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FOR THE DEGREE OF MASTER

Effect of Oral Antioxidants on the Progression of Canine Senile Cataracts: A Retrospective Study

개의 노령성 백내장 진행에 대한 경구 항산화제의 효과

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Effect of Oral Antioxidants on the Progression of Canine Senile Cataracts: A Retrospective Study

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Abstract

This study was conducted to analyze the delaying or preventing effect of oral product containing several antioxidants on canine senile cataracts through retrospective analysis.

Medical records of dogs from January 1, 2015 to July 10, 2020 were reviewed. Dogs that were 8 years of age or older with senile cataracts were included in this study. The dogs were divided into two treatment groups (dogs administered with Ocu-GLO™ supplement and dogs administered with Meni-One Eye R/C™ supplement) and a control group (dogs that were not administered any supplement).

Dogs with incipient and immature cataracts were included in this study. The period of time that cataracts progressed from incipient to immature, and from immature to mature was recorded for each dog.

Altogether, 112 dogs (156 eyes) with incipient cataracts and 60 dogs (77 eyes) with immature cataracts were included. The mean ages of dogs with incipient and immature cataracts were 11.5 ± 2.5 years and 11.8 ± 2.6 years, respectively. Maltese and Yorkshire Terrier were the most prevalent dogs in the two groups, with incipient and immature cataracts, respectively. There was no significant delaying effect on the progression of incipient cataracts. However, Both Ocu-GLO™ (hazard ratio = 0.265, P = 0.026) and Meni-One™ (hazard ratio = 0.246, P = 0.005) significantly delayed the progression of immature cataracts compared to the control group.

Although there was no significant delaying effect of oral antioxidants on incipient cataract progression, antioxidants could be used to delay the progression of senile immature cataract.

Keywords: antioxidant, cataract, dog, Meni-One™, Ocu-GLO™, senile cataract

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Contents

Introduction

Cataracts are the leading cause of impaired vision or blindness in dogs (Williams *et al.*, 2004). There are several classification schemes for canine cataracts (Davidson and Nelms, 2012). Canine cataracts are classified by specific etiology, age at which cataracts develop (congenital, juvenile, and senile cataract), anatomical location of the cataract within the lens (capsular, subcapsular, zonular, cortical, nuclear, sutorial, axial, and equatorial), and the stage of cataract progression (incipient, immature, mature, and hypermature) (Davidson and Nelms, 2012). Although cataract surgery is considered the only definite treatment for cataracts, preventing cataract formation or delaying cataract progression would be beneficial (Williams and Munday, 2006).

Senile cataracts in dogs are known to be caused by oxidative stress by various factors, including ultraviolet radiation, lipid peroxidation, and protein peroxidation (Abdelkader *et al.*, 2015; Williams, 2006; Ferrer *et al*., 1991). Commercially available oral antioxidant supplements and antioxidant eye drops are publicized to prevent or delay cataract formation in dogs and humans. Lipid peroxidation and protein peroxidation have also been confirmed in the lens of canine senile cataracts (Simeonova *et al.*, 2018).

Several antioxidants that have been reported to delay cataract progression include vitamin C, vitamin E, β-carotene, alpha-lipoic acid, astaxanthin, grape seed extract (GSE), and zinc (Abdelkader *et al.*, 2015; Barden *et al.*, 2008; Jacques and Chylack, 1991; Simeonova *et al.*, 2018; Satyam *et al.*, 2014; Toh *et al.*, 2007; Robertson *et al.*, 1991; Varma *et al.*, 1995; Group A-REDSR, 2001; The REACT group, 2002; Ferrer

et al., 1991). Currently, Ocu-GLO™ and Meni-One Eye R/C™ have been widely used as oral antioxidant supplements in veterinary ophthalmology. No studies have evaluated the effect of commercially available oral antioxidants on senile cataract progression in dogs, and only the effects of alpha-lipoic acid and astaxanthin, which are ingredients of Ocu-GLO™ and Meni-One Eye R/C™, respectively, have been evaluated in dogs with diabetic cataracts (Yang *et al.*, 2020; Williams *et al.*, 2015). Although various antioxidant products for delaying or preventing cataracts have been introduced, there have been a few clinical trials and were only conducted for vitamin C, vitamin E, and β-carotene in humans (Mathew *et al.*, 2012; Toh *et al.*, 2007), and no clinical trials have been conducted in dogs. This retrospective study aimed to analyze whether senile cataract progression was delayed in dogs that were administered oral antioxidants (Ocu-GLO™ or Meni-One Eye R/C™) when compared with the control group.

Materials and Methods

1. Antioxidant products

Ocu-GLO™ (Animal HealthQuest, Bellingham, WA, USA) and Meni-One Eye R/C™ (Meni-one, Nagoya, Japan) were prescribed in dogs with incipient and immature cataracts if the owners complied with administering supplements to their dogs. Both products contain several ingredients that have antioxidant effects. Both supplements contain grape seed extract (GSE) and vitamin E, and each supplement contains various other antioxidants. Ocu-GLO™ contains antioxidants such as alpha-lipoic acid and vitamin C, while Meni-One Eye R/C™ contains astaxanthin and curcuminoid (Table 1). All dogs included in this study were administered a supplement at the dosage recommended by the manufacturer.

Table 1. Ingredients of two oral antioxidants, Ocu-GLO™ and Meni-One Eye R/C™

	Ocu-GLO™	Meni-One Eye R/C™
Ingredients	GLO™ proprietary blend (Grape Seed Extract 95%, Lutein 20%, Omega-3 55%) Vitamin C Vitamin E Vitamin B-1 Vitamin B-3 Vitamin B-6 Folate Vitamin B-12 Biotin Pantothenic Acid Zinc Alpha Lipoic Acid Coenzyme Q10 Lycopene from Tomato Extract Green Tea Exract 40% ECGC Other Ingrediants (Gelatin, Glycerin, Beeswax, Lecithic and Soybean Oil)	Hydrogenated Maltose Starch Syrup Grape Seed Extract Curcuminoid (Turmeric Extract) Astaxanthin Vitamin E Starch Dextrin/crystal Cellulose Modified Starch HPC CMC-Ca Calcium Stearate Fine-grain Silicon Dioxide

2. Animals

Medical records of dogs presented to the Division of Ophthalmology, Veterinary Medical Teaching Hospital, College of Veterinary Medicine, Seoul National University in Seoul, Korea from January 1, 2015 through July 10, 2020 were evaluated retrospectively. To assess the effectiveness of antioxidant products on canine senile cataract progression, the following inclusion criteria were established: (1) dogs with incipient or immature cataracts that were 8 years of age or older and (2) dogs that were consistently administered supplements according to the study protocol. Dogs with congenital cataracts and those with other concurrent ocular diseases or systemic co-morbidities that could promote cataractogenesis (uveitis, hyphema, retinal detachment, progressive retinal atrophy, sudden acquired retinal degeneration syndrome, diabetes mellitus, and trauma) were excluded. Ophthalmic examinations were performed for all dogs prior to the study initiation. Neuro-ophthalmic examinations included menace response and dazzle reflex assessment. Tear volumes were quantified by Schirmer tear test 1 (Schirmer tear test®; MSD, NJ, USA) and intraocular pressures (IOP) were estimated (TONOVET®; Finland Oy, Helsinki, Finland) for all eyes. The eyes of the dogs were examined and pictures were made using a slit lamp biomicroscope (Topcon ® -Model SL-D7; Topcon Corp., Tokyo, Japan). The fundus was evaluated using indirect ophthalmoscopy (Keeler Vantage Plus ®; Keeler Ltd, Windsor, Berkshire, UK). Several eyes could be evaluated for cataracts without instillation of mydriatic drugs, but cataracts located in the equatorial region of the lens were evaluated after

