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Short Communication

Effects of Antioxidant Supplement Inducing Superoxide Dismutase for an Aged Dog

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Abstract

The efficiency of an antioxidant supplement developed for humans was investigated in an aged dogs with high plasma malondialdehyde (MDA) level. After administration of this antioxidant supplement for 12 weeks, plasma MDA level in this dog decreased from 4.78 μmol L^{-1} to 1.30 μmol L^{-1}. On the contrary, plasma superoxide dismutase (SOD) activity in this dog increased from 14.5 U mL^{-1} to 30.6 U mL^{-1}. No marked changes in plasma glutathione peroxidase (GSHPx) activities were observed after administration of the supplement for 12weeks. On the basis of changes in plasma parameters levels before and after administration for 12 weeks, this antioxidant supplement is expected to prevent lipid metabolic disorder at early stage by affecting lipid peroxide formation in aged dogs.

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Key words: dog, superoxide dismutase (SOD), malondialdehyde (MDA)

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Abbreviations:

ALP	alkaline phosphatase	LDH	lactate dehydrogenase
ALT	alanine aminotransferase	MDA	malondialdehyde
AST	aspartate aminotransferase	NEFA	non-esterified fatty acid
BCS	body condition score	SOD	superoxide dismutase
BUN	blood urea nitrogen	TC	total cholesterol
CRE	creatinine	TG	triglyceride
GLU	glucose	TP	total protein
GSHP x	glutathione peroxidase		

Introduction

In dogs, the occurrence of lipid metabolic disorders, such as obesity and diabetes, has markedly increased in recent years [1], and hyperlipidemia is regarded as a common factor for obesity in animals [2]. In aged dogs, even if they were clinically healthy, plasma insulin, glucose, and triglyceride concentrations were significantly influenced [3]. Plasma malondialdehyde level, a lipid peroxide stress marker, elevated accompanying with increases in plasma total cholesterol, triglyceride, non-esterified fatty acid, and insulin concentrations in obese dogs [4].

In humans, a correlation between plasma lipid peroxide level and high prevalence of diabetes mellitus has been reported [5,6].To treat lipid metabolic disorder, caused by excess lipid peroxides, a natural and low-molecular weight antioxidant supplement has been developed [7]. The aim of this study was to investigate the effectiveness of the above antioxidant supplement developed for humans in an aged dog.

Materials and methods

Animals

One client-owned aged dog (toy poodle, female (spayed), 15 years old, 4.15kg) was used to evaluate the effectiveness of a natural and low-molecular weight antioxidant supplement developed for humans (UCHIGAWA BIKEN, KURASHI TO KENKOU Inc., Tokyo). The degree of obesity in this dog was assessed as body condition score 3 on the basis of the following five-point scale [1-5]: very thin [1], underweight [2], ideal [3], over- weight [4], and obese [5]. The animal seemed to be clinically healthy.

Antioxidant supplement

A natural, low-molecular weight antioxidant supplement was developed by Niwa et al. The contents of this supplement are as follows: soy beans, sesame, wheat germ, rice bran, Hatomugi (Oriental barley), Yuzu (*Citrus junos*), and Koji fungus. The Koji fungus was mixed to brew soy beans. The soy beans, wheat germ, rice bran, and Hatomugi were crushed to form a powder and roasted at approximately 89 ℃ by infrared rays at a wavelength of 4-14 μm and emulsified in sesame oil obtained from sesame seeds. The dog in this study was given 3g of the antioxidant supplement with ordinary food twice a day.

Blood collection and plasma metabolite and enzyme activities assays

Plasma metabolite and enzyme activities were examined before and after administration of the supplement for 4 weeks, 8 weeks, and 12 weeks, respectively. Blood samples from the dogs' jugular veins after overnight fasting (without any nutrients for ≥ 8 h after the last meal) were collected in heparinized tubes. Plasma was recovered by centrifugation at 4℃ and stored at −25℃ until use. Glucose (GLU), triglycerides (TG), total cholesterol (TC), total protein (TP), blood urea nitrogen (BUN), creatinine (CRE) concentrations and alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) activities were measured using an autoana-

lyzer (JCA-BM2250, JEOL Ltd., Tokyo, Japan) with manufacturer's reagents. Non-esterified fatty acid (NEFA) concentrations were measured using a commercial kit (NEFA-C test, Wako Pure Chemical Industries, Inc., Tokyo, Japan). In addition, plasma MDA concentrations, glutathione peroxidase (GSH-Px) and SOD activities were measured using commercial kits (Northwest Life Science Specialties, LLC, Vancouver).

