Plasma Antioxidant Capacity in Dogs with Naturally Occurring Heart Diseases

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With 1 table

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Summary

The aim of the study was to compare the plasma levels of antioxidants by measuring total antioxidant activity (TAS) and ferric reducing ability of the plasma (FRAP) in healthy dogs and those that are suffering from dilated cardiomyopathy (DCM) or from mitral endocardiosis (ME). Dogs with echocardiographically diagnosed ME (10 dogs) as well as DCM (23 dogs) were sampled. Of dogs with DCM, eight having DCM with sinus rhythm (SR) were included in the DCM-SR group and 15 having DCM with atrial fibrillation (AF) in the DCM-AF group. Total antioxidant levels measured by TAS assay differed neither significantly between the cardiac patients and the control group nor between the heart disease groups. Ferric reducing ability of the plasma in animals with cardiac disease was significantly higher than in the control animals, and the difference was also significant in between the two DCM groups. However, the differences between the antioxidant levels of the DCM and ME groups did not reach significance in none of the antioxidant (TAS and FRAP) tests. Summarizing the results of this study it can be concluded that there is an increased antioxidant reactivity detected by the FRAP, but not by the TAS assay in the blood of dogs with naturally occurring cardiac disorders. The magnitude of this increase seems to be more affected rather by the severity of the cardiac insufficiency and/or by the heart rate or rhythm disorder than by the underlying heart disease itself.

Introduction

Reactive oxygen species (ROS) are constantly formed in human and animal body and exert both beneficial and deleterious effects, dependent on the type and concentration of the ROS and on the antioxidant reserve of the tissues. An increase in ROS level can cause significant damage to cellular proteins, membranes and nucleic acid leading to cellular dysfunction and death. There is an increasing evidence that oxidative stress plays a role in the pathophysiology and progression of heart failure in both humans and animals (Freeman et al., 1999; Byrne et al., 2003). Despite the overwhelming data on this issue obtained during research settings, few studies have evaluated the occurrence of oxidative stress in spontaneous canine cardiac diseases. Only Freeman et al. (1999, 2005) investigated the degree of oxidative stress and plasma antioxidant concentrations in dogs with naturally occurring heart disease, namely idiopathic dilated cardiomyopathy (DCM) and mitral endocardiosis (ME). They found evidence that plasma levels of some markers of oxidative stress and antioxidant system were different between healthy control dogs and dogs suffering from DCM or ME. The aim of the present study was to compare the plasma levels of antioxidants by measuring total antioxidant activity (TAS) and ferric reducing ability of the plasma (FRAP) of healthy dogs with those of dogs suffering from spontaneous DCM or ME.

Materials and Methods

Animals and sampling

Blood samples were collected from dogs that underwent cardiological examination at the Department of Internal Medicine of the Faculty of Veterinary Science, Szent István University during a one-year period (between April 2000 and May 2001). Blood samples were taken from dogs with echocardiographically diagnosed mitral endocardiosis (ME) and with a dilated left atrium (at least 2:1 left atrium to aorta ratio in 2DE short axis view) and dogs with DCM (based on enlarged end diastolic and end systolic cardiac chamber dimensions, reduced fractional-shortening (FS) value of <25% and without other detectable cardiac disease). Dogs with other cardiac problems or with detectable non-cardiac diseases (based on the clinical and laboratory data) were excluded from the study. Healthy control animals without any sign of cardiac or systemic disease based on the history, clinical examination, echocardiography and laboratory work up were also chosen. Dogs that have been already receiving (cardiac or non-cardiac) medications were excluded; however data on the included dogs’ diet or additional vitamin intake was not collected. Briefly, the study populations consisted of healthy control dogs and dogs with ME or DCM without any medical treatment at the time of sample collection.

