

Full length Research Paper

Effect of vitamin C and vitamin E administration on lipoproteins and lipid peroxidation markers in natural diabetic dogs

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Abstract

The effects of vitamin C and vitamin E treatment on the plasma lipoproteins and lipid peroxidation were carried out on 28 clinically diabetic dogs in addition to 10 healthy normal dogs, considered as a control group, their ages ranged from 5-8 years. The diabetic dogs categorized into four groups (7 animals in each). The first one considered as diabetic non treated while the 2nd treated with insulin and 3rd and 4th treated with insulin in combination with vitamin C or vitamin E respectively. Blood samples were collected from control dogs as well as from diabetic ones before and after treatment. All blood samples were collected in heparinized clean tubes and plasma separated for assaying levels of glucose and lipoproteins as well as SOD, CAT, GSH-PX and MDA. The obtained results revealed that, glucose levels returned to normal levels in vitamins treated diabetic dogs. By the same way treatment of diabetic dogs with vitamin C and E significantly decreased lipoproteins levels. Moreover, lipid peroxidations were significantly decreased in vitamins treated groups than diabetic one as indicated by MDA level. We concluded that vitamin C and E treatment may potentiate insulin action on lipid peroxidation in diabetic dogs.

Keywords: Diabetes, lipoproteins, free radicals, antioxidant, dogs.

INTRODUCTION

The dog has been an important medical research model because they share the same environment as humans and develop many of the same chronic diseases (Adams et al., 2000 and Kearns et al., 1999). Much of their biochemical and endocrine mechanisms are similar to humans (Felsburg, 2002 and Kararli, 1995), yet their basal metabolic rate and energy expenditure is 3–8 times greater (Hinchcliff et al., 1997).

Diabetes mellitus is one of the most frequently diagnosed endocrinopathies in cats and dogs. The present classification system divides diabetes into four categories. The most common form of diabetes in companion animals varies with the species. Type 1 diabetes mellitus, previously called insulin dependent diabetes, is most common in dogs. (Expert Committee on

the diagnosis and classification of diabetes Mellitus , 1997). In the same respect, Marmor, et al (1982) and Guptill et al (2003) mention that, diabetes mellitus is one of the most frequent endocrine diseases affecting middle-aged and older dogs, and the prevalence is increasing. The prevalence in some veterinary hospitals had increased threefold to 58 per 10,000 dogs as reported by Guptill et al (2003). At present, there are no internationally accepted criteria for the classification of canine diabetes. No laboratory test is readily available to identify the underlying cause of diabetes in dogs, and diagnosis is generally made late in the disease course. If the criteria established for human diabetes are applied to dogs, at least 50% of diabetic dogs would be classified as type 1, because this proportion has been shown to have antibodies against β -cells (Hoenig, and Dawe, 1992; Davison et al., 2003).

The multiple systemic disturbances in diabetes results from defective utilization of glucose by cells and the

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extensive use of alternate energy-generating metabolic pathways, lipid metabolism was considered (Opie et al., 1979; Bajaj, 1984). In this respect, Darcko et al. (2001) and Bureau et al (2002) found that, diabetes can affect lipoprotein metabolism in many ways. The diabetic's lipoprotein profile is characterized by changes in lipoprotein concentrations and composition, where diabetic patients show increases in triglycerides and decreases in high density lipoprotein (HDL) cholesterol levels.

It has been documented that, oxidative stress considered as a common pathway linking diverse mechanisms for the pathogenesis of complications in diabetes (Shih et al., 2002). In the same side, previous results of Siesjo et al. (1986) approved that, diabetes mellitus tends to increase oxidative stress in both humans and animals, and increased oxidative stress may play a role in the development of late diabetic complications. Mechanisms that contribute to increased oxidative stress in diabetes may include increased nonenzymatic glycosylation, autooxidative glycosylation, and an alteration in sorbitol pathway activity. Glutathione acts as an antioxidant and helps to maintain the normal redox potential within cells. It protects the cell from the toxic effects of reactive peroxides and free radicals, serves in the storage and transport of cysteine moieties, and also participates in catalytic processes and transhydrogenation reactions (Meister, 1985). Glutathione is needed to maintain both the integrity of the cell membrane and the thiol-disulfide status of the cell. A reduction of the glutathione concentration in red blood cells makes them vulnerable to hemolysis, especially in conditions leading to oxidative stress. Other cells are probably also affected under these conditions (Orlowski and Karkowsky, 1976).