Results and Discussion

Based upon our previously reported criteria for canine hyperlipidemia [8], the dog in this study was not regarded as hyperlipidemia. However, plasma MDA concentrations, which have been used as a lipid peroxide stress marker [9], were very high compared to those before administration of the antioxidant supplement inducing SOD activity to the dog. After 12weeks of administration of the supplement, a significant decrease in plasma MDA concentrations (from 4.78 μmol L^{-1} to 1.30 μmol L^{-1}) and an increase in SOD activities (from 14.5 U mL^{-1} to 30.6 U mL^{-1}) were observed as shown in Table 1. No significant changes in plasma GSHPx activities were observed after 12 weeks of the supplement administration. On the basis of changes in plasma parameters levels before and after 12 weeks of administration of the supplement, this antioxidant supplement will be expected to have a marked effect against lipid peroxide stress, and this antioxidant supplement may be effective to prevent early stages of lipid metabolic disorder in dogs. In our previous study, this supplement was administrated to 6 overweight dogs and after 4 weeks of administration, we found that plasma MDA concentrations decreased in 5/6 (83.3%) and plasma SOD activities increased in 3/6 dogs (50%), respectively [10].

SOD and GSHPx are enzymes that ameliorate oxidation stress. If dogs are not affected by oxidative stress, plasma SOD and GSHPx activities are likely to be below the detection limit. In this study, plasma SOD activities may increase because of reaction to

Table 1 Changes in plasma biomarker levels before and after administration of antioxidant supplement to an aged dog

		Pre	4 weeks	8 weeks	12 weeks
Body weight	(kg)	4.15	4.15	4.05	4.1
BCS		3	3	3	2.5
GLU	(mg dL^{-1})	95	97	84	83
TC	(mg dL^{-1})	226	213	275	218
TG	(mg dL^{-1})	129	99	101	62
NEFA	(mEq L^{-1})	0.48	0.53	0.24	0.52
MDA	(μmol L^{-1})	4.78	2.09	2.19	1.30
TP	(g dL^{-1})	6.4	6.2	6.2	6.5
BUN	(mg dL^{-1})	26	24	31	32
CRE	(mg dL^{-1})	1.2	1	1.3	1
AST	(IU L^{-1})	24	19	19	20
ALT	(IU L^{-1})	59	66	46	54
ALP	(IU L^{-1})	901	822	809	657
LDH	(IU L^{-1})	62	49	64	75
SOD	(U mL^{-1})	14.5	11.1	19.6	30.6
GSHPx	(mU mL^{-1})	ND	ND	ND	ND

ND: not detected (under detection limit)

oxidative stress other than lipid peroxidation. Furthermore, it seems that lipid peroxidation precedes the increase in plasma TC, TG, and NEFA levels in aged dogs.

Occurrence of lipid metabolic disorder in dogs may increase with aging, and canine hyperlipidemia may be complicated with pancreatitis, liver disease, atherosclerosis, ocular disease, and seizures [11]. Therefore, it will be important to prevent hyperlipidemic conditions as early as possible using this supplement.

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スーパーオキシドジスムターゼ導入抗酸化サプリメントの老犬への効果

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要　約

血漿高マロンジアルデヒド（MDA）値を示す老犬に対する人用に開発された抗酸化サプリメントの有効性を検証した。本抗酸化サプリメントを 12 週間投与後，この犬の血漿 MDA 値は 4.78 μmol L^{-1} から 1.30 μmol L^{-1} に減少した。一方この犬の血漿スーパーオキシドジスムターゼ（SOD）活性は 14.5 U mL^{-1} から 30.6 U mL^{-1} に高まった。12 週間の投与において血漿グルタチオンペルオキシダーゼ（GSHP x）活性の著しい変化は認められなかった。12 週間の投与前後における血漿生化学値より，この抗酸化サプリメントは過酸化脂質の形成に影響し，老犬における脂質代謝障害を早期に予防することが期待される。

(予防動物医学 5 (2), 89-93, 2013)

キーワード：犬，スーパーオキシドジスムターゼ（SOD），マロンジアルデヒド（MDA）

略語：

ALP	アルカリホスファターゼ	LDH	乳酸脱水素酵素
ALT	アラニンアミノトランスフェラーゼ	MDA	マロンジアルデヒド
AST	アスパラギン酸アミノトランスフェラーゼ	NEFA	非エステル型脂肪酸
BCS	ボディコンディションスコア	SOD	スーパーオキシドジスムターゼ
BUN	尿素窒素	TC	総コレステロール
CRE	クレアチニン	TG	トリグリセライド
GLU	グルコース	TP	総蛋白
GSHP x	グルタチオンペルオキシダーゼ		

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