A DISSERTATION

FOR THE DEGREE OF MASTER

The effect of intravitreal cidofovir injection on end-stage glaucoma in dogs: A retrospective study of 153 eyes from 2016 to 2021

개의 말기 녹내장에서 유리체 내 시도포비어 주사의 효과: 153개 안구의 후향적 연구 (2016~2021)

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The effect of intravitreal cidofovir injection on end-stage glaucoma in dogs: A retrospective study of 153 eyes from 2016 to 2021

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Abstract

This study aimed to evaluate the long-term efficacy, prognostic factors, and complications of intravitreal cidofovir injection in dogs with end-stage glaucoma.

Medical records of 130 dogs (153 eyes) that underwent intravitreal cidofovir injections between 2016 and 2021 were reviewed. A minimum follow-up period of 6 months was required as the inclusion criterion. Signalment, type of glaucoma, pre-injection intraocular pressure (IOP), types of applied glaucoma eye drops, pre-

existing ocular diseases, outcomes, and complications were recorded. Success was defined as IOP of ≤25 mmHg at the 2-week recheck that remained to the 6-month recheck.

The overall success rate of intravitreal cidofovir injection was 91.5% (140/153). The success rate of a single injection was 69.3% (106/153). Forty-two eyes received a 2nd injection, and the success rate was 59.5% (25/42). Fourteen eyes received a 3rd injection, and the success rate was 42.9% (6/14). Six eyes received a 4th injection, and the success rate was 33.3% (2/6). Two eyes received a 5th injection, and the success rate was 50.0% (1/2). IOPs at 6 months post-injection were significantly higher when the injection was repeated, fewer glaucoma eye drops were applied prior to the injection, and cataract stages were advanced at the time of injection ($p < 0.05$). The most common complications were phthisis bulbi (42.5%), cataract progression (30.1%), and intraocular hemorrhage (16.3%). Six eyes were enucleated, and three were enucleated due to corneal perforation.

Intravitreal cidofovir injection had a high long-term success rate in lowering IOP in dogs with end-stage glaucoma.

Keywords: canine, cidofovir, end-stage glaucoma, intraocular pressure, intravitreal injection, pharmacological ciliary body ablation

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Introduction

Canine glaucoma constitutes a group of diseases characterized by increased intraocular pressure (IOP) that results in disruption of axoplasmic flow in the optic nerve head, apoptosis of retinal ganglion cells and their axons, and cupping of the optic disc (Miller, 2018). Glaucoma is one of the leading causes of blindness in dogs, with a prevalence rate of approximately 1–2% (Gelatt and MacKay, 2004a; Gelatt and MacKay, 2004b; Plummer *et al*., 2021). Even with extensive medical and surgical treatment, irreversible blindness and ocular pain are common sequelae in dogs within months of disease onset (Komáromy *et al.,* 2019). Treatment options for end-stage glaucomatous eyes include surgical procedures, such as enucleation and evisceration, and pharmacological ciliary body ablation (CBA). Pharmacological CBA may be preferred in dogs because it often does not require general anesthesia and may be economically favorable. Gentamicin and cidofovir are two intravitreal injections available for pharmacological CBA, and both drugs are known to be safe and effective in decreasing IOP by reducing aqueous humor production (Low *et al.,* 2014; Rankin *et al.,* 2016; Maggs, 2018; Julien *et al.,* 2021).

Cidofovir is an antiviral drug that may have cytotoxic effects on the ciliary body, reduce aqueous humor formation, and decrease IOP when injected intravitreally (Maggs, 2018). Cidofovir-induced ocular hypotony was first noted in humans as an adverse drug effect (Davis *et al.,* 1997). Subsequently, studies determining the effects on IOP and toxicity of intravitreal cidofovir in guinea pig and rabbit models were established (Taskintuna *et al.,* 1997). Dose-dependent decrease in IOP was observed in guinea pigs, while statistically insignificant drop in IOP was

observed in rabbits 2 and 4 weeks after intravitreal cidofovir injection. Corneal epithelial edema, thickening, and vascularization, destruction of ciliary body, intraocular inflammation, outer retinal destruction, and retinal pigment epithelial changes were observed in guinea pigs histologically. Similar findings were observed in the rabbit eyes but at higher doses. More recently, intravitreal injection of cidofovir was reported to reduce IOP in canine patients with chronic glaucoma successfully (Low *et al.,* 2014).

When drugs are injected intravitreally, various factors, including the volume and location of injection, pathophysiological condition of the vitreous body and lens, presence of inflammation and infection, and the molecular size of the drug, affect drug distribution (Regnier, 2021). Studies on immediate efficacy and dose-dependent effect of intravitreal cidofovir injection in dogs are few (Low *et al.,* 2014; Park *et al.,* 2017), and studies on the long-term efficacy and the impact of other factors on intravitreal cidofovir injection have not been conducted. Therefore, this retrospective study aimed to evaluate the long-term efficacy, effects of different prognostic factors on outcomes, and complications of intravitreal cidofovir injection in end-stage glaucoma in dogs.