In diabetes mellitus, an abnormal generation and disposal of plasma glutathione exists (Collier et al., 1990; Hiramastu and Arimori, 1988; Lovan et al., 1986; Mollennan et al., 1991; and oberley, 1988). Previous studies have outlined the relationship between abnormalities of glutathione metabolism and production of free radicals ((Collier et al., 1990; Oberley, 1988) and have postulated free radicals to play a pathogenetic role in the pathophysiology of the β -cell response to glucose (Ammon, et al., 1979; Ammon and Mark, 1985) and in the genesis of chronic complications (Simonelli et al., 1989).

Numerous studies have demonstrated that antioxidant vitamins and supplements can help lower the markers indicative of oxidant stress and lipid peroxidation in diabetic subjects and animals. A number of studies have reported vitamin C and E and beta-carotene deficiency in diabetic patients and experimental animals (Naziroglu et al., 2005 and Penckofer et al., 2002). Although contradictory results have been reported for blood levels during experimental diabetes. The most frequently studied antioxidant vitamins are C and E. It has been

reported by Naziroglu et al. (1995) that, vitamin C is the strongest physiological antioxidant acting in the organism's aqueous environment. It has been shown to be an important antioxidant, to regenerate vitamin E through redox cycling, and to raise intracellular glutathione levels. Thus vitamin C plays an important role in protein thiol group protection against oxidation.

Lykkesfeldt (2007) found that, the plasma levels of Malondialdehyde (MDA) considered as a good and sole biomarker derived from lipid peroxides, that changes in MDA concentration reflects changes in lipid oxidation level and that lipid oxidation is in fact somehow predictive of atherosclerotic events. All these aspects remain subjects of major controversy.

So, the present study aimed to detect the antioxidant biomarker as well as lipid peroxidation marker in normal and diabetic dogs. Moreover, to investigate the effect of vitamin C and E on blood glucose and lipoprotein levels as well as lipid peroxidation biomarkers levels after administration in combination with insulin.

MATERIALS AND METHODS

Animals

The study was carried out on 28 Dogs clinically affected with diabetes mellitus as indicated by regular hyperglycemic blood samples (by rapid chick test strips). Their ages ranged from 5-8 years. In addition 10 clinically healthy dogs were used as control group.

The diseased dogs categorized into 4 groups (7 animal in each), the 1st one was left as diabetic control one and 2nd one was treated with insulin (NPH) in a dose rate of 0.5 unite/kg body weight twice S/C daily while the 3rd one in addition to insulin, it injected with ascorbic acid in a dose rate of 30mg/kg body weight once daily S/c while the 4th group was treated with insulin and vitamin E-selenium (Seletoc ® in a dose rate of 1ml/20Lb body weight once daily s/c . all treatment continuous for one week.

Sampling protocol

Blood samples were collected after 24 hour from the last dose by vein puncture, into heparinized glass-Stoppard tubes for biochemical analysis of selected parameters (Schalm et al., 1986).

Glucose levels

The plasma glucose levels were estimated using commercially available test kits supplied by Spinreact according to the method described by Kaplan (1984)

Table 1 The plasma levels of glucose (mg/dl) ,cholesterol (mg/dl) , HDL(μ mol/dl) and LDL (μ mol/dl) in normal and diabetic after treatment with insulin or insulin combined with vitamin C or vitamin E.

	Glucose (mg/dl)	Cholesterol (mg/dl)	LDL (μ mol/dl)	HDL (μ mol/dl)
Control	86 \pm 2.63a	126.30 \pm 8.86 a	25.30 \pm 0.59a	264.70 \pm 1.79 a
Diabetic	286 \pm 5.20b	277.30 \pm 2.97b	34 \pm 0.47b	338.90 \pm 7.30b
Diabetic insulin treated	180 \pm 4.60c	204.32 \pm 5.36 c	30.21 \pm 1.22c	300.32 \pm 8.43b
Diabetic vitamin E treated	94 \pm 2.21a	184 \pm 4.00c	28.30 \pm 0.63c	273.40 \pm 4.41a
Diabetic vitamin C treated	88.80 \pm 2.33a	170 \pm 2.50c	26.60 \pm 0.37a	260.80 \pm 2.29a
F. Value	869.38 **	147.15**	52.64**	66.16**

** highly significant at p < 0.01

The different letters in the same column are significant

Table 2 The plasma levels of SOD (units/mg Hb) , CAT (units/mg Hb) , GSH (mg/dl) and MDA ((μ mol/l)) in normal and diabetic dogs

	Normal	Diabetic	T. value
SOD	0.067 \pm 0.005a	0.022 \pm 0.002b	8.29**
Cat	0.079 \pm 0.002a	0.046 \pm 0.002b	8.80**
GSH	20.00 \pm o.47a	16.00 \pm 0.50b	6.22**
MDA	16.90 \pm 0.28a	27.36 \pm 0.50b	17.61**

** highly significant at p <

The same letter in the same rows indicate non significant

Table 3 : The plasma levels of SOD (units/mg Hb) , CAT (units/mg Hb) , GSH (mg/dl) and MDA ((μ mol/l)) in normal and diabetic after treatment with insulin or insulin combined with vitamin C or vitamin E.