Cardiological examinations

After history reviewing and physical examination, all dogs underwent standard electrocardiogram (ECG) using a three-channel ECG equipment (Schiller Cardiovit AT-3, Baar, Switzerland) and the six frontal limb systems. Echocardiography (Panther 2002, Bruel and Kjaer, Naerum, Denmark) including two-dimensional (2DE-) and M-mode exams in all animals, colour and pulsed wave Doppler in some dogs was performed in right and left lateral recumbency. Two-dimensional and M-mode cardiac dimensions and indexes were measured according to the literature (Thomas et al., 1993).
Blood sampling

Blood samples were collected from a peripheral vein into a tube containing ethylene diamine tetra acetate acid (EDTA). Samples were centrifuged and plasma was harvested within 60 min. Plasma was then frozen and stored at −20°C. Samples were measured within 2 weeks.

Laboratory work

Routine biochemistry and haematology were performed from simultaneously collected blood samples to disclose concomitant non-cardiac diseases.

TAS assay

Total antioxidant activity was performed as described by Miller et al. (1993). The assay principle:

ABTS** (2,2'-azino-di-(3-ethylbenzthiazoline-6-sulphonate) (Randox Laboratories Ltd, Crumlin, UK) is incubated with a peroxidase (metmyoglobin) and H₂O₂ to produce the artificial radical cation ABTS⁺. This has a relatively stable blue-green colour, which is measured at 600 nm. Antioxidants in the added sample cause suppression of the colour production to a degree, which is proportional to their concentration, so it reflects the total antioxidant capacity of the sample. The assay is performed spectrophotometrically on semi-automated analyser FP 900 (Labsystem Oy., Helsinki, Finland) at 37°C and 578 nm.

FRAP test

The method to measure the FRAP is described in detail by Benzie and Strain (1996). The principle of the method: The Fe³⁺–TPTZ (2,4,6-tripyridyl-s-triazine) complex can be changed by the reducing components of the sample to Fe²⁺–TPTZ at low pH. This end product has violet colour with absorption maximum at 593 nm and the extinction of the solution is proportional to the reducing power of the mainly non-enzymatic antioxidants in the plasma. The procedure was performed on a clinical biochemistry analyser Eppendorf 5040 (Eppendorf GmbH, Hamburg, Germany), programmed at 37°C and 578 nm.

Statistical analysis

Mean values and standard deviations were calculated for age, heart rate, FS, TAS and FRAP results (Microsoft Excel 2002). Statistical analysis was done by testing three independent contrasts (C1: controls versus all dogs with heart disease; C2: ME versus DCM; C3: DCM/SR versus DCM/AF) using Student’s two-sample t-tests allowing for different variances (i.e. Welch-test) by Excel. Differences between the values were considered significant if the P-values were ≤0.05. Sex ratios were compared between the groups by Fisher’s exact test (S-PLUS 2000 Professional Edition for Windows, Release 3, Insightful Corp., Seattle, WA, USA).

Results

Forty-one dogs fulfilling the inclusion criteria, were included in this study. The following four groups were formed according to the disease and cardiac rhythm of the animals:

The ME group consisted of 10 dogs (all males) with mitral valve disease. They all were in SR. Their mean age was 12.0 ± 2.1 years. The represented breeds included three mixed breeds, two Dachshunds, and one of each Poodle, English Cocker Spaniel, Puli, Pekingese and Chihuahua.

The DCM-SR group consisted of eight dogs (seven males, one female) with DCM. All these patients had SR during the ECG examination. Their mean age was 8.5 ± 1.9 years. The mean M-mode FS value was 15.5 ± 5.0%. The breeds represented in this group were three Dobermans, and one of each Hungarian Vizsla, English Cocker Spaniel, Great Dane, German Shepherd and Belgian Shepherd.

The DCM-AF group consisted of 15 animals (11 males, four females) with DCM and persistent atrial fibrillation (AF). Their mean age was 7.2 ± 2.6 years. The mean measured FS value was 12.7 ± 6.0%. The breeds in this group included three Dobermans, two Great Danes, two German Shepherds, two Caucasian Ovtcharkas and one of each Kuvasz, Boxer, Saint Bernard, Leonberger, Hungarian Vizsla and Central Asian Ovtcharka.

