

Detection of Some Biochemical and Lipid Peroxidation Parameters in *Dirofilaria immitis* Infected Dogs

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Abstract: This research has compared the levels of some biochemical and lipid peroxidation parameters between the *Dirofilaria immitis* infected and ivermectin treated dogs. The dogs of the experimental group were treated with 0.05 mg kg⁻¹ ivermectin and blood samples were collected on the 7th day after treatment. The blood samples were analyzed for microfilariae (mf) concentration and the plasma samples were analyzed for MDA and NO levels, serum ALP, ALT, AST, CHOL, GGT, total protein and triglyceride levels on day 0 and 7. The results of this study showed that the MDA and NO levels of the dogs naturally infected with *D. immitis* were higher than those of the control group. It was also demonstrated that ivermectin treatment is effective on *D. immitis* microfilariae. Consequently, heartworm disease causes lipid peroxidation in dogs but ivermectin treatment possesses no regulatory effects on serum biochemical parameters, except for NO and AST.

Key words: Dogs, lipid peroxidation, plasma, *Dirofilaria immitis*, ivermectin, Turkey

INTRODUCTION

Dirofilaria immitis, commonly found in the pulmonary arteries and right ventricle of dogs and other canines is also seen in cats and human beings. Several mosquito species are responsible for transmission of the disease. Adult form of the parasite may cause to severe pathological damages, such as oedema, asthma, heart failure or even death of the infected dogs (Yildirim, 2004). Infected dogs display symptoms of low and medium severity, including lack of endurance during exercise, persistent cough and weight loss and severe symptoms, including right-sided congestive heart failure, acute and/or chronic vena cava syndrome, liver failure and acute pulmonary thromboembolism (McManus and Pulliam, 1984; Polizopoulou *et al.*, 2000).

In recent years, the role of free radicals in both organ and tissue damage and the ethiopathogenesis of various diseases have become popular areas of interest of health researchers. Free radicals may either be the primary cause of any particular disease or play a secondary role (Gani *et al.*, 2000). Helminths that infect humans and animals may also lead to lipid peroxidation (Amanvermez and Celik, 2002; Kilic *et al.*, 2003a; Kolodziejczyk *et al.*, 2005; Saraymen *et al.*, 2004). Malondialdehyde (MDA) is a product generated during

the oxidative decomposition of certain macromolecules and is found in either free form or bound to certain structures in tissues (Valenzuela, 1991). Despite not being a specific or quantitative indicator of the oxidation of fatty acids, it is considered to be the most significant indicator of membrane lipid peroxidation, resulting from the interaction of reactive oxygen species with cell membranes (Benzer and Ozan, 2003; Durak *et al.*, 2002). Nitric acid reacts with oxygen to form peroxynitrite, which in turn, causes tissue damage. Therefore, Nitric Oxide (NO) may also exist in the environment as an oxidant that is generated upon inflammatory processes (Kilinc *et al.*, 2003).

For the treatment of *D. immitis* is being used various adulticide (Thiacetarsamide sodium and Melarsomine dihydrochloride, *e.t.*) and microfilaricide (Levamisole, Milbemycin oxime and Ivermectin, *e.t.*) anthelmintics (Henry and Dillon, 1994; McCall *et al.*, 1996). Ivermectins, depending on the dose administered are drugs that are effective against many parasites and their life cycles. Although, being quite effective against microfilariae, for the treatment of *D. immitis* is being used various adulticide (Thiacetarsamide sodium and Melarsomine dihydrochloride, *e.t.*) and microfilaricide (Levamisole, Milbemycin oxime and Ivermectin, *e.t.*) anthelmintics (Henry and Dillon, 1994; McCall *et al.*, 1996). Ivermectins,

depending on the dose administered, are drugs that are effective against many parasites and their life cycles. Although, being quite effective against microfilariae, administration of the ivermectin at a dose of 0.05 mg kg⁻¹ is not effective against adults of *D. immitis* (Dillon, 1989). This study aimed at determining the levels of some biochemical and lipid peroxidation parameters on the *D. immitis* infected and ivermectin treated dogs and compared the data acquired.

MATERIALS AND METHODS

Field studies, collection of blood samples and establishment of study groups: Blood samples were collected from the *V. cephalica antebrahii* into heparinised (5 mL) and serum (2 mL) vacutainer tubes from randomly selected 180 dogs raised in Kayseri and its vicinity. Following parasitological examination, Group 1 (5 animals) served as noninfective controls, Group 2 (15 animals) served as nonmedicated vehicle-treated controls, group 3 (15 animals) was infected with *D. immitis* and given ivermectin (Ivomec, Topkim®), a single subcutaneous dose of 0.05 mg kg⁻¹. The blood samples taken from the dogs which were determined to be positive upon parasitological examination were served as the positive control. Blood samples were then collected on the 7th day post-administration.

