pISSN 1598-298X / eISSN 2384-0749
J Vet Clin 36(6) : 325-329 (2019)
http://dx.doi.org/10.17555/jvc.2019.12.36.6.325

Journal of Veterinary Clinics
www.ksvc.or.kr

Evaluation on Clinical Application of Osmotic Pump with Dorzolamide in Normal Dogs

Yonghwan Kwon, Jury Kim* and Min-Su Kim[1]**

College of Veterinary Medicine, Chonbuk National University, Iksan 54596, Korea
**Bundang Bright-eye Animal Hospital, Seongnam 13439, Korea*
***Department of Veterinary Clinical Science, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, Gwanak-gu, Seoul 08826, Korea*

(Received: November 17, 2019 / Accepted: November 28, 2019)

Abstract : This study was designed to compare the effects of continuous release of an anti-glaucoma drug applied by an osmotic pump and by conventional eye drop instillation in normal beagle dogs, by measuring drug concentration in the blood and monitoring intra-ocular pressure (IOP). In group 1, an osmotic pump filled with Trusopt® was implanted subcutaneously over the right eye of each dog and the IOP was measured. In group 2, the right eye of each dog was administered with 2% dorzolamide (Trusopt®, Merck, USA) three times per day. Blood was sampled once per week in all groups. The IOP of the end of this study was 16.7 ± 0.58 mmHg in group 2 and 17.7 ± 2.52 mmHg in group 2. Dorzolamide concentration in blood increased continuously over 4 weeks up to 585.8 ± 79.42 μg/L in group 1, and 747.3 ± 27.89 μg/L in group 2. We achieved satisfactory results in the osmotic pump group, which had a similar effect on IOP, and low fluctuations in IOP. Therefore, the results of this study should allow osmotic pumps to be consider as an alternative method to eye drops for the effective, safe, and convenient treatment of glaucoma.

Key words **:** glaucoma, intra-ocular pressure, osmotic pump, dorzolamide, dog.

Introduction

Glaucoma is the second-leading cause of vision loss and visual impairment worldwide, affecting 70 million people (9). It is a devastating disorder that leads to retinal ganglion cell (RGC) degeneration, visual field loss, and eventual blindness (20). In veterinary medicine, glaucoma is a leading cause of irreversible blindness in dogs (7). It has become clear that the relationship between glaucoma and intraocular pressure (IOP) is more complex than previously presumed (16). Although increased IOP is a major risk factor for primary open angle glaucoma (POAG), other concomitant factors affecting the eye are important, including increased glutamate levels, alteration of nitric oxide (NO) metabolism, vascular alteration, and oxidative damage caused by reactive oxygen species (3,14). IOP is determined by the balance between the production rate of the aqueous humor of the ciliary body, the resistance to aqueous outflow at the angle of the anterior chamber, and the episcleral venous pressure (18).

The conventional treatment for glaucoma is the topical application of IOP-lowering drugs. Beta blockers and prostaglandin analogs are the first-line treatments for glaucoma, which reduce the IOP by decreasing the aqueous humor formation and increasing the aqueous humor non-conventional outflow pathway, respectively (5). The second-line treatments of choice for glaucoma are carbonic anhydrase inhibitors (CAIs) and alpha agonists (7).

These topical drugs are the main form of treatment of ocular surface disease, ocular hypertension or glaucoma, and anterior uveitis, and account for 90% of the currently available ophthalmic formulations (10,21). Although eye drops are easy to instill, some problems may be encountered. Initially, the eye appears an ideal, easily accessible target organ for topical treatment. However, the eye actually has effective protection mechanisms against the absorption of foreign materials: first by the eyelids and tear flow, and then by the cornea, which forms a physical-biological barrier. To be effective, a drug must penetrate the eye and achieve an adequate concentration at its site of action (13). Conventional delivery systems often result in poor bioavailability and therapeutic response because high tear fluid turnover and dynamics cause rapid elimination of the drug from the eye (21). Commercial eye drops have a volume of ~30 μL, which is approximately the volume of the conjunctival sac in humans; however, after a single blink, only 10 μL is estimated to remain (19).