The control group was formed by eight healthy dogs (five males, three females). Their mean age was 5.5 ± 2.9 years. All dogs had sinus arrhythmia. The breeds in this group included three Beagles, three mixed breeds, one Boxer and a German Shorthaired Pointer.

The mean TAS, FRAP results and heart rates of the four groups are listed in the Table 1. Each of the heart disease groups had significantly higher mean heart rates when compared with the control animals (192 and 124 beats/min, respectively), moreover, DCM and DCM-AF dogs also had significantly higher mean heart rate when compared with the other disease groups.

The total antioxidant levels measured by the TAS assay were not different between the cardiac patients and the control group. However, mean values of FRAP of dogs with heart disease were significantly higher than in the control animals. Moreover, there were significantly higher antioxidant levels found in the DCM-AF group compared with the DCM-SR group but not between DCM and ME groups in the FRAP test.

Discussion

The aim of our study was to explore the possible relationship between naturally occurring canine cardiac disorders and changes of antioxidant levels of the blood plasma. Therefore, untreated dogs with the two most common spontaneous cardiac diseases were chosen. Because of all the control animals, the dogs with ME and some dogs with DCM had SR, while other dogs with DCM showed persistent AF, two DCM groups were formed according to their cardiac rhythm.

We did not assess the exact stage of cardiac insufficiency of these animals, but all of the sampled dogs had clinically apparent disease (at least NYHA class II). Most probably, the DCM-AF group represented the most severe heart failure patients, which was also reflected by the mean heart rates in the different groups. Although, the groups were not age and sex matched, interestingly, the two DCM groups were similar in age, breed and sex composition, had the same untreated disease with similar FS values. This enabled us to separately assess, whether heart failure severity reflected in increased heart...
rate and in a rhythm disorder causes any difference in the antioxidant status of these animals.

The range of TAS values in all dogs was similar to the findings of Nemec et al. (2000) on healthy beagles. The difference between the findings of the two-antioxidant tests may be explained by methodological reasons or by the different reactivity of the two assays to the various antioxidants. It was already recommended by Schleiser et al. (2002) that at least two methods should be used to measure antioxidant levels because of the relative activities of the individual antioxidants in the different test systems. The significantly higher level of antioxidants in the heart disease groups compared with the control animals measured by the FRAP assay and the significantly higher antioxidant concentration in the DCM-AF group compared with the DCM-SR group in the FRAP test of the present work are similar to the findings of Freeman et al. (1999). They found increased levels of plasma antioxidants, especially erythrocyte glutathione peroxidase activity in dogs with DCM compared with healthy control animals. However, increased glutathione peroxidase level is not suspected to be the explanation for the increased antioxidant levels in our study as this antioxidant is not detected by the FRAP but the TAS assay (Cao and Prior, 1998; Janaszewska and Bartosz, 2002). A possible explanation for the increased FRAP level in our study would be an increased level of uric acid, as uric acid contribute to the 60% of the FRAP value of fresh human blood plasma (Benzie and Strain, 1996). Plasma uric acid level was not measured in this study, but determination of uric acid level would be of special importance in the future when studying free-radical reactions as it may reflect indirect evidence for increased xanthine oxidase (XO) activity, a suspected major source of ROS in the failing heart. Whilst basal cardiac levels of XOs are low, an increase activity was found in pacing induced canine heart failure and in end-stage failing human heart tissues (Leyva et al., 1997; Ekelund et al., 1999).

Even though we are not able to name the antioxidant(s) behind the found elevated FRAP level, the results of Freeman et al. (1999) and of us both contradict the findings of Dhalla et al. (1996) and Prasad et al. (1996). These authors found decreased level of antioxidants in the heart failure groups of animals during experimentally induced heart failure in rodents and dogs. Possible explanation for this contradiction may be that the antioxidant systems react differently during naturally occurring heart diseases from the artificial model of heart failure, or that the antioxidant changes in the blood are not reflecting the true alterations taking place in the heart tissue. However, this latter supposition was contradicted by Lantos et al. (2001), who found that in case of experimentally caused reperfusion injury to the canine heart, peripheral blood samples were also informative regarding the altered balance between ROS production and the antioxidant capacity.