mydriasis using a Finoff transilluminator (Welch Allyn Medical Product, Skaneateles Falls, NY, USA). One of the two diplomates of the Asian College of Veterinary Ophthalmology evaluated and recorded the stage of maturation, anatomical location within the lens, and shape of cataracts. The stages of cataract were classified into incipient, immature, mature, and hypermature. Cataracts with minor or localized opacification involving less than 15% of the lens volume were classified as incipient cataracts. Immature cataract was defined as a stage in which opacification was greater than 15% of the lens volume, the tapetal reflection could be observed through lens opacity, and there was no clinically evident resorption of lens material or capsular wrinkling. Mature cataract was defined as a stage in which the tapetal reflection disappeared due to total opacification. Hypermature cataract was defined as the stage at which proteolysis had progressed with clinically evident resorption of lens material and the lens appeared to be shrunken (Fig. 1) (Ofri, 2017; Davidon and Nelms, 2012). In the retroillumination view, several shots were taken with the light of the weakest intensity as possible, and a picture suitable for evaluation had been selected and saved. Cataract was evaluated by considering not only the retroillumination views and slit lamp biomicroscopy views, but also the equator evaluation with a Finoff transilluminator after dark adaptation. Then, a board-certified ophthalmologist evaluated the cataract. Incipient and immature cataracts were analyzed to evaluate the delaying effect of antioxidant supplements on senile cataracts.

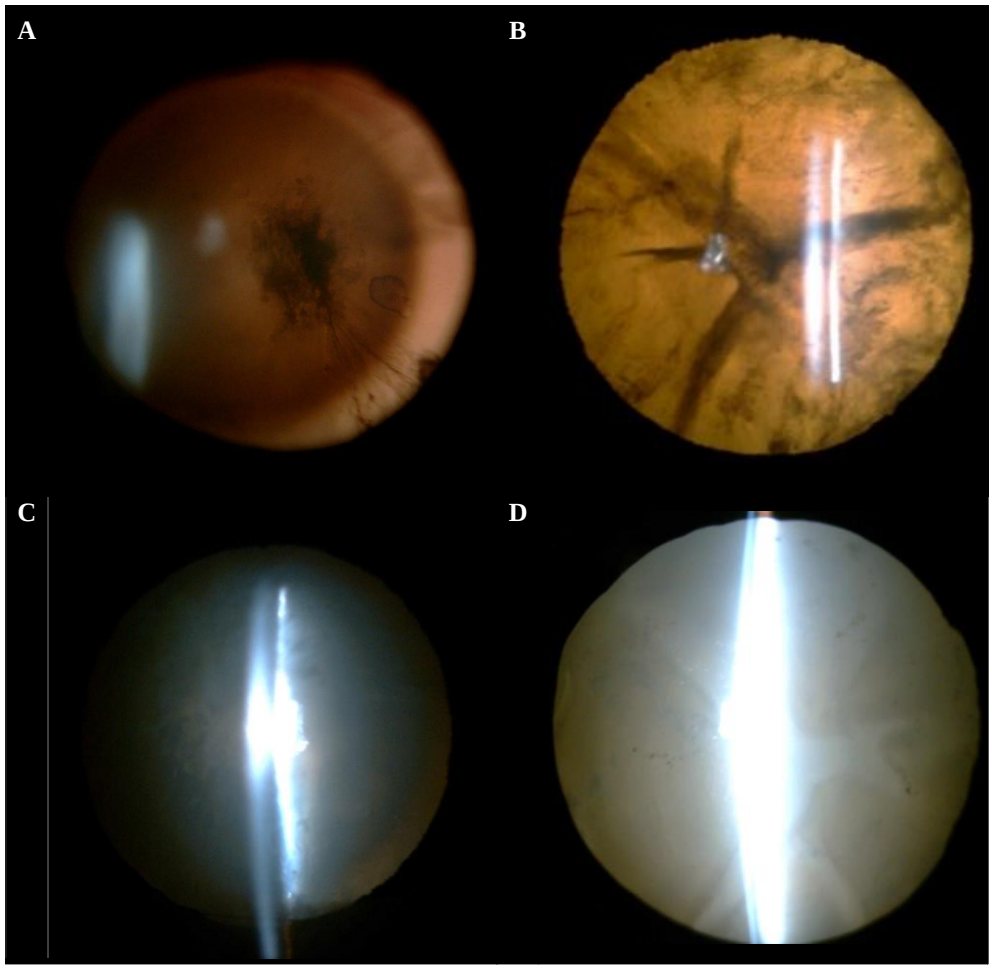

Fig. 1. Stages of cataract in this study. (A) incipient, (B) immature, (C) mature cataract, and (D) hypermature cataract.

3. Incipient cataract progression analysis

Dogs that had incipient cataracts were divided into three groups: dogs that were administered Ocu-GLO™ (group O_1), Meni-One Eye R/C™ (group M_1) and dogs that were not administered any supplement (group C_1). In the C_1 group, the time from the date of diagnosis of incipient cataracts to the date of diagnosis of immature cataracts was recorded. In the O_1 and M_1 groups, the time from the date of supplement administration in the incipient cataract stage to the date of further advancement of cataract and diagnosis of immature cataract was recorded. If the date of supplement administration was earlier than the date of diagnosis of the incipient cataract, the time was calculated in a manner similar to that in the C_1 group. To match the degree of cataract progression at the beginning of follow-up, the slit lamp biomicroscopy images of all dogs were reviewed, and dogs were excluded if the cataract stages were close to immature. The follow-up period was established until a maximum of 800 days was reached in all groups.

4. Immature cataract progression analysis

Dogs with immature cataracts were also divided into the Ocu-GLO™ group (O_2 group), the Meni-One Eye R/C™ group (M_2 group), and the control group (C_2 group). The time from the date diagnosed with immature cataract to the date diagnosed with mature cataract was recorded. To match the degree of cataract progression at the beginning of follow-up, the slit lamp biomicroscopy images of all dogs were reviewed, and dogs were excluded if the cataracts stages were close to mature. The follow-up period was established until a maximum of 800 days was reached in all groups.

5. Statistical analyses

The Kruskal- Wallis test was used to determine the difference in the age variables among the groups. In addition, the Chi-square test was used for the cross-analysis to determine whether there was a difference in sex and species variables among the groups. Fisher's exact test was performed for accurate verification. Kaplan-Meier survival analysis was used for each group to determine the cataract-delaying effect of each antioxidant. The log-rank test was used to evaluate the significance of the differences between the survival curves of the different groups. If there were significant differences in the cross-analysis of age, sex, and breed among the three groups, this could have affected the results. Therefore, the analysis was performed using Cox proportional hazards models that could correct for the effects of variables.

IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA) was used for the statistical analyses, and it was considered statistically significant when $P < 0.05$.

Results

1. Analysis of incipient cataract progression

In total, 156 eyes of 112 dogs were included in the incipient cataract group. Among these, 93 eyes of 72 dogs were included in the C_1 group, 29 eyes of 18 dogs were included in the O_1 group, and 34 eyes of 22 dogs were included in the M_1 group. In the present study, each eye was evaluated independently.

The mean age of all dogs with incipient cataracts was 11.5 ± 2.5 years. The mean ages were 11.9 ± 2.7, 11.1 ± 1.7, and 10.9 ± 2.3 years in the C_1, O_1, and M_1 groups, respectively. No significant difference was observed in the age variables among the three groups (P = 0.139) (Table 2).

There was no significant difference in the sex ratios among the three groups. No significant difference was observed in the sex variables in the cross-analysis performed using the chi-square test and Fisher's exact test (P = 0.559) (Table 3).