Excess drug drains through the nasolacrimal duct into the nose, where it may be absorbed into the systemic circulation; unfortunately, systemic side effects may occur and can be severe. The side effects of ocular drugs should always be considered when a patient presents with new systemic problems (12). In addition, eye drops can only be used for the treatment of the anterior segment disorders, as adequate drug concentrations cannot be reached in the posterior tissues. Therefore, high doses are needed and systemic adverse effects are common such as systemic treatment of glaucoma

[1]Corresponding author.
E-mail : minsukim@snu.ac.kr

with carbonic anhydrase inhibitors (11). Furthermore, each time eye drops are applied, the peak intraocular concentration of drug is rapidly reached and thereafter declines rapidly as time passes. A plot of the intraocular drug concentration versus time presents a series of peaks in drug concentration, which may exceed the toxic threshold of the drug, separated by extended valleys of the drug concentration below that needed to achieve the desired therapeutic efficacy (1).

Novel ophthalmic drug delivery systems have been developed to overcome these problems, including gels, liposomes, niosomes, nanoparticles, iontophoresis, corneal shields, drug-embedded contact lenses, ocular wafers, and films (8,13). Implantable drug delivery systems that have been developed and used include an osmotic mini-pump, a drug pellet coated with polyvinyl alcohol and ethylene acetate, and poly sulfone capillary fibers. The generic osmotic mini pump (ALZET®) is a useful implantable drug delivery system that delivers drug at a constant rate (15). ALZET pumps operate because of an osmotic pressure difference between a compartment within the pump, called the salt sleeve, and the tissue environment in which the pump is implanted. The high osmolality of the salt sleeve causes water to flow into the pump though a semipermeable membrane, which forms the outer surface of the pump. As the water enters the salt sleeve, it compresses the flexible reservoir, displacing the test solution from the pump at a controlled, predetermined rate. The rate of delivery by an ALZET pump is controlled by the water permeability of the outer membrane of the pump. Thus, the delivery profile of the pump is independent of the drug formulation dispensed. Drugs of various molecular configurations, including ionized drugs and macromolecules, can be dispensed continuously in a variety of compatible vehicles at controlled rates. Neither the molecular weight of a compound nor its physical and chemical properties, effect the rate of delivery. The pumps are available with a variety of delivery rates and durations, between 0.11 and 10 μL/h, and 1 day and 6 weeks, respectively (2,8).

The purpose of this study was to apply continuous drug release system in normal beagle dogs to compare the effect of the continuous release of an anti-glaucoma drug, applied to normal beagles by using an osmotic pump, with conventional eye drop instillation, through measurement of the drug concentration in the blood, and monitoring of IOP.

Materials and Methods

Study dogs

Eight healthy beagle dogs were enrolled in this study. The present study was performed in accordance with the guidance of the Ethics Committee for Experimental Animals, Chonbuk National University. They were divided into two groups: 1) an osmotic pump group, in which IOP was measured (n = 4); and 2) a conventional drug instillation group, in which IOP was measured (n = 4). The dogs were housed in individual cages in the departmental animal shed, fed a standard commercial diet, and given free access to water. All dogs were clinically healthy and free from systemic diseases, including in their eyes. Specifically, the eyes of all animals were examined before the experiment, including direct ophthalmoscopy, slit lamp examination, Schirmer tear test (STT), and fluorescein dye staining.

Experimental design

In group 1, an osmotic pump filled with Trusopt® was implanted subcutaneously over the right eye and IOP was measured. In group 2, the animals were administered 2% dorzolamide (Trusopt®, Merck, USA) in the right eye, three times per day. Blood samples were collected once per week in all groups. The recorded measurements included IOP by rebound tonometry (TonoVet®, Tiolat, Finland) and dorzolamide concentration in blood. The study parameters were measured three times daily, at 8 a.m., 4 p.m., and 12 a.m. in the first and second weeks, once every other day for the third and fourth weeks. The study period was 28 days.