Parasitological examination: The membrane filtration technique (25 mm diameter-5 µm pore size; Millipore, TMTP 02500) was used for the detection of microfilariae in heparinised blood samples. Species identification was performed by means of the acid phosphatase histochemical staining method (LEUCOGNOST-SP®, MERCK, 1.16304) indicated by Yildirim (2004). The concentration of microfilariae in the peripheral blood was determined using the membrane filtration test (Martin and Collins, 1985). Parasitic antigens in serum samples were displayed by an ELISA based DiroCHEK® Lab Pack Heartworm Antigen kit (Synbiotics Corp., 96-0230). Results were evaluated upon the reading of ELISA plates in a microplate reader (Bio-Tek Instruments, MicroQuant microplate reader) at a wavelength of 630 nm (Reference wavelength: 450 nm). The cut-off value was calculated by adding 0.020 to the OD value of the negative control.

Biochemical analyses: Plasma MDA levels were determined based on the spectrophotometric measurement of the product generated upon the reaction of MDA with thiobarbituric acid (Yoshioka *et al.*, 1979). NO levels were acquired spectrophotometrically as the

total of nitrite and nitrate, in accordance with the Griess method (Benzer and Ozan, 2003). ALP, AST, ALT, GGT, TRG, total protein and cholesterol values were also measured spectrophotometrically by the indicated method (Tietz, 1995).

Statistical analyses: The statistical analyses were evaluated using the one-way Analysis of Variance (ANOVA). Differences between the groups were determined using Tamhane's T₂ test.

RESULTS

Parasitological examination results: Of the 180 dogs examined, 15 (8.3%) were determined to be positive for *D. immitis* according to the results of membrane filtration and antigen ELISA tests. All of the microfilariae detected displayed staining around the anal and excretory pores, which is specific to *D. immitis* microfilariae upon the application of the polycarbonate filter-acid phosphatase histochemical staining method. The mean microfilariae concentration in the peripheral blood was 3895.04 (8-13933 mf mL⁻¹). The microfilariae concentration reduced to 2.86 (0-14 mf mL⁻¹) (99.9%) 7 days after the administration of ivermectin treatment (Table 1).

Blood microfilariae concentration of the dogs infected with *D. immitis*, measured on the 7th day following the administration of ivermectin at a dose of 0.05 mg kg⁻¹ by SC route, were determined to decrease 99.9%, when compared to day 0 (p<0.05), indicating the strong microfilaricidal activity of the ivermectin.

Biochemical findings: Plasma MDA and NO levels and some serum biochemical values of healthy, infected and ivermectin treated dogs are presented in Table 1.

Compared to the control group, the MDA, NO, ALP, AST, ALT, GGT and TRG levels of the dogs with dirofilariosis were determined to increase (p<0.05). On the other hand, ivermectin treatment was demonstrated not to

Table 1: Some biochemical, lipid peroxidation parameters and blood microfilariae concentrations (mean±SD) of the control, infected and ivermectin treated groups

Parameters	Control group	Infected group day 0	Ivermectin treated group day 7
MF (mf mL ⁻¹)	-	3895.04±4538.58 ^a	2.86±4.88 ^b
MDA (µmol L ⁻¹)	3.10±0.25 ^a	4.25±0.88 ^a	4.85±0.75 ^b
NO (µmol L ⁻¹)	9.95±1.35 ^a	30.24±13.96 ^b	17.28±7.07 ^a
ALP (U L ⁻¹)	82.04±10.27 ^a	135.59±48.02 ^b	116.66±65.61 ^{ab}
ALT (U L ⁻¹)	38.66±19.63 ^a	58.88±5.83 ^b	47.21±17.68 ^{ab}
AST (U L ⁻¹)	33.58±13.16 ^a	49.08±7.58 ^b	36.24±16.79 ^a
CHOL (mg dL ⁻¹)	151.66±27.67 ^a	163.14±25.17 ^a	140.19±30.17 ^a
GGT (U L ⁻¹)	7.60±3.24 ^a	9.93±2.80 ^b	8.74±1.57 ^{ab}
PROT (g dL ⁻¹)	60.52±4.36 ^a	62.98±10.02 ^a	59.77±10.30 ^a
TRG (mg dL ⁻¹)	82.04±10.27 ^a	128.13±32.65 ^b	101.77±59.33 ^{ab}

*The difference between the groups coded with different letters in the same row is significant (p<0.05)

cause any significant change in the serum ALP, ALT, GGT, total protein, cholesterol and triglyceride levels, which were measured on day 7. However, the plasma MDA and NO levels on the day 0 of the infected dogs displayed statistically significant increases, when compared to the control group ($p < 0.05$).

