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Decreased level of serum paraoxonase (PON) activity in dogs with dilated cardiomyopathy (DCM)

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Serum Paraoxonase (PON1) is a high-density lipoprotein associated esterase capable of hydrolyzing numerous organophosphates and protects low-density lipoprotein against peroxidation. PON1 is believed to play a crucial role in the prevention of cardiovascular disease in animals and PON1 activity has been shown to be low after myocardial infarction, liver disease and during oxidative stress. Here in this article we demonstrated that PON1 level is significantly lowered during the dilated cardiomyopathy (DCM) in dogs. This investigation was carried out on 208 canine serum samples. The serum PON/arylestrase activity was measured in 84 healthy dogs and 124 dogs with dilated cardiomyopathy (DCM) of varying severity. Since heart failure is characterized by oxidative stress, inflammation, deficiency in metabolic substrates and lack of blood supply to heart. Decreased PON activity was significantly observed in advanced stages of DCM.

Key words: DCM, Paraoxonase, Phenyl acetate, High-density lipoprotein, NYHA-classification.

INTRODUCTION

Canine dilated cardiomyopathy (DCM) is a primary cardiac disease characterized by chamber dilatation, systolic and diastolic dysfunction mostly affecting the left side of the heart (Kittleson 1998), besides myxomatous valvular disease it is the most common heart disease in dogs, affecting mainly large and medium breed dogs (Fox 1989). Certain breeds, Doberman Pinscher, Great Dane, Irish Wolfhound, Newfoundland, and Cocker Spaniel known to be more affected (Monnet et al. 1995, Sisson et al. 2000) (Fox and Moise 1999). It has become more evident that inflammation is an important factor in the pathophysiology of heart failure, even though cytokine are not considered being the primary reason for heart failure, their enhanced release and production contributes to remodelling and the failing heart (Anker et al.1997, Sisson et al. 1995). Previous reports suggested that distinct enhancement of Reactive oxygen species (ROS) generation was noticed in failing myocardium(Burton et al. 1984). Surprisingly, antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidases (GSHPx) were not affected during the ROS generation, (Tsutsui et al. 2008). ROS subsequently lead to cellular growth, hypertrophy, remodelling, lipid oxidation, inflammation, and cardiomyocyte apoptosis (Byrne et al. 2003). Sorescu and Griendling (2002) reported the role of ROS in end-stage heart failure due to remodelling

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remodelling of the failing myocardium by fibrosis, collagen deposition and metalloproteinase activation.

Serum Paraoxonase 1 (PON 1) is mainly synthesized in liver and tightly associated with High-density lipoproteins (HDL) (Hasset et al., 1991, Förstermann 2010). PON1 is calcium dependent enzyme and exhibit three distinct activities; PON, arylesterase and diazoxonase (Gan et al, 1991; Canales and Sanchez-Muniz, 2003; Bionaz et al, 2007). Besides the ability of PON1 to hydrolyze a variety of organophosphates and oxidized lipids and thus plays a major role in the prevention of cardiovascular disease in animals (Genest et al, 1992) (Aviram et al, 1995, 2000; Oda et al, 2001). The association between low PON1 levels in serum and increased risk of coronary artery disease (CAD) has been well established from last three decades in cardiovascular research (Mackness et al. 1999, Mackness et al. 2001, Azarsiz et al. 2003, Granér et al. 2006). Besides in cardiovascular diseases reduced serum PON activity has been observed in numerous disease conditions and states of increased ROS formation as altered lipid metabolism, inflammation, diabetes, liver failure, chronic renal failure, and neoplasias (Katsuramaki et al, 2002). Our results from this present investigation showed that the levels of PON1 in serum specimen of dogs with DCM were drastically decreased compared to control healthy samples. Interestingly as disease prolongs (NYHA I to IV) the level of PON1 keeps on diminishing, suggesting the possible influence of cardiac disease on anti oxidative capacity in association with severity of heart disease.