Osmotic pump implantation

The osmotic pump (model 2004, Alzet®, USA) was filled with 2% dorzolamide, attached to a flow moderator, and connected with a polyethylene catheter to obtain a continuous infusion of the drug (Fig 1A). The pump was primed for 40 h before use. The pumps had an average flow rate of 0.25 μL/h and the drug was infused for a period of 28 days. The pump with flow moderator was weighed before the implantation and at the end of experiment to check that the drug was released completely.

Beagles were anesthetized with combination of medetomidine (Domitor®, Pfizer, Finland) and tiletamin and zolazepam (Zoletil®, Virbac, France) 0.02 mL/kg. At the incision site, the skin at the superior-lateral orbital rim was aseptically prepared and an incision of approximately 1 cm was made. The skin was then dissected to make a pocket to place the pump by using Metzenbaum scissors. The pump was connected to the catheter and then inserted in the subcutaneous pocket (Fig 1B). The catheter was then placed in the lat-

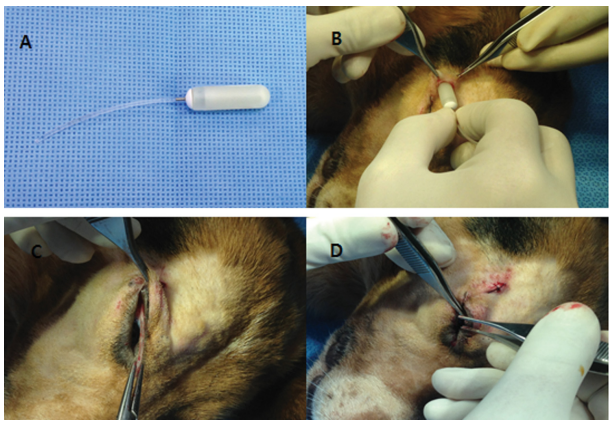

Fig 1. Osmotic pump implantation. (A) The osmotic pump (model 2004, Alzet ®, USA) was filled with 2% dorzolamide, attached to a flow moderator, and connected with a polyethylene catheter. (B) The pump connected to the catheter was then inserted to the subcutaneous pocket. (C) The catheter was then placed in the lateral fornix through a stab incision and cut to the required length. (D) The catheter was then fixed with 3-0 polyglactin 910 (Vicryl ®) and the skin was closed by simple interrupted stitches.

eral fornix through a stab incision and cut to the required length (Fig 1C). The catheter was then fixed with 3-0 polyglactin 910 (Vicryl®) and the skin was closed by simple interrupted stitches (Fig 1D). An Elizabethan collar was applied around the neck of the dogs to prevent self-trauma. Cefazolin (Cefazolin sodium, Chong Kun Dang Pharm, Korea) was administered intravenously, as a prophylactic antibiotic, every 12 h (q12h) for 7 days after the surgery. The pump was removed under general anesthesia after 28 days and then weighed.

Collection of the blood samples

During the study, blood samples were obtained once per week from animals in each group. Blood was collected in heparinized polypropylene tubes and a sample of the whole blood was frozen at −70°C before screening. No glass material was used at any time in the collection, transfer, or storage of specimens to avoid adsorption.

Sample preparation

The blood samples were analyzed after dilution. In brief, aliquots of the blood samples were placed in an Eppendorf microfuge tube. Methanolic zinc sulphate solution was added to the samples to give a 10-fold dilution, releasing compounds from the matrix by causing hemolysis and disrupting protein binding. The samples were then vortexed for 10 min and centrifuged at 13,000 rpm for 5 min. The precipitated proteins and cell debris were deposited at the bottom of the tube, leaving the compounds of interest in the clear supernatant above. The clear supernatant (300 μL) was transferred into suitable vials for liquid chromatography-tandem mass spectrometry (LC-MS/MS) vials and analyzed.

Statistical analysis

Comparisons of the results were computed by SPSS (SPSS 12.0 Chicago, IL, USA) using repeated measures ANOVA (Tukey). Within each test period, the average measurements for IOP and dorzolamide concentration in blood were compared with the subsequent measurements to detect significant changes ($P < 0.05$) by using Tukey tests for repeated measurements.