There were 17 breeds included in the incipient cataract group. Maltese (n = 47, 30.1%) was the most common breed, followed by Poodle (n = 20, 12.8%), and Cocker Spaniel (n = 19, 12.2%). There was a significant difference in breed variables in the cross-analysis performed using the chi-square test and Fisher's exact test (P = 0.006) (Table 4).

The difference in rates of cataract progression for each group was confirmed through representative retroillumination images (Fig. 2). Based on the results of the Kaplan-Meier survival curves for the three groups, the

survival rates of the O_1 and M_1 groups were higher until 400 days. However, there was no significant difference among the survival rates of the three groups according to the log-rank test (C_1 – O_1 groups, P = 0.161; C_1 – M_1 groups, P = 0.450; O_1 – M_1 groups, P = 0.459) (Fig. 3).

As the distribution of breeds was significantly different among the three groups, the analysis was performed using the Cox proportional hazards models. The hazard-ratio (HR) of the cataract progression compared to Maltese was significant in Shih-Tzu (HR = 6.316, P = 0.010) (Table 5). The variable due to the difference in breed distribution was corrected using Cox proportional hazards models. However, even though the variable was corrected, the group administered supplements did not show a significant difference in HR compared to the control group (C_1 – O_1 groups, HR = 0.460, P = 0.143; C_1 – M_1 groups, HR= 0.320, P = 0.133) (Table 6).

Table 2. Mean ages of the three groups in dogs with incipient cataract

Groups	Mean age (years)	SD	*P*
C_1	11.9	2.7	
O_1	11.1	1.7	.139
M_1	10.9	2.3	
Total	11.5	2.5	

C_1, dogs with incipient cataracts administered no supplements; O_1, dogs with incipient cataracts administered Ocu-GLO™; M_1, dogs with incipient cataracts administered Meni-One Eye R/C™; SD, standard deviation.

Table 3. Sex distribution of the three groups in dogs with incipient cataract

Sex	Groups			Total	*P*
	C_1	O_1	M_1		
F	12 (7.7%)	1 (0.6%)	3 (1.9%)	16 (10.3%)	.559
FS	39 (25.0%)	13 (8.3%)	13 (8.3%)	65 (41.7%)	
M	5 (3.2%)	0 (0.0%)	3 (1.9%)	8 (5.1%)	
MC	37 (23.7%)	15 (9.6%)	15 (9.6%)	67 (42.9%)	

F, female; FS, female spayed; M, male; MC, male castrated.

Table 4. Breed distribution of the three groups in dogs with incipient cataract

Breeds	Groups			Total	*P*
	C_1	O_1	M_1		
Maltese	25 (16.0%)	12 (7.7%)	10 (6.4%)	47 (30.1%)	
Poodle	8 (5.1%)	5 (3.2%)	7 (4.5%)	20 (12.8%)	
Cocker Spaniel	17 (10.9%)	0 (0.0%)	2 (1.3%)	19 (12.2%)	
Shih-tzu	9 (5.8%)	1 (0.6%)	6 (3.8%)	16 (10.3%)	
Yorkshire Terrier	8 (5.1%)	2 (1.3%)	4 (2.6%)	14 (9.0%)	
Mixed	8 (5.1%)	3 (1.9%)	1 (0.6%)	12 (7.7%)	
Pomeranian	4 (2.6%)	0 (0.0%)	1 (0.6%)	5 (3.2%)	
Schnauzer	1 (0.6%)	3 (1.9%)	0 (0.0%)	4 (2.6%)	0.006*
Miniature Pinscher	4 (2.6%)	0 (0.0%)	0 (0.0%)	4 (2.6%)	
Bichon Frise	0 (0.0%)	0 (0.0%)	2 (1.3%)	2 (1.3%)	
Boston Terrier	2 (1.3%)	0 (0.0%)	0 (0.0%)	2 (1.3%)	
Chihuahua	2 (1.3%)	0 (0.0%)	0 (0.0%)	2 (1.3%)	
Labrador Retriever	0 (0.0%)	2 (1.3%)	0 (0.0%)	2 (1.3%)	
Weimaraner	2 (1.3%)	0 (0.0%)	0 (0.0%)	2 (1.3%)	
Dachshund	1 (0.6%)	0 (0.0%)	0 (0.0%)	1 (0.6%)	
Pekingese	0 (0.0%)	1 (0.6%)	0 (0.0%)	1 (0.6%)	

C_1, dogs with incipient cataracts administered no supplements; O_1, dogs with incipient cataracts administered Ocu-GLO™; M_1, dogs with incipient cataracts administered Meni-One Eye R/C™.

*There was significant difference in breed variables in the cross-analysis performed using the chi-square test and Fisher's exact test ($P < 0.05$).

Table 5. The hazard ratio (HR) of each breeds compared to Maltese under the Cox proportional hazard model in dogs with incipient cataract

Breeds	HR (95% CI)	*P*
Maltese	1	
Poodle	4.060 (0.966 – 17.056)	0.056
Cocker Spaniel	2.159 (0.430 – 10.853)	0.350
Shih-Tzu	6.316 (1.543 – 25.857)	0.010*
Yorkshire Terrier	4.266 (0.858 – 21.211)	0.076
Mixed	Unable to measure	-
Pomeranian	Unable to measure	-
Schnauzer	6.626 (0.685 – 64.111)	0.102
Miniature Pinscher	Unable to measure	-
Other breeds†	5.014 (0.993 – 25.308)	0.051

HR, hazard ratio; CI, confidence interval.

*The hazard ratio of the cataract progression was significant compared to the Maltese ($P < 0.05$).

†If there were two or fewer dogs in one breed, they were classified as 'Other breeds' (Beagle (n = 2), Bichon Frise (n = 2), Boston Terrier (n = 2), Chihuahua (n = 2), Labrador Retriever (n = 2), Weimaraner (n =2), Dachshund (n = 1), Pekingese (n = 1)).

Table 6. The hazard ratio (HR) and *P*-value under the Cox proportional hazards model compared to the C_1 group

Groups	HR (95% CI)	*P*
O_1	0.460 (0.163-1.299)	.143
M_1	0.320 (0.072-1.414)	.133

C_1, dogs with incipient cataracts administered no supplements; O_1, dogs with incipient cataracts administered Ocu-GLO™; M_1, dogs with incipient cataracts administered Meni-One Eye R/C™; HR, hazard ratio; CI, confidence interval.