Materials and Methods

1. Patients

The medical records of client-owned dogs with ophthalmic diseases referred to the Seoul National University Veterinary Medical Teaching Hospital (SNU-VMTH) between 2016 and 2021 were retrospectively evaluated. Dogs that received intravitreal cidofovir injections after being diagnosed with chronic end-stage glaucoma were included in the study. Informed consent was obtained from the owners of all animals described in this work for the procedures and therapy undertaken and the use of data. The study conformed to the Guidelines for Ethical Research in Veterinary Ophthalmology (GERVO).

All dogs underwent a complete ophthalmic examination, including neuro-ophthalmic examination, slit-lamp biomicroscopy (SL-D7, Topcon, Tokyo, Japan), indirect ophthalmoscopy (Vantage Indirect Ophthalmoscope®, Keeler, Windsor, UK), and rebound tonometry (Icare® Tonovet, Icare, Helsinki, Finland). Chronic end-stage glaucoma was diagnosed based on an IOP of $\geq$25 mmHg, absence of menace response and dazzle reflexes, and clinical signs consistent with chronic glaucoma, including buphthalmos, episcleral injection, Haab's striae, corneal edema, and lens dislocation. Optic nerve atrophy, tapetal hyperreflectivity, and retinal vessel attenuation were included as posterior changes observed in end-stage glaucoma. Eyes were excluded based on the following criteria: (1) follow-up period of less than 6 months, (2) previous history of glaucoma surgery, or (3) suspected ocular mass. Ocular ultrasonography was performed on all eyes suspected of ocular mass before intravitreal cidofovir injection, especially when direct ophthalmic examination of the

posterior segment was unavailable, to ensure no injected eyes had masses. Dogs that did not allow the procedure with manual restraint were sedated for the procedure due to ethical concerns, and they were excluded from the study.

2. Glaucoma eye drops

The glaucoma eye drops included in this study were 0.005% latanoprost (Xalatan®, Pfizer, NY, USA), 0.03% bimatoprost (Lumigan®, Allergan, CA, USA), 2% dorzolamide (Trusopt®, Merck, NJ, USA), a combination of 2% dorzolamide hydrochloride/0.5% timolol maleate (Cosopt®, Merck, NJ, USA), 1.0% brinzolamide (Azopt®, Alcon Laboratories Inc, TX, USA), and 0.5% timolol (Timolol Maleate Ophthalmic Solution USP; Alcon Laboratories Inc, TX, USA). Different types of eye drops (range: 1–3 types of eye drop per dog) were prescribed according to the patient's glaucoma status.

3. Intravitreal cidofovir injection

After application of 0.5% proparacaine hydrochloride ophthalmic solution (Alcaine®, Alcon Laboratories Inc, TX, USA) on the site of aqueous humor paracentesis and intravitreal injection with a cotton swab for 1 min, the eye was aseptically prepared with 0.5% povidone-iodine solution. None of the dogs were under sedation or anesthesia during the procedure. Aqueous humor paracentesis was performed through the peripheral cornea with a 30-g needle and a plunger-less syringe to ambient pressure. A volume of 0.12 mL (600 μg) of cidofovir was injected intravitreally with an insulin syringe 5–8 mm posterior to the dorsolateral limbus, in the area of pars plana, directed toward the optic nerve to avoid hemorrhage or

damage of the lens. A combination of 4 mg triamcinolone acetonide (Dongkwang, Seoul, Korea) and 4 mg gentamicin sulfate (Shinpoong, Seoul, Korea) was injected with a 30-g needle underneath the dorsal subconjunctiva. All dogs were prescribed topical antibiotics and anti-inflammatory drugs to prevent infection and manage intraocular inflammation post-injection. Prescribed post-procedure antibiotics and anti-inflammatory eye drops were 0.5% moxifloxacin (Vigamox®, Alcon Laboratories Inc, TX, USA), 1% prednisolone acetate (Pred Forte®, Allergan, CA, USA), neomycin/polymyxin B/dexamethasone (Maxitrol®; NOVARTIS, Puurs, Belgium), and 0.03% flurbiprofen sodium ophthalmic solution (Bausch & Lomb, FL, USA). Eye drops were chosen based on complete ophthalmic examination and clinical signs, and the frequency and period of application were decided accordingly. Glaucoma eye drops were continued for 4 days post-injection and discontinued 10 days before the 2-week recheck. Re-injection was considered when IOP was ≥25 mmHg and the patient expressed clinical signs of high IOP, including episcleral and conjunctival injections, corneal edema, blepharospasm, third eyelid elevation, and changes in dog's behavior. Glaucoma eye drops were re-prescribed when IOP was ≥25 mmHg and when the owner did not want re-injection.

4. Clinical data

A complete ophthalmic examination was performed during each re-examination. Clinical data at the time of injection and after injection (second week, sixth month, and final examination) were collected. Clinical data included signalment, IOP, type of glaucoma, pre-existing ocular diseases, number of different glaucoma eye drop types applied before and after the procedure, and complications. Procedure success

was defined as an IOP of ≤25 mmHg at the 2-week recheck that remained to the 6-month recheck. Primary glaucoma was diagnosed when the glaucomatous eye showed no clinical signs of underlying ocular diseases that may trigger an increase in IOP at the onset of the disease. Breed predisposition along with morphological findings of the iridocorneal angle and ciliary cleft via gonioscopy and ultrasound biomicroscopy of the contralateral eye were also considered. Secondary glaucoma was diagnosed when the eye had other concurrent ocular diseases that could have resulted in glaucoma. Ocular conditions observed at the time of injection included intraocular hemorrhage, lens displacement, chronic uveitis, and vitreal degeneration. Previous intraocular surgery was also recorded.