It is also interesting that those dogs that had more severe form of heart disease (group DCM-AF) show higher FRAP values than those of less severe cases (group DCM-SR). This suggest that antioxidant system of dogs having more possible risk for hypoxia/reperfusion injury respond to this stimulus more intensively, than others.

We found some similarities with the findings of Freeman et al. (2005). Nevertheless, they measured eight antioxidant parameters and biomarkers of oxidative stress, and we measured only two. In their study some of them showed a

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<th>Table 1. Measured antioxidant parameters of dogs with cardiac disorders</th>
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<tr>
<td>Controls</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Sex (male:female)</td>
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<tr>
<td>8.5 ± 3.5</td>
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<tr>
<td>5:3</td>
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<tr>
<td>87 ± 308</td>
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<tr>
<td>1.13 ± 0.23</td>
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<tr>
<td>0.64 ± 0.16</td>
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<tr>
<td>P-value* (controls versus heart disease)</td>
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<tr>
<td>FRAP (l mol/l)</td>
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<tr>
<td>602 ± 105</td>
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<tr>
<td>HR (beats/min)</td>
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<td>124 ± 13</td>
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*Comparisons were made by Student’s t-test for independent samples allowing for unequal variances except for sex, where Fisher’s-exact test was applied.

ME, mitral endocarditis; DCM-SR, dilated cardiomyopathy and sinus rhythm; DCM/AF, dilated cardiomyopathy and atrial fibrillation; TAS, total antioxidant status; FRAP, ferric reducing ability of the plasma; HR, heart rate.
decreased (GSH, GSSG and vitamin E concentrations) and some an increased [oxygen radical absorbance capacity (ORAC) and vitamin C concentrations] antioxidant defence in congestive heart failure group compared with controls. Our results gained by the FRAP assay revealed an increase in the groups with heart disease, moreover, there was an increase in FRAP in the more severe cases. So, ORAC and vitamin C values in Freeman’s observation changed in the same direction as FRAP in our experiment. In chronic diseases we can expect an increased synthesis of some antioxidants (i.e. vitamin C) as a consequence of the long term, but low-level free radical attack, while some antioxidants (vitamin E, GSH) may show exhaustion due to the same reason. As vitamin C is an important reducer of the ferric iron, it is not a surprise why FRAP assay show similar change with vitamin C. However, the net increasing and decreasing effects of the free radicals on some antioxidant elements may cause no change in TAS value.

Finally, we suspect that our results give relevant information of the possible changes of antioxidant status of dogs with heart diseases in spite of that we did not measure ROS, and enzymatic or non-enzymatic members (i.e. glutathione-peroxidase, superoxide dismutase, reduced and oxidized glutathione) of the antioxidant system in the blood or tissue of our patients. Further limitations of our study were the relatively small sample sizes and the fact that no information was gained about the vitamin intake and food type fed by the dogs.

Summarizing the results of this study it can be concluded that there is an increased antioxidant reactivity detected by the FRAP, but not by the TAS assay in the blood of dogs with naturally occurring cardiac disorders. The magnitude of this increase seems to be more affected rather by the severity of the cardiac insufficiency and/or by the heart rate or rhythm disorder than by the underlying heart disease itself. It can be suspected that the increased antioxidant concentration is secondary to long term increased ROS formation. Long-term heart diseases can cause periodical hypoxia/reperfusion injury and as a consequence, increased formation of free radicals. This process can stimulate the enzymatic and non-enzymatic antioxidant system to eliminate these radicals and is called ‘pre-conditioning’. (Becker, 2004). Further research on naturally occurring canine cardiac disease is warranted. In these future studies both direct and indirect measurement of ROS formation, ROS reaction products and the antioxidant systems should be simultaneously investigated. The suggested role of increased cardiac XO reactivity in spontaneous canine cardiac disorders should be also confirmed in future studies.

References