MATERIALS AND METHODS

Study design and criteria of selection

We selected serum samples from healthy dogs (control) and dogs with DCM. DCM – dogs were classified according to New York Heart Association (1994) scheme (NYHA I to IV) depending on the severity of heart disease. PON1 levels in all these samples were measured by using the synthetic compound phenyl acetate as a substrate for Paraoxonase (PON1). 124 client-owned dogs with DCM were enrolled into this prospective multicenter study. Between May, 2005 and November, 2007, 41 dogs were presented to the Small Animal Hospital, University of Leipzig, 38 to the Small Animal Hospital Kaiserberg (Duisburg), 12 to the Small Animal Hospital Huettig (Reutlingen) and 33 to the Small Animal Hospital Vollmar (Wissen/Bonn) (Tab. 1) were included in this study. DCM was diagnosed with echocardiography and measurements were made in right parasternal views. Dogs were excluded if other underlying systemic diseases such as infectious, gastro-intestinal, endocrine (except hypothyroidism), neoplastic, auto-immune diseases or fever of unknown origin were present. All experimental dogs were monitored with thorough medical history and physical examination, followed by echocardiography and a standard 6 - lead electrocardiogram to assess heart rate and diagnose cardiac arrhythmias. For echocardiography, the unsedated patients were either in right lateral recumbency or in standing position, with simultaneous ECG-recording, using accepted techniques (Thomas et al. 1993, Chetboul et al. 2005). Measurements of left ventricular (LV) end-systolic diameter, end-diastolic diameter, LV free wall and septal thickness in diastole and systole were done on 2D-guided M-mode recordings. LV % fractional shortening (FS) and ejection fraction (EF) were calculated by using Teichholz' equation. E – Point septal separation (EPSS) was obtained in M – mode. Left atrial (LA) and aortic diameter were measured in short axis 2D mode and the LA/Ao ratio was calculated (Bonagura 1983, Thomas 1984). In order to determine DCM, criteria stated by the ESVC Taskforce for Canine Dilated Cardiomyopathy were used (2003). The degree of heart disease was classified according to NYHA recommendations (Criteria Committee, 1994). The control group consisted of 72 medium- to large- sized blood donor dogs of the University of Leipzig and 12 Irish Wolfhounds who underwent DCM screening (Table 1). All dogs received a thorough physical examination**,** prior to taking the blood samples. The Irish Wolfhounds were found normal through a cardiologic examination (echocardiography, ECG).

Blood sampling

All dog's blood samples were allowed to clot for 45 minutes at room temperature and centrifuged at 3000 rpm for 15 minutes and the isolated serum was stored at -20 °C for further analysis.

Paraoxonase /arylestrase activity assay

Paraoxonase/arylestrase activity was measured by spectrophotometrically using phenyl acetate as a substrate. The assay reaction buffer was prepared using 2mM phenyl acetate dissolved in 20mM Tris-HCl buffer pH 8.0 containing 2mM calcium chloride, and 20 µl isopropyl alcohol was added for the total reaction volume to facilitate complete dissolution of phenyl acetate in the buffer. Total volume of 3ml reaction mixture was used for the assay. The reaction was initiated by adding 10µl of serum into 2990 µl of buffer substrate. The increased absorbance at 270nm was read for 3 minutes continuously. Blanks were included to correct the spontaneous hydrolysis of phenyl acetate. Enzyme activity was calculated using molar extinction coefficient 1310 M^{-1} cm^{-1}. The Paraoxonase/arylestrase enzyme activity was expressed in U/L (Prasad et al, 2009).

Statistical analysis

The statistic evaluation of the data was performed with the program SPSS. (SPSS Inc. Illinois 60606). The examination of normal distribution was done with Kolmogorov Smirnov test. The data was further analyzed for statistically significant differences between groups with the Mann -Whitney U test, with a level of significance set at $p< 0.05$ or higher.

RESULTS

From the statistical evaluation data of PON activity, a significant difference was accomplished due to the significant deviations from the normal distribution with the non-parametric Kruskal Valais test and U-test after Mann Whitney. The normal PON1 activities in control samples were between 65,000 U/L to 96,000 U/L. No reference values have been published in veterinary medicine except studies done by Turk et al. (2004, 2005, 2007) in dairy cows where normal PON activity was ranging between 60,000 to 80,000 U/L. Table 1 summarizes the baseline characteristics for study participants. The average

Table 1. Client-owned dogs with DCM enrolled.