Complications included corneal ulceration, corneal degeneration, intraocular hemorrhage, progression of cataracts, development of ocular mass, endophthalmitis, and phthisis bulbi. Phthisis bulbi was diagnosed when IOP was below 5 mmHg, and the eye showed clinical signs, including shrinkage and disorganization, characterized by a smaller globe size, folding of Descemet's membrane, opaque and thickened cornea, neovascularization of the iris, and retinal detachment (Tripathy *et al.,* 2018).

5. Statistics

All data are presented as mean ± standard deviation (SD). Statistical analyses were performed using statistical software (SPSS® ver.26 for MAC, IBM, USA). Collected data were analyzed using a paired-sample t-test. Multiple linear regression analysis was performed to evaluate the association between post-injection IOPs and other variables and the association between the time to phthisis bulbi and other variables. Statistical significance was set at $p < 0.05$.

Results

A total of 153 eyes from 130 dogs were included in this study (oculus dexter: 78 eyes; oculus sinister: 75 eyes). The mean ± standard deviation (SD) follow-up duration was 19 ± 12 months (range: 6–67 months). The age was 10.8 ± 3.6 years (range: 1–18 years). Fifty-six dogs were spayed females, 50 were neutered males, 19 were intact females, and 5 were intact males. Fourteen breeds were included, and Shih Tzu (51/130, 39.2%) was the predominant breed followed by the American Cocker Spaniel (30/130, 23.1%), Maltese (16/130, 12.3%), Poodle (10/130, 7.7%), mixed breed (5/130, 3.8%), and French Bulldog (4/130, 3%). Other breeds included Bichon Frise, Jindo, Pekingese, Samoyed, Schnauzer, White Terrier, Boston Terrier, and Spitz (Table 1).

TABLE 1. Breeds included in the study and the success rate of intravitreal cidofovir injection in each breed

Breeds	**Number of dogs (%)**	**Number of eyes (%)**	**Success (%)**	**Single injection (%)**
Shih Tzu	51 (39.2)	60 (39.2)	51 (85.0)	39 (65.0)
American Cocker Spaniel	30 (23.1)	36 (23.5)	35 (97.2)	22 (61.1)
Maltese	16 (12.3)	19 (12.4)	19 (100.0)	16 (84.2)
Poodle	10 (7.7)	12 (7.8)	12 (100.0)	11 (91.7)
Mixed	5 (3.8)	6 (3.9)	6 (100.0)	6 (100.0)
French Bulldog	4 (3.0)	4 (3.0)	3 (75.0)	4 (100.0)
Others	14 (10.8)	16 (10.5)	14 (87.5)	12 (75.0)

One hundred eyes (100/153, 65.4%) had primary glaucoma, and 44 (44/153, 28.8%) had secondary glaucoma. Diagnoses of primary or secondary glaucoma in nine eyes (9/153, 5.9%) were limited because they were referred to the clinic in the late stages of the disease, and it was unable to differentiate between the two. The pre-injection IOP was 47.6 ± 21.6 mmHg (range: 5–99, median: 48), 48.3 ± 24.6 mmHg (range: 5–96, median: 46.5), 45.3 ± 22.0 mmHg (range: 11–90, median: 41), 54.0 ± 17.4 mmHg (range: 34–76, median: 53), and 54.0 ± 18.4 mmHg (range: 41–67, median: 54) in groups that received a single, 2^{nd}, 3^{rd}, 4^{th}, and 5^{th} injection, respectively (Table 2). In eyes with IOP lower than 25 mmHg, cidofovir was injected because IOP spikes were observed once glaucoma eye drops were discontinued, and the owners wanted to withdraw the eye drops. The number of glaucoma eye drop types applied before injection was 2.0 ± 0.6 (range: 0–3) in the success group. The prevalence of the ocular conditions assessed at the time of injection is listed in Table 3. The pre-existing conditions included chronic uveitis, cataracts, vitreal degeneration, lens dislocation, and intraocular hemorrhage. Chronic uveitis was observed in 108 eyes. Ninety eyes had cataracts, including 36 incipient cataracts, 9 immature cataracts, 15 mature cataracts, and 30 hypermature cataracts. The evaluation of cataract stages in 26 eyes was limited due to ocular surface diseases, including severe corneal edema and corneal pigmentation, severe miosis due to uveitis or drug, or posterior lens luxation. Aphakia was observed in 10 eyes due to previous intracapsular lens extraction or phacoemulsification. Vitreal degeneration was observed in 74 eyes, although ocular diseases of the anterior segment hindered diagnosis in some cases. Lens dislocation was diagnosed in 49 eyes, including 23 lens subluxation, 16 posterior lens luxation, 9 anterior lens luxation, and 1 subluxated

intraocular lens after phacoemulsification with intraocular lens implantation. Intraocular hemorrhages, including hyphema, intravitreal hemorrhages, and blood clots, were observed in 19 eyes. Ten eyes had previous intraocular surgery; seven eyes underwent phacoemulsification with or without intraocular lens implantation, and three eyes underwent intracapsular lens extraction.