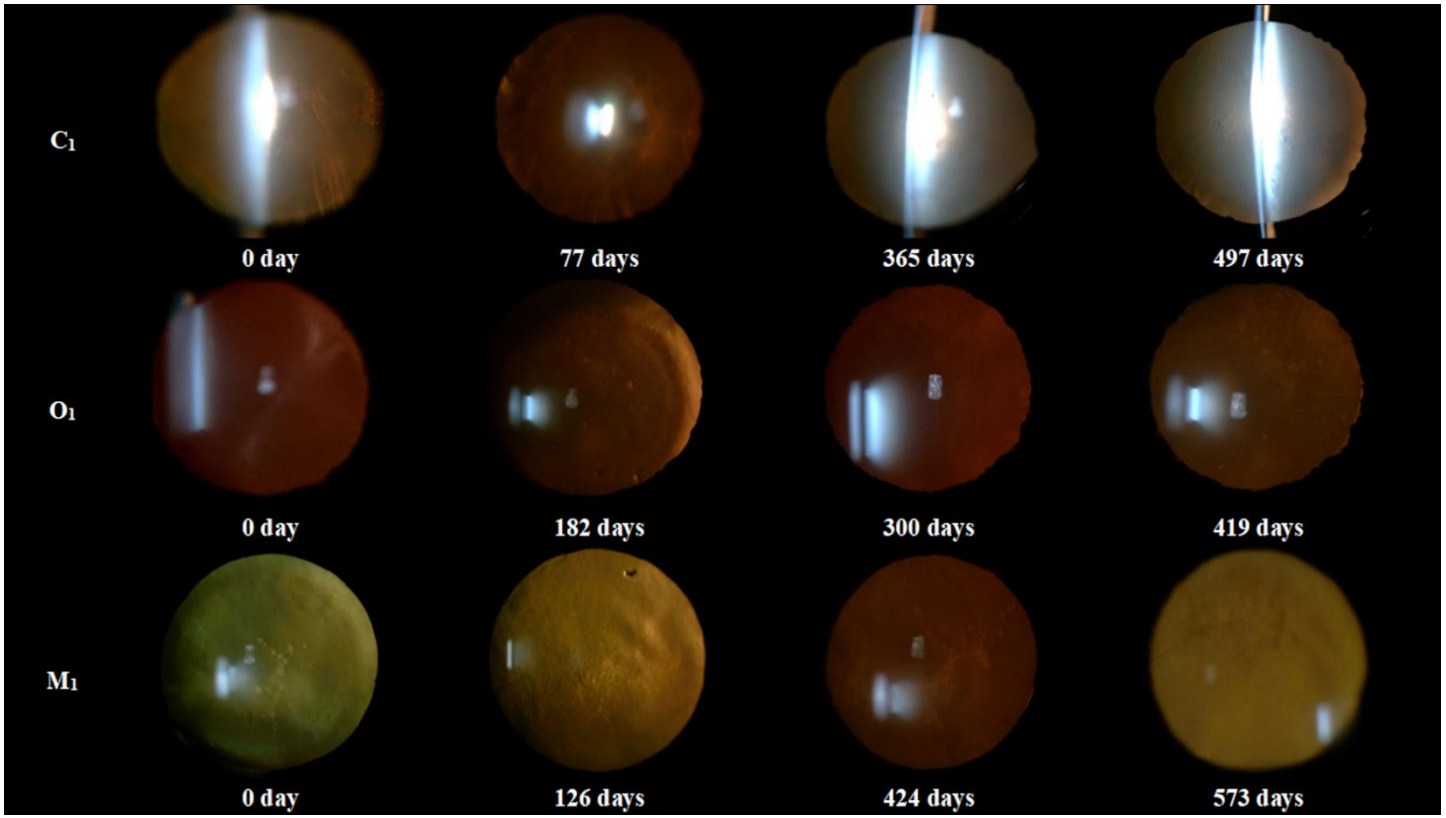

Fig. 2. Representative retroillumination images of incipient cataract progression in three groups; C_1, dogs with incipient cataracts administered no supplements; O_1, dogs with incipient cataracts administered Ocu-GLO™; M_1, dogs with incipient cataracts administered Meni-One Eye R/C™.

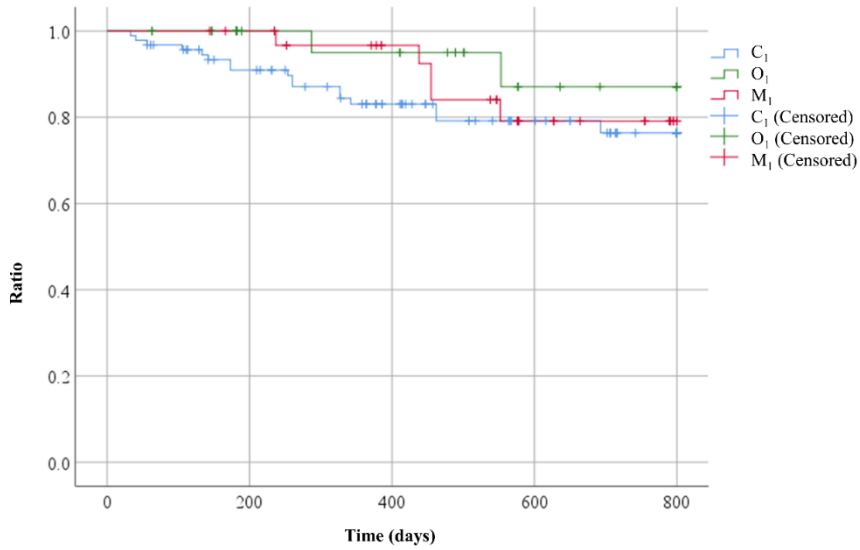

Fig. 3. Kaplan-Meier survival curves in dogs with incipient cataracts; C_1, dogs with incipient cataracts administered no supplements; O_1, dogs with incipient cataracts administered Ocu-GLO™; M_1, dogs with incipient cataracts administered Meni-One Eye R/C™. There was no significant difference among the survival curves of the three groups.

2. Analysis of immature cataract progression

There were 77 eyes of 60 dogs from the immature cataract group that were analyzed including 41 eyes of 34 dogs in the C_2 group, 20 eyes of 13 dogs in the O_2 group, and 16 eyes of 13 dogs in the M_2 group. Each eye was evaluated independently.

The mean age of all dogs with immature cataracts was 11.8 ± 2.6 years. The mean ages of the C_2, O_2, and M_2 groups were 12.0 ± 2.6 years, 12.5 ± 2.6 years, and 10.8 ± 2.3 years, respectively. No significant difference was observed in the mean age among the three groups ($P = 0.093$) (Table 7).

The difference in sex distribution among the three groups was not statistically significant (chi-square test and Fisher's exact test, $P = 0.145$) (Table 8).

There were 12 breeds included in the immature cataract group. The Yorkshire Terrier (19.5%) was the most common in the immature cataract group, followed by Maltese and Poodle (18.2%), and Cocker Spaniel (15.6%). There was significant difference in breed variables in the cross-analysis performed using the chi-square test and Fisher's exact test ($P = 0.002$) (Table 9).

The difference in the rate of cataract progression in each group could be confirmed through representative retroillumination images (Fig. 4). Based on the Kaplan-Meier survival plots, the survival rate of the O_2 and M_2 groups was higher than that of the C_2 group. However, there was no significant difference between the Kaplan-Meier survival curves of the M_2 and C_2 groups in the log-rank test, whereas there was a significant difference

between the survival curves of the O_2 and C_2 (C_2 – O_2 groups, $P = 0.032$; C_2 – M_2 groups, $P = 0.067$; O_2 – M_2 groups, $P = 0.979$) (Fig. 5).

Table 7. Mean ages of three groups in dogs with immature cataract

Groups	Mean age (years)	SD	*P*
C_2	12.0	2.6	
O_2	12.5	2.6	.093
M_2	10.8	2.3	
Total	11.8	2.6	

C_2, dogs with incipient cataracts administered no supplements; O_2, dogs with incipient cataracts administered Ocu-GLO™; M_2, dogs with incipient cataracts administered Meni-One Eye R/C™; SD, standard deviation.

Table 8. Sex distribution of the three groups in dogs with immature cataract

Gender	Groups			Total	*P*
	C_2	O_2	M_2		
F	2 (2.6%)	4 (5.2%)	0 (0.0%)	6 (7.8%)	.145
FS	17 (22.1%)	10 (13.0%)	11 (14.3%)	38 (49.4%)	
M	3 (3.9%)	0 (0.0%)	0 (0.0%)	3 (3.9%)	
MC	19 (24.7%)	6 (7.8%)	5 (6.5%)	30 (30%)	

F, female; FS, female spayed; M, male; MC, male castrated.