DISCUSSION

Dirofilariosis is a life threatening disease of dogs (Niwetpathomwat *et al.*, 2007). Adult *D. immitis* can be found in the right ventricle of the heart, pulmonary artery, caudal vena cava, peritoneal cavity, brain, eyes and other tissues (Martin and Collins, 1985; Polizopoulou *et al.*, 2000; Simpson and Jackson, 1985). Sharma and Pachauri (2005) have reported increase in the serum total bilirubin, globulin, chlorine, phosphor, ALT and AST levels and decrease in the albumin levels of the dogs with dirofilariosis. Balikci and Sevgili (2005) has reported statistically significant increases in the serum ALT, AST, CK and AP values of dogs with dirofilariosis. Kitoh *et al.* (1994) have determined the serum ALT, AST, AP and LDH activities of the dogs administered a *D. immitis* extract to increase. Niwetpathomwat *et al.* (2007) have also demonstrated *D. immitis* infection to cause increase in the serum ALP, AST and ALT levels. Likewise, the ALP, AST, ALT, GGT and TRG levels pertaining to the dogs with dirofilariosis of the study increased significantly, as compared to the control group ($p < 0.05$). Changes in the parameters of the dogs with dirofilariosis of the study suggest *D. immitis* infections to cause especially liver failure. Reports carried out on the ivermectin against *D. immitis* microfilariae, have shown to exhibit strong ivermectin activity against microfilariae (McCall *et al.*, 1996; Venco *et al.*, 2004). However, it has been indicated to be not effective against adult *D. immitis* (Dillon, 1989). Therefore, adult worms may be present in ivermectin treated dogs. In the present study, despite the reduction by 99.9% of the blood microfilariae concentration of dogs infected with *D. immitis* on the 7th day following the administration of ivermectin, the absence of any significant change in the serum ALP, ALT, GGT, total protein, cholesterol and triglyceride levels ($p < 0.05$) and the increase in plasma MDA and NO values, when compared to the control group ($p < 0.05$) might be explained with the lasting of the failures seen in the liver caused by adult worms. In recent years, the role of free radicals in both organ and tissue damages and the ethiopathogenesis of various diseases have become popular areas of interest of researchers. Free radicals may either be the primary cause of a disease or play a secondary role in certain diseases (Gani *et al.*, 2000). Helminths that infect humans and animals may also lead to lipid peroxidation. It has been demonstrated to occur in

rats by Kolodziejczyk *et al.* (2005) and in sheep by Benzer and Ozan (2003) in case of *Fasciola hepatica* infection. It has also been displayed in *Toxocara canis* infection in mice by Yarsan *et al.* (2002), in *Trichuris trichuria* infection in humans by Saraymen *et al.* (2004) and in a *scaris lumbricoides* infection in humans by Kilic *et al.* (2003b). Likewise, lipid peroxidation has been reported in cases of hydatid cysts in humans by Amanvermez and Celik (2002) in *Schistosomia mansoni* infection in mice by Sokkary *et al.* (2002) and humans by Facundo *et al.* (2004) and in *Trichinella spiralis* infection in mice by Grudzinski *et al.* (2001). MDA (1990) and NO (1995) levels are important determinants to evaluate the lipid peroxidation in biological samples. The increase of MDA and NO levels are thought to be an indicator of increased free radicals and lipid peroxidation in dogs infected with *D. immitis* in the present study. Free radicals may also be generated as a result of the use of various drugs. Gurgoze *et al.* (2003) have reported ivermectin to cause the generation of free radicals in sheep. The inexistence of any meaningful difference between the plasma MDA levels and values pertaining to day 0 in the dogs and the serum NO levels elevated when compared to the control group, may be related to the induction of the generation of free radicals by adult parasites present in the body as well as the administration of ivermectin.

CONCLUSION

D. immitis infections were determined to cause lipid peroxidation in dogs and ivermectin treatment, despite exhibiting strong activity against the microfilariae of the parasite was demonstrated not to cause any significant change in biochemical parameters except for AST.

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