Parameter	Mean ±SD				
	NYHA I (n-30)	NYHA II (n-23)	NYHA III (n-30)	NYHA VI (n-41)	Control (n-84)
Age (years)	6.3±0.5	7.6±0.6	7.3±0.4	7.6±0.4	4.3±2.5
Sex					
Female	11	7	9	9	44
Male	19	16	21	32	40
Weight (kg)	64.9 kg±3	46.4 kg±4.7	47.4 kg±3.9	51.3 kg±3	37.1 kg±15.1
Heart rate (beats/minute)	120±6	131±7	135±9	174±7	90±12
Breeds	Bouvier (n-1)	Afghan (n-1)	Austr. Shepherd (-1)	AC Spaniel (n-1)	Barsoi (n-1)
	Dalmatian (n-1)	Am. C. Spaniel (n-1)	B. Collie (n-1)	Bobtail (n-2)	B. Collie (n-2)
	Dobermann (n-2)	Bouvier (n-1)	C. Spaniel (n-1)	Bullterrier (n-1)	Dalmation (n-1)
	GSH (n-1)	Briard (n-1)	Deerhound (n-1)	Dalmatian (n-1)	Dobermann (n-8)
	Great Dane (n-2)	Bullmastiff (n-1)	Dobermann (n-4)	Dobermann (n-10)	Dogue de Bordeaux (n-3)
	IW (n-20)	Dalmatian (n-1)	Great Dane (n-2)	Great Dane (n-11)	G. Shorthair (n-1)
	Labr. Retriver (n-1)	Great dane (n-1)	Engl. Setter (n-3)	G. Retriever (n-1)	G. Retriver (n-7)
	NFL (n-1)	GSH (n-1)	G. Retriver (n-1)	Hovawart (n-1)	Great Dane (n-2)
	Rottweiler (n-1)	I. Terrier (n-1)	Hovawart (n-1)	I. Setter 9 (n-1)	Greyhound (n-1)
	-	IW (n-9)	IW (n-10)	IW (n-2)	GSH (n-10)
	-	Labr. retriver (n-2)	Labr. Retriever (n-1)	Mongrel (n-2)	Hovawart (n-1)
	-	Mongrel (n-1)	Mongrel (n-1)	NFL (n-2)	IW (12)
	-	NFL (n-1)	NFL (n-1)	G. Schnauzer (n-2)	Labr. Retrievr (n-14)
	-	W. Shephered (n-1)	Port. Water Dog (n-1)	Rottweiler (n-1)	Malinois (n-5)
	-	-	Min. pinscher (n-1)	St. Bernard (n-3)	Mongrel (n-3)
	-	-	-	-	Rhod. Ridgback (n-2)
	-	-	-	-	Rottweiler (n-3)
	-	-	-	-	Munsterlander (n-1)
	-	-	-	-	St. Bernard (n-2)
	-	-	-	-	Vizsla (n-1)
	-	-	-	-	Weimaraner (n-1)

AC Spainel- American Cocker Spaniel, B. Collie- Border Collie, C. Spaniel-Cocker Spaniel, I.Setter- Irish Setter, I.Terrier- Irish Terrier, IW-Irish Wolfhound, G.

age, body weight and heart rate of the control dog group was 4.3 ± 2.5 years, 37.1 ± 15.1kg and heart rate 90 ± 12BPM, respectively and among these 52.4% were female. Dogs in NYHA I were 6.3 ± 0.5 years of age, 64.9 ± 3kg, heart rate 120 ± 6BPM, and 36.7% were female. Dogs in NYHA II were 7.6 ± 0.6 years, 46.4-±4.7kg body weight, and heart rate 131 ± 7BPM at an average with 30.4%female amongst them. In dogs with NYHA III mean age, body weight and heart rate were 7.3 ± 0.4 years, body weight 47.4 ± 3.9kg, and heart rate 135 ± 9, and 30% female. NYHA IV-classified