	SOD	Cat	GSH	MDA
Control	0.07 \pm 0.005a	0.08 \pm 0.003a	20.00 \pm 0.47a	16.90 \pm 0.31a
Diabetic	0.02 \pm 0.002b	0.05 \pm 0.003b	16.00 \pm 0.34b	27.50 \pm 0.54b
Diabetic insulin treated	0.03 \pm 0.005b	0.045 \pm 0.002b	18.50 \pm 0.33b	25.90 \pm 0.76c
Diabetic vitamin C treated	0.05 \pm 0.002c	0.07 \pm 0.003c	18.50 \pm 0.30c	17.50 \pm 0.30a
Diabetic vitamin E treated	0.05 \pm 0.002c	0.06 \pm 0.002c	18.60 \pm 0.37c	19.70 \pm 0.36c
F. value	29.26**	29.19**	14.52 **	153.34 **

** highly significant at p < 0.01 %

The different letters in the same column are significant

LDL, HDL and Cholesterol

Cholesterol, LDL and HDL test were carried out using commercially available test kits supplied by Quimica Clinica Aplicicad S. A. according to the method described

by Flegg (1973).

Super oxide dismutase (SOD)

The activity of SOD in whole blood was carried out

using commercially available test kits supplied by Biodiagnostic Egypt according to the method described by Nishikimi et al (1972).

Catalase (CAT)

The catalase enzyme was determined using commercially available test kits supplied by Biodiagnostic Egypt according to the methods described by Aebi (1984).

Glutathion peroxidase

The activity of GSX-px in whole blood was carried out using commercially available test kits supplied by Biodiagnostic Egypt according to the method described by Beutler et al (1963).

MDA estimation

MDA levels were estimated using commercially available test kits supplied by Biodiagnostic Egypt according to the methods described by Satoh, (1978) and Ohkawa et al., (1979).

Statistical analysis

The obtained data were statistically analyzed using ANOVA test according to Snedecor and Cochran (1980).

RESULTS

Table (3) showed the plasma levels of glucose and lipoproteins in diabetic and treated groups. The results showed a significant decrease in the glucose level in treated groups than diabetic dogs that reaching nearly to healthy level (86 ± 2.63 mg/dl). Moreover, treatment of diabetic dogs with insulin in combined vitamin C or Vitamin E significantly ($p < 0.01\%$) decreased cholesterol, LDL and HDL levels in plasma.

Table (2) showed a significant ($p < 0.01\%$) decrease in the plasma levels of SOD in diabetic dogs (0.022 ± 0.002) when compared with the normal one (0.067 ± 0.005). By the same manner, serum levels of CAT as well as plasma levels of GSH were significantly reduced in diabetic dog than control normal one. However, the MDA levels were significantly increased (27.36 ± 0.50) in diabetic dog than normal one (16.90 ± 0.28).

Table (3) showed plasma levels of SOD, CAT, GSH and MDA in normal, diabetic and in diabetic after treat-

ment with insulin combined with vitamin C or vitamin E. The result showed that, treatment either by vitamin C or E was significantly increased the plasma levels of SOD, CAT and GSH if compared with diabetic dogs but it is not returned to the normal levels in the non diabetic dogs. The MDA level was significantly increased (27.50 ± 0.54) in diabetic dogs. While vitamin C treatment would return it nearly to the level of non diabetic dog (17.50 ± 0.30).

DISCUSSION

Vitamin E, alpha-tocopherol, is a fat-soluble antioxidant whereas vitamin C, ascorbic acid, is a water soluble antioxidant (Cay et al., 2001; Halliwell et al., 1996). A number of studies have reported the existence of vitamins C and E deficiency in diabetic patients (Ferber, et al., 1999; Czernichow and Hercberg, 2001). The present results showed that, vitamin C and vitamin E treatment of diabetic dogs return the blood glucose to basal control level (table 1). This result could be explained by Paolisso et al (1992) who approved the positive correlation between vitamin E and glutathione directly potentiated insulin secretion in subjects with insulin resistance and impaired glucose tolerance. Moreover, Ceriello et al (1991) found that, glutathione infusions affected the β -cell response to glucose and improved insulin action. On the same respect, treatment of diabetic dogs with vitamin C and Vitamin E could be significantly decreased the plasma level of cholesterol, LDL and HDL as presented in (table 3). These data could be conceded with the previous data of Nazirglu, et al (2004) in their results in postmenopausal diabetic woman.