Table 9. Breed distribution of the three groups in dogs with immature cataract

Breeds	Groups			Total	*P*
	C_2	O_2	M_2		
Yorkshire Terrier	6 (7.8%)	9 (11.7%)	0 (0.0%)	15 (19.5%)	
Maltese	5 (6.5%)	2 (2.6%)	7 (9.1%)	14 (18.2%)	
Poodle	12 (15.6%)	1 (1.3%)	1 (1.3%)	14 (18.2%)	
Cocker Spaniel	5 (6.5%)	6 (7.8%)	1 (1.3%)	12 (15.6%)	
Shih-Tzu	6 (7.8%)	0 (0.0%)	2 (2.6%)	8 (10.4%)	
Chihuahua	1 (1.3%)	2 (2.6%)	4 (5.2%)	7 (9.1%)	0.002*
Beagle	2 (2.6%)	0 (0.0%)	0 (0.0%)	2 (2.6%)	
Bichon Frise	1 (1.3%)	0 (0.0%)	0 (0.0%)	1 (1.3%)	
Boston Terrier	1 (1.3%)	0 (0.0%)	0 (0.0%)	1 (1.3%)	
Malamute	1 (1.3%)	0 (0.0%)	0 (0.0%)	1 (1.3%)	
Miniature Pinscher	0 (0.0%)	0 (0.0%)	1 (1.3%)	1 (1.3%)	
Pomeranian	1 (1.3%)	0 (0.0%)	0 (0.0%)	1 (1.3%)	

C_2, dogs with incipient cataracts administered no supplements; O_2, dogs with incipient cataracts administered Ocu-GLO™; M_2, dogs with incipient cataracts administered Meni-One Eye R/C™.

*There was a significant difference in breed variables in the cross-analysis performed using the chi-square test and Fisher's exact test ($P < 0.05$).

Table 10. The hazard ratio (HR) of each breeds compared to Yorkshire Terrier under the Cox proportional hazard model in dogs with immature cataract

Breeds	**HR (95% CI)**	***P***
Yorkshire Terrier	1	
Maltese	0.374 (0.112 – 1.244)	0.109
Poodle	0.288 (0.085 – 0.973)	0.045*
Cocker Spaniel	0.239 (0.072 – 0.790)	0.019*
Shih-Tzu	0.133 (0.024 – 0.736)	0.021*
Chihuahua	1.046 (0.284 – 3.856)	0.947
Other breeds†	0.536 (0.146 – 1.965)	0.347

HR, hazard ratio; CI, confidence interval.

*The hazard ratio of cataract progression was significant compared to the Yorkshire Terrier ($P < 0.05$).

†If there were two or fewer dogs in one breed, they were classified as 'Other breeds' (Beagle (n = 2), Bichon Frise (n = 1), Boston Terrier (n = 1), Malamute (n = 1), Miniature Pinscher (n = 1), Pomeranian (n = 1)).

Table 11. The hazard ratio (HR) and *P*-value under the Cox proportional hazards model compared to the C_2 group

Groups	HR (95% CI)	*P*
O_2	0.265 (0.082-0.853)	.026*
M_2	0.246 (0.093-0.650)	.005*

C_2, dogs with incipient cataracts administered no supplements; O_2, dogs with incipient cataracts administered Ocu-GLO™; M_2, dogs with incipient cataracts administered Meni-One Eye R/C™; HR, hazard ratio; CI, confidence interval.

*The hazard ratio (HR) was significantly lower than that of the control group ($P < 0.05$).

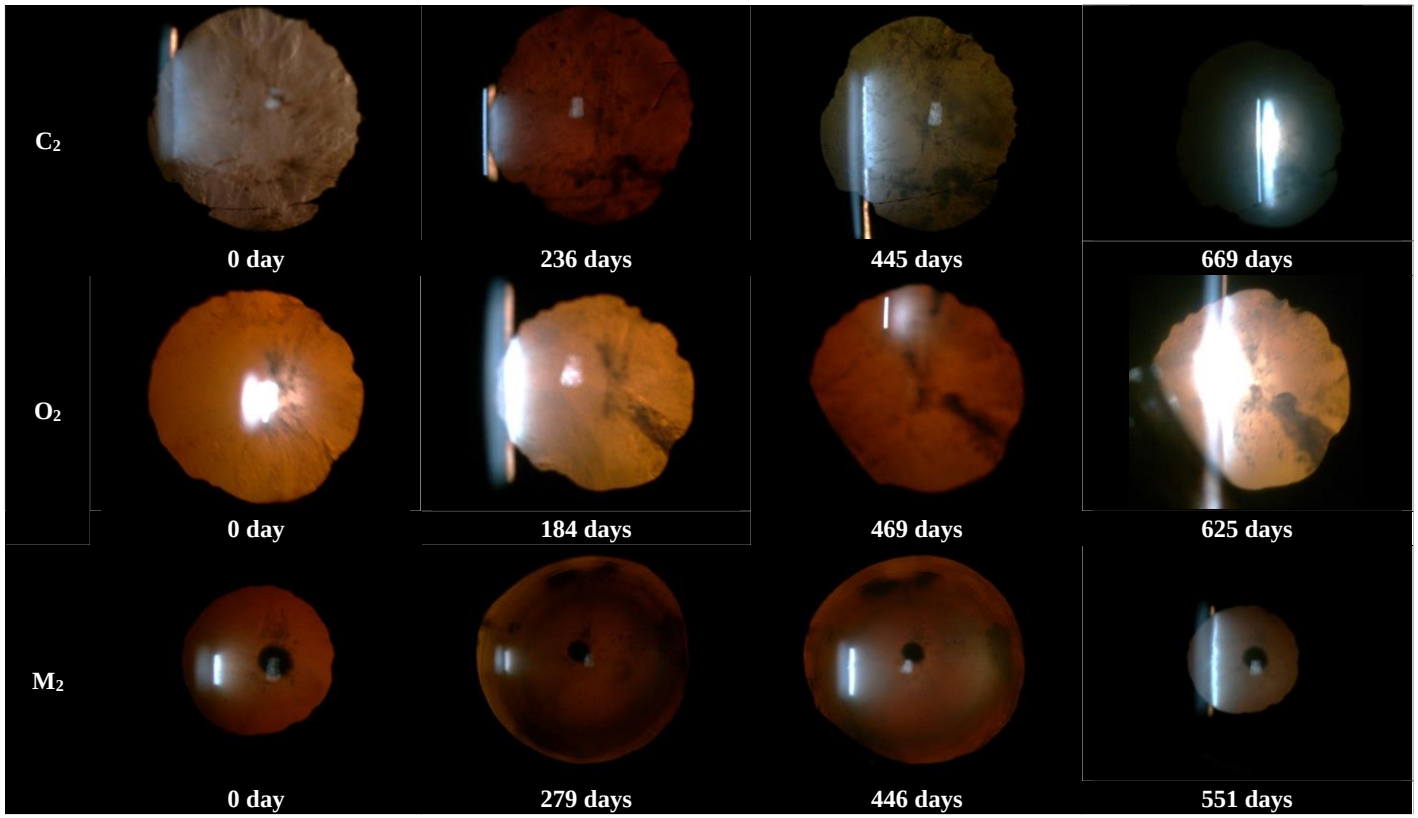

Fig. 4. Representative retroillumination images of immature cataract progression in three groups; C_2, dogs with incipient cataracts administered no supplements; O_2, dogs with incipient cataracts administered Ocu-GLO™; M_2, dogs with incipient cataracts administered Meni-One Eye R/C™.