Results

Intraocular pressure

The mean ± SD changes in IOP for groups 1 and 2 are described in Fig 2. The most notable change was the more extensive reduction in IOP in group 1 than group 2, especially on the first and second days after implantation; the IOP was 13.0 ± 1.0 mmHg and 15.7 ± 0.58 mmHg, respectively. Because the IOP reduction was considered from inflammation, cefazolin (Cefazolin sodium, Chong Kun Dang Pharm, Korea) was administered as prophylactic antibiotic intravenously q12h for 7 days after the surgery. The IOP gradually increased and was similar degree to that in group 3 on the fourth day after implantation. The IOP of the end of this study (the 28th day after surgery), was 16.7 ± 0.58 mmHg in group 1 and 17.7 ± 2.52 mmHg in group 2. It was of interest that a diurnal variation in IOP was noted in groups 1 and 2. The greatest daily fluctuation in IOP was 13.7 ± 2.08 mmHg in group 1 and 16.7 ± 4.93 mmHg in group 2. The comparison between the highest and lowest point of IOP each day revealed the greatest daily difference occurred in group 2, of 4.1 ± 1.74 mmHg, and the lowest daily difference in group 1, of 1.7 ± 0.87 mmHg. The decrease in IOP from baseline was significant for the treated eye and also for the untreated eye in all groups except the control group. The difference in the decrease in IOP between the treated and untreated eyes was significant in both groups 1 and 2 ($P < 0.05$).

Dorzolamide concentration in blood samples

The mean ± SD changes in the concentration of dorzolamide in whole blood for groups 1 and 2 are shown in Fig 3. The concentration of dorzolamide in the first week was 235.7 ± 58.77 μg/L in group 1. In the conventional drug instillation group, the blood concentration of dorzolamide

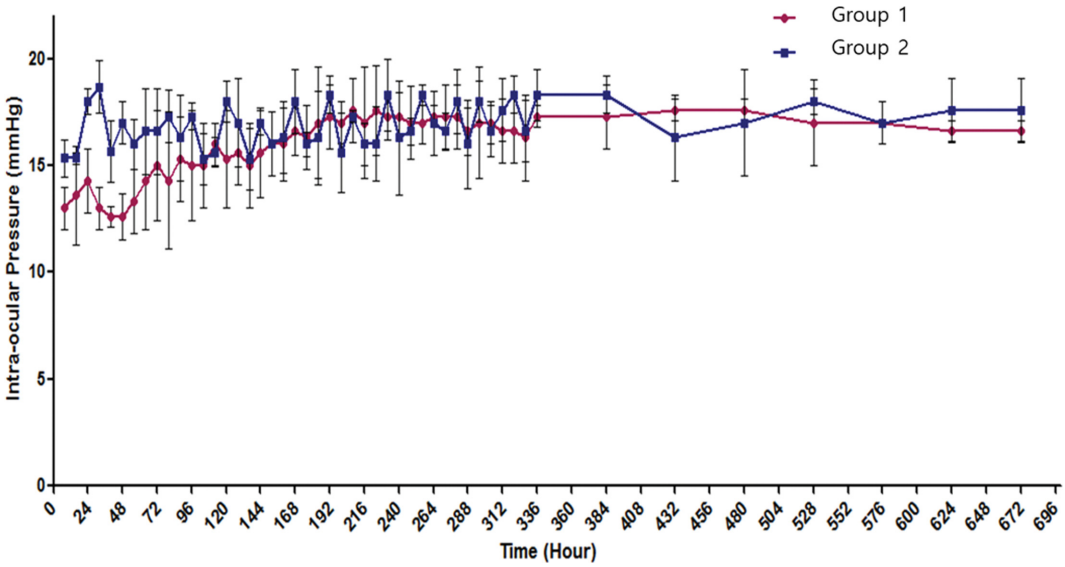

Fig 2. IOP change over 672 hours (28 days).