TABLE 2. The number of intravitreal cidofovir injection and the success rate

Number of injections	Number of eyes (%)	Success (%)	Pre-injection IOP (mmHg)	Mean interval to the next injection (days)
1	153 (100.0)	106 (69.3)	47.6 ± 21.6	59.0 ± 78.0
2	42 (27.5)	25 (59.5)	48.3 ± 24.6	79.0 ± 72.0
3	14 (9.2)	6 (42.9)	45.3 ± 22.0	28.0 ± 13.0
4	6 (3.1)	2 (33.3)	54.0 ± 17.4	39.5 ± 16.3
5	2 (1.5)	1 (50.0)	54.0 ± 18.4	None

IOP, intraocular pressure.

TABLE 3. Ocular conditions at the time of intravitreal cidofovir injection

Ocular conditions	Specific type	Number of eyes
Chronic uveitis		108
Cataracts	Hypermature	30
	Mature	15
	Immature	9
	Incipient	36
Vitreal degeneration		74
Lens dislocation	Lens subluxation	23
	Posterior lens luxation	16
	Anterior lens luxation	9
	IOL subluxation	1
Intraocular hemorrhage		19
Previous intraocular surgery		10

IOL, Intraocular lens.

Procedure success was defined as an IOP of ≤25 mmHg at the 2-week recheck that remained to the 6-month recheck. The overall success rate of intravitreal cidofovir injection was 91.5% (140/153). The success rate of a single injection was 69.3% (106/153). The second injection increased the success rate to 85.6% (131/153), the third to 89.5% (137/153), the fourth to 90.8% (139/153), and the fifth to 91.5% (140/153). Forty-two dogs received a 2nd injection, and the success rate was 59.5% (25/42). Fourteen dogs received a 3rd injection, and the success rate was 42.9% (6/14). Six dogs received a 4th injection, and the success rate was 33.3% (2/6). Two dogs received a 5th injection, and the success rate was 50.0% (1/2). The mean interval between the first and second, second and third, third and fourth, and fourth and fifth injection was 59.0 ± 78.0 days (range: 14–371 days), 79.0 ± 72.0 days (range: 14–232 days), 28.0 ± 13.0 days (range: 14–42 days), and 39.5 ± 16.3 days (range: 28–51 days), respectively (Table 2). The single-injection success rates were lower in American Cocker Spaniel (61.1%, 22/36) and Shih Tzu (65.0%, 39/60) than in other breeds (Table 1). The IOP 2 weeks after injection was 9.5 ± 10.2 mmHg in the success group and 32.3 ± 13.7 mmHg in the failure group. In 95.0% (133/153) of the patients in the success group, IOP was controlled without topical glaucoma medications. The number of glaucoma eye drop types prescribed after injection reduced considerably to 0.1 ± 0.4 from 2.0 ± 0.6 in the success group ($p < 0.001$). Glaucoma eye drops were continued after injection in 10 dogs in the failure group (n=13 dogs). In three dogs, medication was continued until the final examination. The average time to discontinuation of medication in seven dogs was 354.7 ± 169.0 days (range: 226–723 days). When prognostic variables were statistically evaluated, repetition of the procedure, cataract stages, and the number of glaucoma eye drops

applied were significantly correlated with IOPs 6 months after the injection. Dogs that received repeated injections, applied fewer glaucoma eye drops before injection, and had more advanced cataract stages at the time of injection, had significantly higher IOPs 6 months after the final injection ($p < 0.05$) (Table 4). Other pre-injection variables, including age, sex, breed, pre-injection IOP, previous intraocular surgery, intraocular hemorrhage, lens dislocation, and vitreal degeneration, did not significantly correlate with post-injection IOP.

TABLE 4. The association between prognostic variables and IOP (intraocular pressure) 6 months post-injection

Prognostic variables	Number of eyes	IOP 6 months post-injection (mean ± SD mmHg)
Frequency of injection (n = 153)		
1	111	5.3 ± 4.2
2	28	8.7 ± 7.7
3	8	11.0 ± 6.0
4	4	15.8 ± 15.4
5	2	11.0 ± 9.9
Number of glaucoma eye drops (n = 153)[+]		
0	2	16.0 ± 1.4
1	35	9.4 ± 9.3
2	100	5.7 ± 4.2
3	16	4.1 ± 2.5
Cataract stages (n = 113)[++]		
0	23	4.7 ± 3.6
1	45	5.8 ± 3.8
2	45	8.2 ± 8.1

IOP, intraocular pressure; SD, standard deviation. Cataract stages: 0 = no cataract, 1 = incipient and immature cataracts, 2 = mature and hypermature cataracts.

[+]The number of glaucoma eye drops applied until the injection.

[++]The cataract stages at the time of injection.