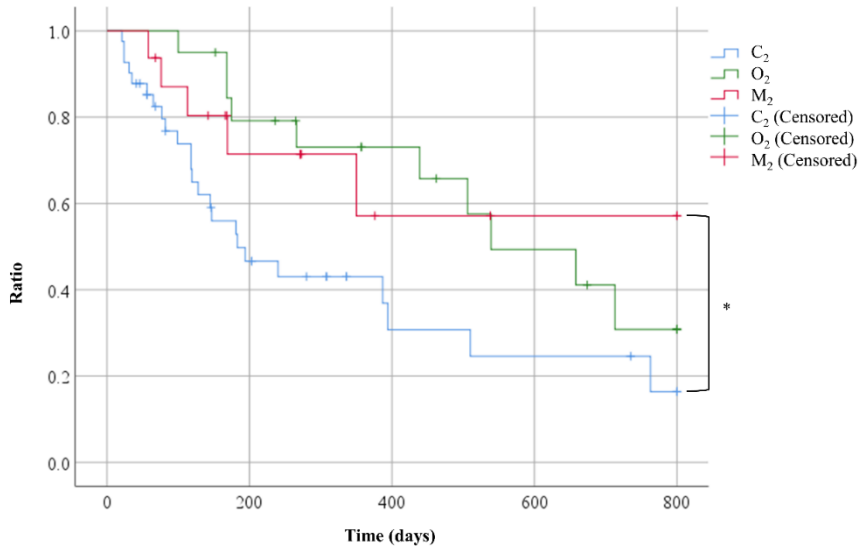

Fig. 5. Kaplan-Meier survival curves in dogs with immature cataracts; C_2, dogs with incipient cataracts administered no supplements; O_2, dogs with incipient cataracts administered Ocu-GLO™; M_2, dogs with incipient cataracts administered Meni-One Eye R/C™.

*There was significant different between O_2 and C_2 groups ($P = 0.032 < 0.05$).

Discussion

Cataracts are responsible for the reduced vision in a large number of aged dogs (Williams *et al.*, 2004; Davidson and Nelms, 2012). A previous study including 2000 dogs in the United Kingdom reported that half of the dogs had cataracts at 9.4 ± 3.3 years of age (Williams *et al.*, 2004). It was also reported that all dogs over 13.5 years of age had some degree of cataracts (Williams *et al.*, 2004). In the study reported here, the mean age of onset of incipient and immature cataracts was 11.2 ± 2.3 years and 11.4 ± 2.5 years, respectively. In the present study, dogs over 8 years of age were analyzed for senile cataracts. In veterinary medicine, the age of onset of senile cataract has not been clearly defined (Davidson and Nelms, 2012). It was reported that if cataracts develop in dogs above 7 years of age, a hereditary basis would be unlikely (Davidson and Nelms, 2012). As each owner might have a different way of counting the age of their dog, dogs over 8 years of age were included to decrease the likelihood of a hereditary basis for cataracts substantially in the present study. The onset of senile cataracts occurs after 10 years of age in small breed dogs and after 6 years of age in large breed dogs (Ofri, 2017). In addition, senile cataracts tended to be more common in large breeds than in small breeds from the same age group (Urfer *et al.*, 2011). As most of the dogs in the present study were small breed dogs, the age criteria for senile cataracts were not established according to their size.

The causes of senile cataract vary and have not been clearly elucidated in dogs and humans (Varma *et al.*, 1995). However, oxidative damage has been considered as one of the primary factors in cataractogenesis (Abdelkader *et al.*, 2015; Barden *et*

al., 2008; Varma *et al.*, 1995; Ferrer *et al.*, 1991). Factors involved in the mechanism of cataractogenesis in humans and mice include reactive oxygen which induces oxidation of lens proteins, DNA damage, and lipid peroxidation (Abdelkader *et al.*, 2015; Williams, 2006; Varma *et al.*, 1995; Group A-REDSR, 2001). Peroxidation of lipids and proteins has also been reported in the lens of dogs with senile cataracts in vitro (Simeonova *et al.*, 2018 #15)

Recently, several dogs have been administered various antioxidant products to delay the progression of cataracts. Previous studies have shown that age-related cataracts are associated with oxidative stress (Abdelkader *et al.*, 2015; Bfabizhayev and Yegorov, 2014; Toh *et al.*, 2007; Williams, 2006; Varma *et al.*, 1995; Ferrer *et al.*, 1991). A previous study reported that glutathione was a critical substance for maintaining the homeostasis of the lens, and vitamin C, vitamin E, β-carotene, and acetyl-salicylic acid could prevent the cataractogenesis and the aging of the lens in vitro (Ferrer *et al.*, 1991). It was reported that vitamin C could prevent cataract formation in vitro, and vitamin E also partially inhibited lipid peroxidation and cataract formation in vitro experiment using the lens of rats (Varma *et al.*, 1995). In addition, it had been shown that glutathione and vitamin C, endogenous antioxidants, play important roles in the lens of humans and rats with senile cataracts (Abdelkader *et al.*, 2015; Robertson *et al.*, 1991). Grape Seed Extract (GSE), one of the antioxidant components of Ocu-GLO™ and Meni-One Eye R/C™, was reported to have antioxidant effects on canine lens epithelial cells in vitro (Barden *et al.*, 2008; Miller *et al.*, 2018). In addition, it was reported that GSE and Zincovit tablet (nutritional food supplement) were effective in delaying or preventing selenite-induced rat cataracts in a rat model (Satyam *et al.*, 2014). However, no clinical trials

have analyzed the delaying effects of GSE in senile cataract in humans or dogs. Alpha-lipoic acid and astaxanthin, which are components of Ocu-GLO™ and Meni-One Eye R/C™, respectively, were found to have a reversal effect on lens opacification and a delaying effect on canine diabetic cataracts (Yang *et al.*, 2020; Williams *et al.*, 2015). The delaying effect of astaxanthin on cataract progression was also reported in selenite-induced rat cataract model (Liao *et al.*, 2009). However, the effects of alpha-lipoic acid and astaxanthin on senile cataracts have not been elucidated. Despite several positive results of antioxidants, the effect of oral antioxidants on the development and progression of senile cataracts is controversial in human clinical trials. Conflicting studies have reported that vitamin C, vitamin E, and β-carotene may delay the increase in opacity of senile cataracts in human clinical trial (The REACT Group, 2002), whereas other clinical trials reported that these supplements were ineffective in delaying or preventing senile cataracts (Mathew *et al.*, 2012; Toh *et al.*, 2007; Group A-REDSR, 2001; McNeil *et al.*, 2004). In addition, there have been no clinical trials to determine the effect of antioxidants on senile cataracts in dogs.

In the study reported here, the effect of Ocu-GLO™ and Meni-One Eye R/C™ on delaying the progression of incipient and immature cataracts was investigated. There was no significant delaying effect on the incipient cataract progression in either the Kaplan-Meier survival analysis or the Cox proportional hazards model. However, in the analysis of immature cataract progression, Kaplan-Meier survival curves of the O_2 and M_2 groups decreased more slowly than that of the C_2 group, and there was a significant difference between the survival curves of the O_2 and C_2 groups. Although there was no significant difference between the survival curves of the M_2 and C_2

groups, the survival curves of the M_2 groups showed a tendency to decrease more slowly than that of the control group. In addition, the Cox proportional hazards model, which corrected the differences of in breed distribution, confirmed that the cataract progression of the O_2 and M_2 groups was significantly delayed.

