reaction based on oxidation of mainly unsaturated fatty acids, leading to production of lipid peroxides and other intermediates. These intermediates may influence the properties of cell membranes, and one of the most common of these intermediates is MDA [8, 20]. In this study, plasma MDA concentrations were found to be increased in the dogs with coccidiosis compared to the control group, while decreases of GSH concentrations and CAT activities were observed. As GSH and CAT are involved in the conversion of radicals into less effective metabolites, these changes coupled to the increase of MDA concentrations, suggest that an excessive ROS production occurred during disease caused by *Isospora canis* and *Isospora ohioensis*. By contrast, GSHPx activities were not significantly altered compared to control values, although this enzyme activity was slightly lower in the infected group. Furthermore, the stress oxidative intensity evidenced by increase of plasma MDA concentrations coupled to decreases of CAT activities and GSH concentrations was associated with the clinical severity of coccidiosis.

The vitamin E (in the form of α-tocopherol) is the major lipid-soluble antioxidant of lipoproteins and in biomembranes [22, 26, 42], and the vitamin C (ascorbic acid) acts as a potent water soluble chain-breaking antioxidant in the biological fluids, but it cannot scavenge radicals within the membrane lipid region [11]. The synergy between ascorbic acid and α-tocopherol in the inhibition of lipid peroxidation is well known: vitamin C enhances the antioxidant activity of vitamin E by recycling the α-tocopheroyl radical back to α-tocopherol and the depletion of the α-tocopherol is markedly reduced [1, 14, 24, 33]. The β-carotene and retinol (vitamin A) are quenchers of the singlet oxygen and exhibit the ability to react directly with the peroxy radicals involved in lipid peroxidation [5, 6]. In this study, the serum concentrations of vitamins E, C and A, which are responsible for protecting cells from damage caused by lipid peroxidation, were found significantly lower in the infected group than in the control group, while there was no significant change in the concentrations of β-carotene. The decrease of serum concentrations of antioxidant vitamins (except for β-carotene) could result to their over utilisation due to the disease related oxidative stress, and these antioxidant depletion may be exacerbated by insufficient intake due to the appetite loss. In addition, the positive correlation recorded between the vitamin A concentrations and the CAT activities suggests that these 2 antioxidants were used synchronously during the oxidative stress situation whereas vitamin C negatively correlated with CAT activities and with vitamin A and β-carotene concentrations: the ascorbic acid would be firstly recruited before the other antioxidant systems then its concentrations decreased because of its involvement in regenerating radical scavengers (vitamin A and β-carotene, for example).

In conclusion, this study has highlighted the occurrence of an oxidative stress associated with marked depletion of major enzymatic (CAT) and non enzymatic (GSH, vitamins E, C and A) antioxidants in dogs infected by *I. canis* and *I. ohioensis*. As the oxidative stress can be considered as an aggravating factor of the coccidiosis, antioxidant supplementation may be an efficient adjuvant therapy to the classical treatment procedures for eradicating this disease.

References