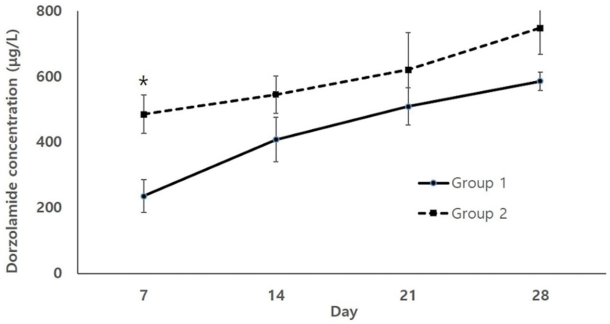

Fig 3. Dorzolamide concentration in the blood over 28 days.

increased dramatically compared with the other groups in first week, to 484.6 ± 49.92 μg/L. In groups 1, and 2, dorzolamide concentrations increased continuously over the 4-week experimental period. Dorzolamide accumulated in the blood over 4 weeks, with concentrations of 585.8 ± 79.42 μg/L in group 1, and 747.3 ± 27.89 μg/L in group 2.

Discussion

Conventional treatment for glaucoma is the topical application of IOP-lowering drugs. Oral CAIs have been used to lower IOP for the past 40 years. A higher risk of adverse events with systemically administered agents means that topical CAIs have now almost completely replaced systemically administered CAIs (1). Topically administered CAIs are not associated with systemic adverse effects, but may cause periocular dermatitis, conjunctivitis, or keratitis as a result of hypersensitivity reactions (17). Conventional topical eye drops provide a massive dose that may surge to peak drug concentration with every eye drop instillation (15). As IOP will rise in the nighttime in patients with glaucoma, it is important to follow an accurate dosing schedule; however, in reality, it is hard to give eye drops. Moreover, the administration of eye drops to animals is often difficult to achieve.

One of the drug delivery systems used is a mini osmotic pump that operates owing to a difference in osmotic pressure between a compartment within the pump, called the salt sleeve, and the tissue environment in which the pump is implanted. The pump we used in this study was an ALZET® osmotic pump model 2004; with a release rate of 0.25 μL/h over 28 days. The total volume supplied by the pump was 6 μL per day; in contrast, the conventional topical eye drop volume of 2% dorzolamide averages 90 μL per day. After implantation, IOP was dramatically decreased until 11 mmHg. For the first few days, the low IOP was suggested to be a result of mild uveitis. The average IOP gradually increased and was similar to the eye drop instillation group. Clinical trials of glaucoma treatment have demonstrated the efficacy of IOP reduction in the retardation of glaucoma progression. However, a related issue has recently surfaced and been discussed, which is the relationship between IOP fluctuation and glaucoma. Although not all studies have shown a link between glaucoma progression and IOP fluctuation, prospective and retrospective studies have suggested that long-term IOP fluctuation was significantly related to the rate of visual field loss (3). Therefore, the management of glaucoma should not only include robust IOP reduction in patients at risk of visual field loss, but also aim to reduce IOP fluctuation, particularly in patients who progress at lower pressures (6). In this study, the osmotic pump would be a possible mechanism to lower the IOP fluctuation and reduce IOP. In addition, the osmotic pump did not affect IOP of the untreated eye, whereas IOP was reduced in both eyes in the conventional drug instillation group. The dorzolamide concentration in the blood samples was similar in the osmotic pump group and conventional drug instillation group. In first week, the concentration of dorzolamide in the conventional drug instillation group was twice as high as that in the osmotic pump group. There was no significant difference in dorzolamide level between the osmotic pump groups and the conventional drug instillation group in the 2nd, 3rd and 4th week.

In this study, the analysis of blood sample was performed every week to check the drug release from the osmotic pump. The results of the analysis indicated that the osmotic pump was operating continuously, but the desired amount of drug was not released at the beginning of study. We were satisfied with the results showing similar effects on IOP, low IOP fluctuation, and a relatively lower effect compared with conventional eye drop instillation. The possibility of inflammation due to the pump was also monitored during the study; however, conjunctival hyperemia, aqueous flare, ocular discharge, and discomfort were not observed. This study requires further research to determine an osmotic pump implantation site that replaces other site where different osmolality is around eye. In addition, another problem in this study is that all of the eyes studied were clinically normal therefore an effect on glaucomatous eyes was not assessed. Therefore, further study is planned to apply the pumps to glaucomatous eyes. To ensure the study is accurate, more pump models with variable size and release rate, and other implantation sites, should be studied. The results of this study should allow osmotic pumps to be consider as an alternative method to eye drops for the effective, safe, and convenient treatment of glaucoma.