There was no significant effect of the antioxidant supplements in several human clinical trials (Mathew *et al.*, 2012; Group A-REDSR, 2001; McNeil *et al.*, 2004), and it was not effective in delaying the progression of incipient cataracts in this study. A previous study reported that 70.1% of age-related cataracts did not progress and 29.9% progressed slowly, and the proportion in which cataracts did not progress was higher in incipient cataracts than in immature cataracts in dogs (Fischer *et al.*, 2018). Because incipient cataracts did not progress or progressed very slowly, the significant delaying effect of the supplement on incipient cataracts might not have been identified in this study and human clinical trials. Therefore, to analyze the progression of the incipient cataract, it is necessary to follow up for a sufficient time or devise a method to quantify the cataract area. In human clinical trials, retroillumination images were analyzed, visual acuity was evaluated, and questionnaires were conducted to analyze microscopic changes (Mathew *et al.*, 2012; Group A-REDSR, 2001; The REACT Group, 2002; McNeil *et al.*, 2004). However, as tapetum exists in dogs, the area of the cataract may vary depending on the direction in which the retroillumination view is taken. It would be important to find a method to quantify the cataract area in dogs reliably.

In this study, it was found that antioxidants effectively inhibit the progression of immature cataracts. The immature cataract tends to progress in 72.7% and progresses faster than other cataracts (Fischer *et al.*, 2018). Because of these features, this study,

immature cataracts might have more significant results than incipient cataracts in this study, which evaluated whether the cataract progressed to the next stage. In addition, unlike the human clinical trial, which investigated supplements containing only vitamin C, vitamin E, and β-carotene, the two supplements compared in this study contained several other major antioxidants. This may be due to because of the effects of other antioxidants.

Senile cataracts in humans are associated with several biochemical changes, such as ionic imbalance (high sodium, calcium and low potassium), enzymatic modification (thiol transferase, glutathione reductase, endopeptidases, and aldose reductase), and non-enzymatic or post-translational modification (conformation changes, denaturation, cross-linking, aggregation, deamidation, and glycation) (Abdelkader *et al.*, 2015). Similarly, as the development and progression of senile cataracts might be associated with various mechanisms in dogs, there is no conclusive evidence that antioxidants are not effective in preventing or delaying senile cataracts.

Eye drops containing antioxidants, as well as oral antioxidants, have been widely used in humans and dogs. Pirenoxine antioxidant eye drops were reported to suppress the progression of presbyopia in rats and also reduce lens opacity and delayed cataract progression in dogs (Asakura *et al.*, 1993; Hu *et al.*, 2011; Tsuneyoshi *et al.*, 2017). Additionally, the instillation of eye drops containing N-acetyl carnosine, another antioxidant, resulted in the delayed progression of senile cataracts and subtle reduction in the opacification of the lens in humans and dogs (Babizhayev and Yegorov, 2014; Williams and Munday., 2006; Babizhayev *et al.*, 2002; Babizhayev *et al.*, 2004; Babizhayev *et al.*, 2009; Babizhayev *et al.*, 2001). Therefore, further

studies comparing the delaying effects of oral and topical antioxidants on senile cataracts are needed.

There are several limitations to this retrospective study. Re-evaluation intervals varied among dogs or groups. Long-term re-evaluations might have led to an erroneous conclusion that cataract progression was delayed in dogs with progressive cataracts. Conversely, analysis of dogs with short-term re-evaluations might have led to an inaccurate conclusion of rapid cataract progression. However, because the incipient cataract rarely progresses or progresses very slowly, the re-evaluation interval would not have a significant effect on the results. Dogs with immature cataracts were usually re-evaluated regularly to prevent lens-induced uveitis or to plan surgery. Most of the dogs were re-evaluated at regular intervals, and only few dogs were re-evaluated at short or long-term intervals.

Image analysis programs have been used in previous studies (Williams and Munday, 2006; Babizhayev *et al.*, 2002) to quantify cataract progression, but were not used in the present study as progression to the next stage of cataract maturation was established as a criterion that did not require documentation of subtle changes in lens opacification. If the degree of cataract opacity had been analyzed and quantified in 2D or 3D, it would have been possible to analyze subtle changes, such as the progression of incipient cataracts. However, as there is a tapetum in dogs, it could make and artifacts and errors when measuring the cataract area using the retroillumination view. There is a need for a method of calculating the cataract area or volume without these artifacts or errors.

The locations of the cataracts were not discussed in the present study and the tests were performed without instillation of the mydriatic agent each time. Capsular and

subcapsular cataracts typically do not progress or progress very slowly over time, whereas cataracts located in the anterior, posterior, and equatorial cortex typically progress (Martin *et al.*, 2009). Progression of the cataract varies by location within the lens (Maraini *et al.*, 1994; Magno *et al.*, 1993). Documenting the specific detailed location of cataracts within the lens may have revealed correlations regarding cataract progression based on location. Although every examination focused on the location of the previous cataract and determining whether it progressed, this study did not analyze the subtle changes of cataracts, but evaluated whether they had progressed to the next stage. The progression of the cataract to immature or mature cataracts could be assessed even if mydriatic agents had not been instilled.

In this study, the number of dogs that were administered supplements was low. Analysis of a larger number of dogs may have yielded more accurate and significant results. A controlled prospective clinical trial considering these factors would be beneficial.

Conclusion

In conclusion, despite its limitations, the present study could be meaningful, as it clinically analyzed the effect of antioxidants on senile cataract progression in dogs for the first time. Although there was no significant difference in incipient cataract progression, Ocu-GLO™ and Meni-One Eye R/C™ might have delayed the progression of immature cataract in this study. Considering these results, antioxidant supplements could be beneficial in delaying the progression of immature cataracts in canine senile cataracts and could be prescribed to delay the progression of cataracts in dogs that could not undergo cataract surgery for several reasons.

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References

Abdelkader H, Alany RG, and Pierscionek B. Age-related cataract and drug therapy: opportunities and challenges for topical antioxidant delivery to the lens. Journal of Pharmacy and Pharmacology 2015;67:537-550.

Asakura S, Ohta M, Takimoto Y, Hara K, and Nishi M. Effect of pirenoxine opthalmic solution on senile incipient cataract in dogs. Journal of the Japan Veterinary Medical Association 1993;46:952-957..

Babizhayev MA, Deyev AI, Yermakova VN, Semiletov YA, Davydova NG, Doroshenko VS, Zhukotskii AV, and Goldman IM. N-Acetylcarnosine, a natural histidine-containing dipeptide, as a potent ophthalmic drug in treatment of human cataracts. Peptides 2001;22:979-994.

Babizhayev MA, Deyev AI, Yermakova VN, Semiletov YA, Davydova NG, Kurysheva NI, Zhukotskii AV, and Goldman IM. Efficacy of N-acetylcarnosine in the treatment of cataracts. Drugs in R & D 2002;3:87-103.

Babizhayev MA, Deyev AI, Yermakova VN, Brikman IV, and Bours J. et al. Lipid peroxidation and cataracts. Drugs in R & D 2004;5:125-139.

Babizhayev MA, Burke L, Micans P, and Richer SP. N-Acetylcarnosine sustained drug delivery eye drops to control the signs of ageless vision: glare sensitivity, cataract amelioration and quality of vision currently available treatment for the challenging 50,000-patient population. Clinical Interventions in Aging 2009;4:31.

Babizhayev MA, Yegorov YE. Biomarkers of oxidative stress and cataract. Novel drug delivery therapeutic strategies targeting telomere reduction and the expression of telomerase activity in the lens epithelial cells with N-acetylcarnosine lubricant eye drops: anti-cataract which helps to prevent and treat cataracts in the eyes of dogs and other animals. Current Drug Delivery 2014;11:24-61.

Barden CA, Chandler HL, Lu P, Bomser JA, and Colitz CM. Effect of grape polyphenols on oxidative stress in canine lens epithelial cells. American Journal of Veterinary Research 2008;69:94-100.