The most common complication was phthisis bulbi. Phthisis bulbi occurred within 6 months in 65 eyes (42.5%), and the time to phthisis bulbi was 234.2 ± 232.6 days (range: 18–1068). The next most common complication was cataract progression (30.1%), followed by intraocular hemorrhage (16.3%), corneal ulceration (7.2%), corneal degeneration (6.5%), and corneal perforation (2.0%). A significant positive correlation was found between the time to phthisis bulbi and the frequency of injections, and negative correlations were found between the time to phthisis bulbi and age and the number of glaucoma eye drops administered before injection ($p < 0.05$) (Table 5).

TABLE 5. The association between prognostic variables, development of phthisis bulbi, and time to phthisis bulbi

Prognostic variables	Number of eyes	Number of eyes with phthisis bulbi (%)	Time to phthisis bulbi (mean ± SD days)
Frequency of injection (n = 153)			
1	111	88 (79.3)	171.4 ± 166.7
2	28	17 (60.7)	456.1 ± 298.6
3	8	3 (37.5)	586.7 ± 330.1
4	4	1 (25.0)	714.0 ± 0.0
5	2	1 (50.0)	449.0 ± 0.0
Age (years) (n = 130)[+]			
0-5	9	5 (55.6)	247.6 ± 326.6
6-10	48	34 (70.8)	222.6 ± 222.4
11-15	66	53 (80.3)	226.6 ± 208.9
16-20	7	6 (85.7)	142.3 ± 71.5
Number of glaucoma eye drops (n = 153)[++]			
0	2	0 (0.0)	Never
1	35	22 (62.9)	253.8 ± 247.3
2	100	63 (80.8)	237.3 ± 232.5
3	16	14 (87.5)	186.9 ± 219.1

SD, standard deviation.

[+]The age at the time of injection.

[++]The number of glaucoma eye drops applied until the injection.

A total of six globes were enucleated, and of these, three were enucleated due to a grossly enlarged globe and suspected mass of the posterior segment on ocular ultrasonography. In one eye, an ill-defined hypo-echoic structure located behind the sclera was observed, and extraocular myositis, retrobulbar tumor, or retrobulbar abscess was suspected. In another dog, isoechoic mass attached to the medial aspect of the lens and connected to the iris was observed. In the third dog, a hyperechoic mass in the vitreous chamber with moderate blood signal was observed. All three enucleated eyes underwent histopathological examination, and there was no evidence of neoplasia. Two eyes were enucleated after 1 year, and one eye after 3 years. Histopathological evaluation revealed severe chronic-active diffuse suppurative endophthalmitis and fibrinosuppurative endophthalmitis. The remaining three eyes were enucleated due to corneal perforation. One eye was enucleated 2 days after cidofovir injection because of limbal perforation and suppurative inflammation at the site of aqueous humor paracentesis. The patient had a clinical history of severe exposure keratitis and keratoconjunctivitis sicca. The other eye perforated 4 days after the procedure because of deep corneal ulceration and endophthalmitis. The third eye was enucleated 5 months after experiencing recurrent deep corneal ulcerations. All the enucleated eyes received a single injection of cidofovir.

Twelve dogs displayed a transient but nondurable decrease in IOP. Three dogs had IOPs higher than 25 mmHg at 6-month recheck after a transient decrease in IOP and were included in the failure group. In 5 dogs, IOPs decreased to below 25 mmHg after repetition of cidofovir injection or addition of glaucoma eye drops. In 4 dogs, decrease in IOP was observed post-injection after a transient but nondurable decrease

without additional treatment or medication.

Discussion

Cidofovir is a cytidine nucleotide analog antiviral drug used to treat cytomegalovirus retinitis in humans (Safrin *et al.,* 1997). Dose-dependent iritis and ocular hypotony were noted in humans as ocular adverse effects of the drug (Davis *et al.,* 1997; Safrin *et al.,* 1997). Consequently, studies evaluating the IOP and safety after intravitreal administration of the drug in normal guinea pigs and rabbits were conducted, and the results showed species-dependent and dose-dependent effects (Taskintuna *et al.,* 1997). The research showed that with higher doses, decrease in IOP, intraocular inflammation, and damage of ciliary body and retina were observed. In a study evaluating the dose-dependent effect of intravitreal cidofovir injection in 18 canine globes for 4 weeks, while there was no significant change in IOPs when 100 μg was injected, injection of 500 μg and 1,000 μg resulted in a permanent decrease in IOPs starting from 3 days after the injection (Park *et al.,* 2017). Both groups had the lowest IOP on day 10, and there was no difference between the groups that received 500 μg and 1,000 μg.

In a previous study evaluating the efficacy of 562.5 μg cidofovir in canine patients with chronic glaucoma, the overall success rate was 97% (Low *et al.,* 2014). The overall success rate of intravitreal gentamicin injection was 95% (Julien *et al.,* 2021). In the current study, the overall success rate was 91.5%, comparable to but lower than the success rates of the previous studies. However, the current study reflected the long-term outcome of the procedure because all eyes required a minimum follow-up period of 6 months from the time of injection. In contrast, the minimum follow-up period was only 2 weeks in the previous study on cidofovir and

3 months in the study on gentamicin (Low *et al.,* 2014; Julien *et al.,* 2021). In the present study, the single-injection success rate was 69.3%, and when the injection was repeated up to five times, the success rate increased to 91.5%. Repetition of cidofovir injection was performed in these patients because most of the patients had underlying diseases that increased the risk of sedation or anesthesia. When high concentration gentamicin is unavailable, sedation is necessary to inject a large volume of the drug, and anesthesia is necessary for enucleation surgery. In 95.0% of the patients in the success group, IOP was controlled without topical glaucoma medications, and the average number of glaucoma eye drop types applied decreased significantly after the injection. Topical antibiotics and anti-inflammatory eye drops were prescribed based on complete ophthalmic examination and clinical signs, and the frequency and period of application were decided accordingly.