Diabetes mellitus tend to increase oxidative stress in both humans and animals, and increased oxidative stress may play a role in diabetic complications (Baynes, 1991). The results presented in this study in this study revealed a significant decrease in the activity of the total antioxidant capacity in plasma of diabetic dog than normal subject. These reductions were resulted from decreased activities of antioxidant enzymes, super oxide dismutase (SOD), catalase (CAT) and glutathione (GSH) as appear in table (1). these data were agreed with the previous results of Kedziora et al (2000) and Vessby et al., (2002) who reported that, antioxidant capacity in plasma of type -I diabetics rats was shown to be 16% lower than that of the normal animals, and they returned this reduction to decreased in the activity of antioxidant enzymes.

Most of the body cells have an enzyme system to eliminate active oxygen species, because some of these active species are toxic. SOD, CAT and GSH comprise a major defense system against oxygen toxicity (Mahmoud et al., 2002). They added that, SOD functions

in cellular defense against the active species so study of this enzyme is therefore of potential clinical interest.

In the present study, diabetic condition reduce the super oxide radicals in blood, indicating their role in complications of diseases, these data were previously approved by Janigushi (1992). Although several criteria are without doubt required to adequately describe a biomarker, the entire basis of the biomarker is the measurement of compound that directly reflects certain biological events related to pathogenesis of a disease or condition (Lykkesfeldt, 2007). Thus the rational of MDA as a biomarker relies both that it is derived from lipid peroxide, that changes in lipid oxidation levels reflects the changes in MDA concentration.

The increased level of MDA in diabetic in this study reflected the increase in lipid peroxidation due to disease.

This principle was previously observed by Rahimi et al., (2005) who approved the increases in lipid peroxidation were usually accompanied diabetic patient.

Numerous studies have demonstrated that antioxidant vitamins and supplements can help lower the markers indicative of oxidant stress and lipid peroxidation in diabetic subjects and animals (Naziroglu et al., 2005; Penckofer et al., 2002). In the present study, we observed that vitamin C or vitamin E supplementation to diabetic dog could improve the lipid peroxidation process a compound diabetic condition as appeared in the reduction in the levels of MDA as oxidative damage biomarker (table 2). These results could be explained by the previous observation of Naziroglu et al (2005) who found that, vitamin C is the strongest physiological antioxidant acting in the organism's aqueous environment. It has been shown to be an important antioxidant, to regenerate vitamin E through redox cycling, and to raise intracellular glutathione levels. Thus vitamin C plays an important role in protein thiol group protection against oxidation. It has been proposed that, Vitamin C recycles Vitamin E by a non-enzymatic reaction. Additional interactions have been also reported between Vitamin C and other antioxidants. Vitamin C is associated with the recycling of an important cellular antioxidant, the glutathione and functions with it as a redox couple (Winkler et al., 1994). Glutathione is also involved in the recycling of Vitamin E by an enzymatic mechanism (McCay 1985; Chan 1993). Thus, antioxidants form an interacting system where one antioxidant may modulate the activity of many others. These mechanism could explain the most potent effect of vitamin C supplementation in the reduction of free radicals than vitamin E supplemented dogs.

In non-insulin-dependent diabetic patients, an exaggerated free radical activity (Oberley,1988; Collier et al., 1990) and lipid peroxidation (Simonelli, 1989) associated with a reduction in plasma vitamin E (Kerpen, et al., 1985), SOD, plasma thiol concentrations (Lyons,1991), and the ratio of plasma and erythrocyte GSSG to GSH (Costagliola, 1990) have been demon-

strated. Such enhanced oxidative stress has been correlated with the metabolic control (Ceriello et al., 1991a) and the presence of microangiopathy ((Oberley,1988; Collier,et al., 1990). By contrast, daily oral vitamin E supplements have been found to be useful in reducing oxidative stress and protein glycosylation (Ceriello,et al., 1991b). In the present study we confirmed the presence of reduced plasma free radicals biomarker (MDA) concentrations in diabetic dogs treated with vitamin E, indicating its protective role against oxidative stress. We also demonstrated that daily oral vitamin E may improve insulin action.

Based on these findings, antioxidant could be recommended of help in the treatment of diabetes mellitus to reduce the effect of increasing free radicals. Vitamin C and vitamin E which are an effective nutritional antioxidant, could be a valuable possibility in the treatment of diabetes mellitus.

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