Davidson MG and Nelms SR. Veterinary Ophthalmology 5th edn. Ames, Iowa: John Wiley & Sons Inc; 2013:1203-1222.

Ferrer J, Sastre J, Pallardó FV, Federico V, Asensi M, Antón V, Estrela J, Vina J, and Miquel J. Senile cataract: a review on free radical related pathogenesis and antioxidant prevention. Archives of Gerontology and Geriatrics 1991;13:51-59.

Fischer MC and Meyer-Lindenberg. Progression and complications of canine cataracts for different stages of development and aetiologies. Journal of Small Animal Practice 2018;59:616-624

Group A-REDSR. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E and beta carotene for age-related cataract and vision loss: AREDS report no. 9. Archives of Ophthalmology 2001;119:1439.

Hu CC, Liao JH, Hsu KY, Tsai MH, Wu WH, Wei TT, Huang YS, Chiu SJ, and Chen HY. Role of pirenoxine in the effects of catalin on in vitro ultraviolet-induced lens protein turbidity and selenite-induced cataractogenesis in vivo. Molecular Vision 2011;17:1862.

Jacques PF and Chylack Jr LT. Epidemiologic evidence of a role for the antioxidant vitamins and carotenoids in cataract prevention. The American Journal of Clinical Nutrition 1991;53:352S-355S.

Liao J-H, Chen C-S, Maher TJ, Liu CY, Lin MH, Wu YH, and Wu SH. Astaxanthin interacts with selenite and attenuates selenite-induced cataractogenesis. Chemical Research in Toxicology 2009;22:518-525.

Magno BV, Datiles M, and Lasa SM. Senile cataract progression studies using the Lens Opacities Classification System II. Investigative Ophthalmology & Visual Science 1993;34:2138-2141.Mathew MC, Ervin AM, Tao J, et al. Antioxidant vitamin supplementation for preventing and slowing the progression of age-related cataract. Cochrane Database of Systematic Reviews 2012;6.

Maraini G, Belpoliti M, Bonacini M, Graziosi P, Tomba MC, Battaini C, Ferdenzi C, Tencani C, Williams S, and Sperduto R. Incidence and progression of cortical, nuclear, and posterior subcapsular cataracts. American Journal of Ophthalmology 1994;118:623-631.

Martin CL, Pickett JP, and Speiss BM. Ophthalmic Disease in Veterinary Medicine 2nd edn. Boca Raton: CRC Press Inc; 2019:538-549.

McNeil JJ, Robman L, Tikellis G, Sinclair MI, McCarty CA, and Taylor HR. Vitamin E supplementation and cataract: randomized controlled trial. Ophthalmology 2004;111:75-84.

Miller EJ, Gemensky-Metzler AJ, Wilkie DA, Wynne RM, CurtoEM, and Chandler HL. Effects of grape seed extract, lutein, and fish oil on responses of canine lens epithelial cells in vitro. American Journal of Veterinary Research 2018;79:770-778.

Ofri R. Slatter's Fundamentals of Veterinary Ophthalmology 6th edn. St Louis Missiouri: Elsevier Inc; 2018:312-326.

Robertson JM, Donner AP, and Trevithick JR. A possible role for vitamins C and E in cataract prevention. The American Journal of Clinical Nutrition 1991;53:346S-351S.

Satyam SM, Bairy LK, Pirasanthan R, and Vaishnav RL. Grape seed extract and zinc containing nutritional food supplement prevents onset and progression of age-related cataract in Wistar rats. The Journal of Nutrition, Health & Aging 2014;18:524-530.

Simeonova G, Nedev V, and leiman M. Evidence of local oxidative stress in canine senile cataract. Revue de Médecine Vétérinaire 2018;169:7-9.

The REACT Group, Chylack LT, Brown NP, et al. The Roche European American Cataract Trial (REACT): a randomized clinical trial to investigate the efficacy of an oral antioxidant micronutrient mixture to slow progression of age-related cataract. Ophthalmic Epidemiology 2002;9:49-80.

Toh TY, Morton J, Coxon J, and lder MJ. Medical treatment of cataract. Clinical & Experimental Ophthalmology 2007;35:664-671.

Tsuneyoshi Y, Higuchi A, Negishi K, and Tsubota K. Suppression of presbyopia progression with pirenoxine eye drops: experiments on rats and non-blinded, randomized clinical trial of efficacy. Scientific Reports 2017;7:1-7.

Urfer SR, Greer K, and Wolf NS. Age-related cataract in dogs: a biomarker for life span and its relation to body size. Age 2011;33:451-460.

Varma SD, Devamanoharan P, and Morris S. Prevention of cataracts by nutritional and metabolic antioxidants. Critical Reviews in Food Science & Nutrition 1995;35:111-129.

Williams D, Heath M, and Wallis C. Prevalence of canine cataract: preliminary results of a cross-sectional study. Veterinary Ophthalmology 2004;7:29-35.

Williams DL and Munday P. The effect of a topical antioxidant formulation including N-acetyl carnosine on canine cataract: a preliminary study. Veterinary Ophthalmology 2006;9:311-316.

Williams DL. Oxidation, antioxidants and cataract formation: a literature review. Veterinary Ophthalmology 2006;9:292-298.

Williams D, Fitchie A, and Colitz C. An oral antioxidant formulation delaying and potentially reversing. International Journal of Diabetes and Clinical Research 2015;2:023.

Yang M, Chen Y, Zhao T, and Wang Z. Effect of astaxanthin on metabolic cataract

in rats with type 1 diabetes mellitus. Experimental and Molecular Pathology 2020;113:104372.

국문 초록

개의 노령성 백내장 진행에 대한 경구 항산화제의 효과

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본 연구는 몇 가지 항산화제 성분이 포함되어 있는 경구 항산화제가 개의 노령성 백내장 진행을 지연시키거나 예방할 수 있는지를 알아보기 위해 실행되었다.

2015년 1월 1일부터 2020년 7월 10일까지 서울대학교 동물병원에 내원한 개의 의료기록을 수집하였다. 노령성 백내장이 있는 8살 이상의 개를 선정하였으며, 개들을 Ocu-GLO™, Meni-One Eye R/C™를 섭취

한 두 실험군과 아무 것도 섭취하지 않은 대조군으로 구분하였다. 각 군에 속한 개가 초기 백내장 또는 미성숙 백내장으로 진단받은 시점부터 그 다음 단계의 백내장으로 진단된 시점까지의 날짜를 계산하였다.

초기 백내장이 있는 112마리의 개(156개 안구)와 미성숙 백내장이 있는 60마리의 개(77개 안구)가 본 연구에 포함되었다. 초기백내장과 미성숙백내장이 있는 개의 평균 나이는 각각 11.5 ± 2.5세, 11.8 ± 2.6세였다. 초기 백내장이 있는 개 중에서는 Maltese가 가장 많았으며, 미성숙 백내장이 있는 개 중에서는 Yorkshire Terrier가 가장 많았다. 초기 백내장의 진행을 유의미하게 지연시키는 효과는 확인되지 않았다. 하지만 Ocu-GLO™ (위험비, hazard ratio = 0.265, P = 0.026)와 Meni-One Eye R/C™ (위험비, hazard ratio = 0.246, P = 0.005)을 복용했을 때 미성숙 백내장의 진행이 대조군에 비해 유의적으로 지연되는 것을 확인하였다.

이상의 결과로부터 초기 백내장 진행에 대한 경구용 항산화제의 유의적인 지연 효과를 확인할 수 없었지만, 항산화제를 복용함으로써 미성숙 백내장의 진행을 지연시킬 것으로 예측할 수 있다.

주요어: 개, 노령성 백내장, 백내장, 항산화제, Meni-One Eye R/C™, Ocu-GLO™

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