When prognostic variables were statistically evaluated, dogs that received multiple injections had significantly higher IOPs when evaluated 6 months after the injection. Dogs unresponsive to the initial intravitreal cidofovir injection might show a continuously reduced decrease in IOPs. Furthermore, dogs with more advanced cataract stages at the time of injection also had higher postprocedural IOPs. Lens-induced uveitis (LIU), or phacolytic uveitis, may be present in eyes with cataracts (Wilcock and Peiffer, 1987). In the presence of LIU, breakdown of blood ocular barrier occurs, and the rate of the elimination of drugs from the vitreous may be greater than in normality (Kane *et al.,* 1981; Ben-Nun *et al.,* 1989). Although LIU may be present in all stages of cataracts, it is most often associated with advanced stages (Dziezyc *et al.,* 1997). Therefore, the higher elimination rate in advanced cataract stages may have altered the postprocedural IOPs. However, further studies

on the correlation between drug clearance rate and the effect of cidofovir in normal and inflammatory eyes should be conducted.

The most prevalent complication of intravitreal cidofovir injection in this study was phthisis bulbi (42.5%). In previous studies, phthisis bulbi occurred in 70% of patients 6 months after cidofovir injection and in 59.2% of patients 3 months after intravitreal gentamicin injection (Low *et al.,* 2014; Julien *et al.,* 2021). In the current study, none of the eyes exhibited any clinical signs that required additional treatment. Phthisis bulbi occurred within 6 months in 65 eyes, and the average time to phthisis bulbi was 234.2 days. The time to phthisis bulbi was significantly longer in dogs that received multiple injections and fewer pre-injection glaucoma eye drops. This finding is consistent with the aforementioned results. To summarize, the post-injection IOP was significantly higher in dogs with repeated injections and fewer glaucoma eye drops; similarly, phthisis bulbi occurred later in dogs with repeated injections and fewer eye drops. In contrast, phthisis bulbi occurred within a shorter period in older dogs. Lower IOPs with aging have been observed in both humans and dogs (Gelatt and MacKay, 1998; Nomura *et al.,* 2002). Decreased aqueous humor formation with aging has been observed in various human studies, and presumably, occurs due to the senile changes of non-pigmented ciliary body epithelium (Gärtner, 1971; Gaasterland *et al.,* 1978; Brubaker *et al.,* 1981; Toris *et al.,* 1999). Furthermore, with aging, an increase in particle diffusion due to vitreous liquefaction may occur when drugs are injected intravitreally (Hendrix *et al.,* 2021). These senile changes in the canine eye may explain the faster occurrence of phthisis bulbi after cidofovir injections in older dogs.

Six eyes were enucleated, and none showed evidence of neoplasia. In a study

by the Comparative Ocular Pathology Laboratory of Wisconsin (COPLOW), when 48 canine globes with a history of intravitreal gentamicin injection were submitted for histopathological evaluation, 19 globes were diagnosed with primary ocular tumors and showed malignancy and invasiveness (Duke *et al.,* 2013). In another retrospective study of intravitreal cidofovir injection, one globe out of 167 globes was enucleated and revealed the development of uveal melanoma 4 months after the injection (Low et al., 2014). These studies demonstrate that the occurrence of ocular tumors after cidofovir injection is lower than after gentamicin injection. Furthermore, no neoplasia was observed post-injection in the current study. However, the possibility of development of neoplasia after intravitreal injection might not be totally excluded, and periodic monitoring would be recommended.

Although three eyes were enucleated due to corneal diseases, the development of corneal ulceration post-injection in this study was low. In the current study, corneal ulceration occurred in 7.2% of patients. The prevalence of canine ulcerative keratitis reported in a retrospective study including 8877 dogs was 11.5% (Iwashita *et al.,* 2020). After intravitreal gentamicin injection, 22.3% of patients had ulcerative keratitis (Julien *et al.,* 2021), and 2.4% developed superficial corneal ulceration after intravitreal cidofovir injection (Low *et al.,* 2014). Hence, intravitreal cidofovir injection did not increase the occurrence of corneal ulceration. In most cases, the pre-existing corneal ulceration resolved after cidofovir injection following the resolution of cornea exposure resulting from buphthalmos.