References

1. Agrahari V, Mandal A, Agrahari V, Trinh HM, Joseph M, Ray A, Hadji H, Mitra R, Pal D, Mitra AK, Agrahari V, Mandal A. A comprehensive insight on ocular pharmacokinetics. Drug Deliv Transl Res. 2016; 6: 735-754.
2. Bae JH, Plummer CE, Kim J, Kim MS, Kim NS. Continuous ophthalmic treatment using an osmotic pump in a bull calf following surgical removal of an ocular dermoid: a case report. Vet Med Czech 2015; 60: 282-287.
3. Bergea B, Bodin L, Svedbergh B. Impact of intraocular pressure regulation on visual fields in open-angle glaucoma. Ophthalmology 1999; 106: 997-1004.
4. Bertaud S, Aragno V, Baudouin C, Labbé A. Primary open-angle glaucoma. Rev Med Interne 2019; 40: 445-452.
5. Conlon R, Saheb H, Ahmed II. Glaucoma treatment trends: a review. Can J Ophthalmol 2017; 52: 114-124.
6. Hong S, Seong GJ, Hong YJ. Long-term intraocular pressure fluctuation and progressive visual field deterioration in patients with glaucoma and low intraocular pressures after a triple

procedure. Arch of Ophthalmol 2007; 125: 1010-1013.
7. Huang AS, Minasyan L, Weinreb RN. Glaucoma-Intraocular Pressure Reduction. Handb Exp Pharmacol 2017; 242: 181-207.
8. Kim J, Kim MS. The evaluation of osmotic pump as glaucoma drug delivery system in normal dogs. Pak Vet J 2015; 35: 239-241.
9. Lee DA, Higginbotham EJ. Glaucoma and its treatment: a review. Am J Health Syst Pharm 2005; 62: 691-699.
10. Lee SS, Hughes P, Ross AD, Robinson MR. Biodegradable implants for sustained drug release in the eye. Pharm Res 2010; 27: 2043-2053.
11. Lomelino C, McKenna R. Carbonic anhydrase inhibitors: a review on the progress of patent literature (2011-2016). Expert Opin Ther Pat 2016; 26: 947-956.
12. Lusthaus J, Goldberg I. Current management of glaucoma. Med J Aust 2019; 210: 180-187.
13. Mainardes RM, Urban MC, Cinto PO, Khalil NM, Chaud MV, Evangelista RC, Gremiao MP. Colloidal carriers for ophthalmic drug delivery. Curr Drug Targets 2005; 6: 363-371.
14. McMonnies C. Reactive oxygen species, oxidative stress, glaucoma and hyperbaric oxygen therapy. J Optom 2018; 11: 3-9.
15. Patel A, Cholkar K, Agrahari V, Mitra AK. Ocular drug delivery systems: An overview. World J Pharmacol 2013; 2: 47-64.
16. Phulke S, Kaushik S, Kaur S, Pandav SS. Steroid-induced glaucoma: An avoidable irreversible blindness. J Curr Glaucoma Pract 2017; 11: 67-72.
17. Pizzirani S. Definition, classification, and pathophysiology of canine glaucoma. Vet Clin North Am Small Anim Pract 2015; 45: 1127-1157.
18. Roy Chowdhury U, Hann CR, Stamer WD, Fautsch MP. Aqueous humor outflow: dynamics and disease. Invest Ophthalmol Vis Sci 2015; 56; 2993-3003.
19. Rupenthal ID, Daugherty AL. Ocular drugs and drug delivery systems - Current trends and future perspectives. Drug Discov Today 2019; 24: 1425-1426.
20. Russo R, Varano GP, Adornetto A, Nucci C, Corasaniti MT, Bagetta G, Morrone LA. Retinal ganglion cell death in glaucoma: Exploring the role of neuroinflammation. Eur J Pharmacol 2016; 15: 134-142.
21. Sheshala R, Kok YY, Ng JM, Thakur RR, Dua K. In situ gelling ophthalmic drug delivery system: An overview and its applications. Recent Pat Drug Deliv Formul 2015; 9: 237-248.