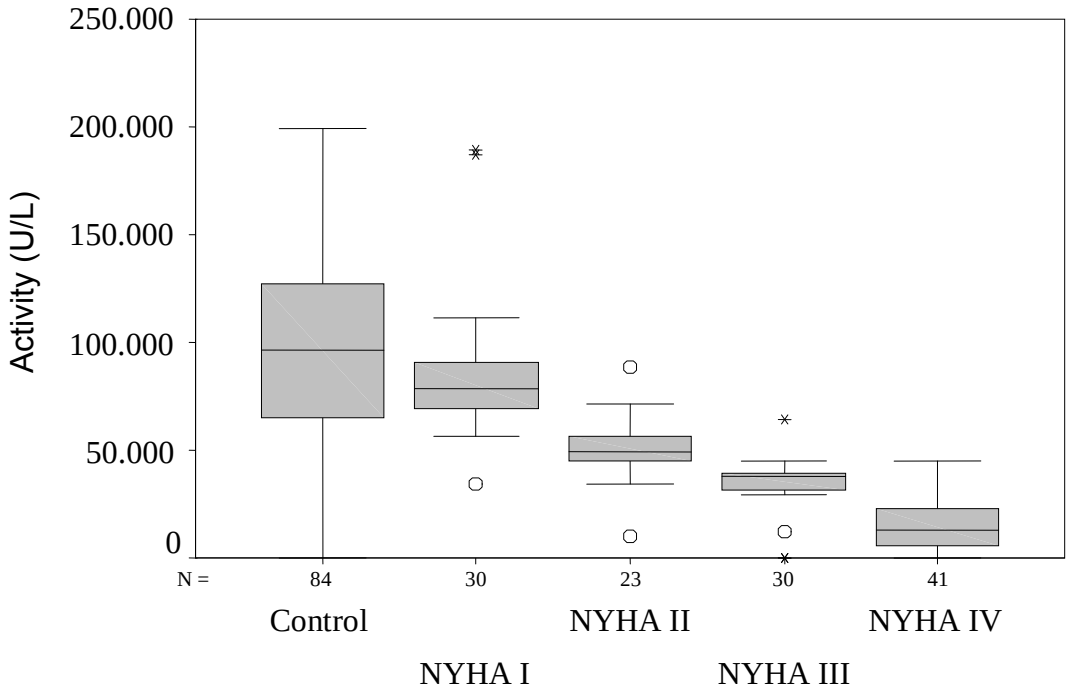

Figure 1. Paraoxonase 1 activity in association of severity of heart disease.

female. classified dogs were 7.6 ± 0.4 years of age, with a weight of 51.3 ± 3kg, heart rate 174 ± 7, and 22% females. The PON1 activity of the serum samples from dogs of NYHA I – IV and control dogs are shown in Figure 1. Median PON levels in control dogs were 96663.5 U/L (65191.8, 127384.8 U/L), in NYHA I 78756 U/L (68689.5, 90850 U/L), NYHA II 49129 (44657, 56783 U/L), NYHA III 37505.5 U/L (31068.8, 39376 U/L), and NYHA IV 12901 U/L (5236, 23000.5 U/L). Median PON concentrations were significantly higher in control group and dogs classified with NYHA I compared with dogs in NYHA class II – IV ($p = 0.0001$).

DISCUSSION

The potential role of reactive oxygen species (ROS) has been discussed on several occasions in scientific literature. With progression of congestive heart failure (CHF) unrelated to etiology increases in ROS a reduction of antioxidant levels (Keith et al. 1998) takes place. Animal models of CHF did not only show excessive ROS generation only, but also impaired myocardial defense mechanisms (Dhalla et al. 1996, Hill et al. 1997). Over the time chronic oxidative injury augments impaired myocardial function and as a consequence also heart failure (Mak and Newton 2001). Considering oxidative stress, production of free radicals, inflammation and genetic factors in cardiomyopathy (Jarvik et al. 2002, Schrier et al 1988), our results from present investigation suggests that oxidative stress and production of free radicals in different stages of heart failure is directly related to the PON1 activity in dogs. However, there was no notable alteration in PON1 activity in dogs with NYHA I, may be due to lack of appearance of clinical symptoms during the initial stage. Hence we assume that Patho physiological mechanisms contributing to de-compensation and ROS formation are activated to a minor degree at NYHA I, whereas in NYHA II, III and IV gradual increase in disease conditions decreases PON activity gradually, suggesting the impor-tance of PON1 in heart disease. Minimal PON activity was shown in dogs classified as NYHA IV, which allows the conclusion of an association between severity of heart disease and anti oxidative capacity. Other stu-dies were able to establish this relationship as well (Keith 1998, Mallat 1998). Other investigators have reported previously that decrease in PON levels in cardiovascular disease and CHF (Watson et al 1995, Xie and Zhao 2002, Aviram et al. 2008) indicating the potential effect of oxidative stress and inflammation on PON1 activity. Hence we could speculate that the lowered level of PON1 is due to the tissue injury and inflammation during the disease.

Previously, Feingold et al. (1998) showed that rapid and sustained decrease of PON1 mRNA level in the liver after LPS administration and now its more evident that our findings also clearly show that PON1 decreases at protein level in the serum, Suggesting that PON1 level is decreased both in post translationally (at protein level) as well as at transcript level (m-RNA level). Hence the results of our study suggested that the anti oxidative ca-pacity of dogs with DCM is impaired with lowered PON1

levels.

Conclusion

Finally we conclude that there is a clear correlation of PON1 activity and severity of heart disease, even though PON1 has been reported so far mostly in regards to cardiovascular disease and protection of HDL and LDL which is not reported in respect to DCM, PON1 activity as a marker of the status of the anti oxidative capacity and heat disease in dogs is very much alluring. Further, studies should be conducted to understand PON1 structural changes during the diseases condition in dogs, also research has to be carried out to understand the mechanism of increasing PON levels in dogs by dietary supplement to cope up with the heart disease and normal physiological conditions. Thus, this novel report on DCM and its correlation with antioxidant enzyme PON1 during pathological condition will open up new era of Paraoxonase research in animals for their better health.

LIMITATIONS

Only 12 dogs out of 84 were used as controls did not receive an echocardiographic evaluation. Possible occult stages of DCM cannot be excluded.

Conflict of Interest

The author(s) have not declared any conflict of interests

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