Another factor to consider when evaluating the post-injection IOP is the condition of the ocular surface. Corneal fibrosis, observed in several cases in this study, might have resulted in the overestimation of IOPs due to altered corneal

hysteresis and thickness. Several eyes in the current study had IOPs ≥ 25 mmHg even after repeated injections, yet had no relevant clinical signs of elevated IOP, including episcleral and conjunctival injections, corneal edema, blepharospasm, third eyelid elevation, and changes in dog's behavior. In all cases, corneal fibrosis and thickening were examined via slit-lamp biomicroscopy. In humans and dogs, IOP measurements are affected by the biomechanical properties of the cornea (Park *et al.,* 2011; Kang *et al.,* 2020). In dogs, central corneal thickness is reported to alter the measurement of IOPs with both rebound tonometry and applanation tonometry (Park *et al.,* 2011). In humans, three cases of false-positive diagnosis of glaucoma due to corneal fibrosis have been reported (Kang *et al.,* 2020).

In a previous study evaluating the success of intravitreal gentamicin, the success rate decreased as the dog's body weight increased (Marchione *et al.,* 2011). In the current study, each dog received the same volume of cidofovir, and no association between success rate and body weight was discovered. However, a prospective study should be conducted to evaluate the exact association between body weight and the use of dose. The dose-dependent effect of intravitreal injection of gentamicin and cidofovir has been confirmed (Taskintuna *et al.,* 1997; Rankin *et al.,* 2016). However, future studies on the precise evaluation of success rate and complications of each injected dose should also be conducted to establish the optimal dosage for successfully lowering IOP with minimal complications.

The limitations of this study include its retrospective nature. Not all variables were controlled for, and certain breeds were overrepresented. Furthermore, only dogs with a minimum follow-up period of 6 months were included in this study. The possibility of dogs experiencing IOP spikes after 6 months cannot not be eliminated.

Finally, there was a lack of histopathological assessment because only the globes in which enucleation was necessary were subjected to histopathological examination.

Conclusion

In this study, the long-term efficacy of intravitreal cidofovir injection for end-stage glaucoma was demonstrated in dogs. Post-injection IOPs were higher when the injection was repeated, more types of glaucoma eye drops were applied prior to the injection, and cataract stages were advanced at the time of injection. Phthisis bulbi was the most prevalent complication, but it did not require additional treatment.

References

Ben-Nun J, Joyce DA, Cooper RL, *et al.* Pharmakokinectics of Intravitreal Injection: Assessment of a Gentamicin Model by Ocular Dialysis. *Invest Ophthalmol Vis Sci* 1989; 30:1055-1061.

Brubaker RF, Nagataki S, Townsend DJ, *et al.* The effect of age on aqueous humor formation in man. *Ophthalmology* 1981;88: 283-288.

Davis JL, Taskintuna I, Freeman WR, *et al.* Iritis and hypotony after treatment with intravenous cidofovir for cytomegalovirus retinitis. *Arch Ophthalmol* 1997;115:733-737.

Duke FD, Strong TD, Bentley E, *et al.* Canine ocular tumors following ciliary body ablation with intravitreal gentamicin. *Vet Ophthalmol*. 2013;16:159-162.

Dziezyc J, Millichamp NJ, Smith WB. Fluorescein concentrations in the aqueous of dogs with cataracts. *Vet Comp Ophthalmol* 1997;7:267-270.

Gaasterland D, Kupfer C, Milton R, *et al.* Studies of aqueous humour dynamics in man. VI. Effect of age upon parameters of intraocular pressure in normal human eyes. *Exp Eye Res*. 1978;26:651-656.

Gärtner J. Aging changes of the ciliary epithelium border layers and their significance for intraocular pressure. *Am J Ophthalmol*. 1971;72:1079-1093.

Gelatt KN, MacKay EO. Distribution of intraocular pressure in dogs. *Vet Ophthalmol.*

1998;1:109-114.

Gelatt KN, MacKay EO. Prevalence of the breed-related glaucomas in pure-bred dogs in North America. *Vet Ophthalmol*. 2004a;7:97-111.

Gelatt KN, MacKay EO. Secondary glaucomas in the dog in North America. *Vet Ophthalmol*. 2004b;7:245-259.

Hendrix DV, Thomasy SM, Gum GG. Physiology of the Eye. In: Gelatt KN, ed. *Veterinary Ophthalmology*. 6th ed. Wiley Blackwell 2021;151-153.

Iwashita H, Wakaiki S, Kazama Y, *et al.* Breed prevalence of canine ulcerative keratitis according to depth of corneal involvement. *Vet Ophthalmol*. 2020; 23: 849-855.

Julien ME, Schechtmann SA, Michau TM, *et al.* Pharmacologic ciliary body ablation for chronic glaucoma in dogs: A retrospective review of 108 eyes from 2013 to 2018. *Vet Ophthalmol*. 2021;24:125-130.

Kane A, Barza M, and Baum J. Intravitreal injection of gentamicin in rabbits: Effect of inflammation and pigmentation on half-life and ocular distribution. *Invest Ophthalmol Vis Sci* 1981; 20:593-597.

Kang BS, Jeoung JW, Oh JY. Inaccuracy of intraocular pressure measurement in congenital corneal opacity: three case reports. *BMC Ophthalmol*. 2020;20:3-7.

Komáromy AM, Bras D, Esson DW, *et al.* The future of canine glaucoma therapy. *Vet Ophthalmol*. 2019;22:726-740.

Low MC, Landis ML, Peiffer RL. Intravitreal cidofovir injection for the management of chronic glaucoma in dogs. *Vet Ophthalmol*. 2014;17:201-206.

Maggs DJ. Ophthalmic Medications and Therapeutics. In: Maggs DJ, Miller PE, Ofri R, eds. *Slatter's Fundamentals of Veterinary Ophthalmology,* 6th ed. Elsevier Suanders; 2018:51-84.

Marchione B, daSilvaCuriel J, Zhou Y, *et al*. Effectiveness of gentamicin for pharmacologic ablation to treat end-stage glaucoma in dogs. *42nd Annual Conference of the American College of Veterinary Ophthalmologists.* Hilton Head, SC, 2011.

Miller PE. The glaucomas. In: Maggs DJ, Miller PE, Ofri R. *Slatter's Fundamentals of Veterinary Ophthalmology,* 6th ed. Elsevier Suanders; 2018:213-253.

Nomura H, Ando F, Niino N, *et al.* The relationship between age and intraocular pressure in a Japanese population: the influence of central corneal thickness. *Curr Eye Res*. 2002;24:81-85.

Park S, Kang S, Park S, *et al.* Retinal Function Analysis and Histopathologic Assessment after Intravitreal Cidofovir Injection in Normal Beagle Dogs. *J Vet Clin*. 2017;34:341-346.

Park YW, Jeong MB, Kim TH, *et al.* Effect of central corneal thickness on intraocular pressure with the rebound tonometer and the applanation tonometer in normal dogs. *Vet Ophthalmol*. 2011;14:169-173.

Plummer CE, Komáromy AM, Gelatt KN. Canine Glaucomas. In: Gelatt KN, ed.

Veterinary Ophthalmology. 6th ed. Wiley Blackwell 2021;1173.

Rankin AJ, Lanuza R, KuKanich B, *et al.* Measurement of plasma gentamicin concentrations postchemical ciliary body ablation in dogs with chronic glaucoma. *Vet Ophthalmol*. 2016;19:57-62.

Regnier A. Clinical Pharmacology and Therapeutics. In: Gelatt KN, ed. *Veterinary Ophthalmology*. 6th ed. Wiley Blackwell 2021;370

Safrin S, Cherrington J, Jaffe HS. Clinical uses of cidofovir. *Rev Med Virol*. 1997;7:145-56.

Taskintuna I, Banker AS, Rao NA, *et al.* An animal model for cidofovir (HPMPC) toxicity: intraocular pressure and histopathologic effects. *Exp Eye Res*. 1997;64:795-806.

Toris CB, Yablonski ME, Wang YL, *et al.* Aqueous humor dynamics in the aging human eye. *Am J Ophthalmol*. 1999;127:407-412.

Tripathy K, Chawla R, Temkar S, *et al.* Phthisis Bulbi – a clinico- pathological perspective. *Semin Ophthalmol*. 2018;33:788-803.

Wilcock BP, Peiffer RL. The pathology of lens-induced uveitis in dogs. *Vet Pathol* 1987; 24:549-553.

국문 초록

개의 말기 녹내장에서 유리체 내 시도포비어 주사의 효과: 153개 안구의 후향적 연구 (2016~2021)

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본 연구는 개의 말기 녹내장에서 유리체 내 시도포비어(cidofovir) 주사의 장기적인 효과, 예후 인자, 그리고 복합증을 평가하기 위해 실행되었다.

2016년에서 2021년 사이 유리체 내 시도포비어 주사를 받은 130마리 개(153개 안구)의 의료기록을 검토하였다. 시술 후 재진 기간이 최소 6개월 이상인 환자들을 포함하였다. 환자 정보, 녹내장 종류, 시술 전

안압, 점안된 녹내장 안약의 개수, 기저 안과 질환, 결과 및 복합증을 기록하였다. 시술의 성공은 2주일 후 안압이 25 mmHg 이하이며, 6개월동안 지속될 경우로 정의하였다.

유리체 내 시도포비어 주사의 총 성공률은 91.5% (140/153)로 확인되었다. 1회 주사의 성공률은 69.3% (106/153)였으며, 40마리의 개에서 2회 시술이 반복되었고, 성공률은 59.5% (25/42)였다. 시술이 3회 반복된 안구는 14개였으며, 성공률은 42.9% (6/14)였다. 시술이 4회 반복된 안구는 6개였으며, 성공률은 33.3% (2/6)였다. 시술이 5회 반복된 안구는 2개였으며, 성공률은 50.0% (1/2)였다. 시술이 반복된 경우, 시술 전 더 적은 수의 녹내장 안약이 점안된 경우, 그리고 시술 당시 백내장 단계가 더 높았을 경우 시술 6개월 후 안압이 유의적으로 높게 확인되었다($p<0.05$). 가장 흔한 복합증은 안로(42.5%), 백내장의 진행(30.1%), 그리고 안내 출혈(16.3%)로 확인되었다. 여섯 개의 안구에서 안구 적출술을 진행하였으며, 그 중 세 개의 안구는 각막 천공이 발생하여 안구 적출술을 진행하였다.

유리체 내 시도포비어 주사는 말기 녹내장 개에서 장기적으로 안압을 낮추는데 효과적이었다.

주요어: 개, 말기 녹내장, 시도포비어, 약물적 모양체 파괴술, 초자체 내 주사

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