Efficacy and safety of bimatoprost 0.01% formulated in tight junction modulation technology compared to marketed benzalkonium chloride preserved bimatoprost 0.01% ophthalmic solution in healthy beagle dogs

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ABSTRACT

Background: This study was undertaken to compare the efficacy and safety of the new technology tight junction modulation (TJM) bimatoprost 0.01% (TJM-bimatoprost), containing polyhexamethylene biguanide hydrochloride as a preservative, and marketed bimatoprost 0.01% (BKC-bimatoprost) in healthy beagle dogs.

Methods: This was a cross-over study and all animals in the study were assigned to one of two treatment arms to receive either TJM-bimatoprost (n=6) or BKC-bimatoprost (n=6) ophthalmic solution. Dosing for period 1 was started on day 3 (8 am everyday) and it continued till day 12. Assessments were carried out every day at 8 am, 9 am, 2 pm and 8 pm throughout the study period till day 17.

Results: For the pooled analysis (n=12 in each group) of period 1 and 2, there was a significant decrease (p<0.001) in mean intra-ocular (IOP) 1 hour post administration as compared to the baseline and this trend continued all throughout the study in both treatment arms. Twenty fours after last dose, on day 12, IOP measurements were 14.20±1.59 mmHg and 13.89±1.5 mmHg in the TJM-bimatoprost and the BKC-bimatoprost group respectively. The analysis of the primary end point revealed that 95% confidence interval for the between group differences in mean IOP values were well within the pre-defined equivalence margin of ±1.5 mmHg. In terms of safety, there was no difference in mean pupillary diameter in the TJM-bimatoprost and BKC-bimatoprost group.

Conclusions: The results of this study enhance our understanding of the proprietary TJM technology by establishing efficacy and safety of TJM-bimatoprost in animal models.

Keywords: Tight Junction modulation technology, Bimatoprost 0.01%, Intraocular pressure, Polyhexamethylene biguanide hydrochloride, Glaucoma

INTRODUCTION

One of the most effective ways of lowering intra-ocular pressure (IOP) for glaucoma patients has been the use of prostaglandin (PG) analogs that exert their action via FP receptors.1,2 Latanoprost, bimatoprost and travoprost are the molecules belonging to the PG class of drugs. Although the exact mode of action of these molecules are not known, receptors present in the ciliary body and Schlemm’s canal point out towards increased aqueous
humor outflow. PG analogs cause 6.4–6.5 mmHg reduction in IOP, which is approximately 2 mmHg more compared to other class of drugs. For their superior IOP lowering properties, they are the natural choice for use as a first line agent. Safety wise, they are well tolerated but are known to cause adverse events such as eye irritation, increased iris pigmentation, distichiasis and conjunctival hyperaemia.

The United States Food and Drug Administration (USFDA) first approved bimatoprost 0.03%, which contained 0.005% benzalkonium chloride (BKC). With this strength, there were reports of increased adverse events attributable to the class effect of FP receptors. Understandably, the company which first marketed bimatoprost decided to lower the strength of the active ingredient to 0.01% but increased the concentration of BKC to 0.02%, in order to increase penetration into the ocular tissue. The fact that BKC improved the spacing of the cell–cell tight junctions in biological models was taken advantage in the revised formulation. This hypothesis was proven in a randomized study, wherein lower strength bimatoprost 0.01% with BKC 0.02% was found to be equivalent in its IOP lowering effect as compared to the higher strength older formulation of bimatoprost 0.03% with BKC 0.005%. Although the ocular side effects were reduced, conjunctival hyperaemia was still persistent in subsequent reports. One reason could be because BKC, apart from being a preservative, is highly corrosive and its deterrent effect leads to tear film instability by evaporating the aqueous layer of the tear film. A closer look at the literature also reveals that BKC causes proapoptotic changes on conjunctival cell lines, decrease in antioxidants, increase in inflammatory mediators in lens epithelial cells and DNA damage. Clinically, reports of increased conjunctival hyperaemia, pruritis and dry eye have been attributed to BKC.

With this background, we have developed a new drug delivery system based on the principles of improved retention and permeation combining our proprietary gel free reservoir (GFR) and transient tight Junction modulation (TJM) technologies. It has been hypothesized that the two compounds present in TJM technology will help to modulate the corneal tight junction to improve the penetration of bimatoprost. While TJM-1 is a negatively charged surfactant with an ability to solubilize claudin binding calcium and phosphorylate tight junction proteins, TJM-2 is a positively charged polymeric molecule which can attach to the negatively charged phospholipid head of bilayer cell lining to increase the fluidity of the membrane (Figure 1). Additionally, it transiently attaches to the phosphorylated TJ proteins to delay the claudin remodelling. This effect leads to drug molecule permeation. In addition, GFR technology, containing two water soluble polymers in an interpenetrating gel network, increases the mean residence time of the drug molecule on the ocular surface and provide lubrication. The polymers are well known for their use in artificial tears to provide relief to the patients in case of ocular surface pathological symptoms. Therefore, these two technologies complement each other to benefit ocular absorption and safety of bimatoprost, as compared to BKC.

One of the modulators in the new formulation is polyhexamethylene biguanide hydrochloride (PHMB). The other one is sodium lauryl sarcosine. Apart from being modulators of tight junction, these chemicals also act as preservative. In addition, PHMB is used as a microbicide, excipient in cosmetic preparations, antiseptic in wound healing and orthopedic surgery and cleansing solution for contact lens. Sodium lauryl sarcosine has been used as a co-preservative in ophthalmic preparations. The first ophthalmic use of PHMB was approved by European Medicines Agency (EMEA) in 2007 for Acanthamoeba keratitis. However, as preservative ingredients in the TJM technology drug delivery system, the preservative antibacterial effectiveness test is done as per USP chapter 51. A pharmacokinetic study conducted in rabbits (study no: BRP17038NG, unpublished data) has shown that maximum concentration and the area under the curve achieved with TJM technology bimatoprost 0.01% is equivalent to low strength bimatoprost 0.01% marketed formulation. Therefore, to further our understanding of this new technology, the present study was undertaken to compare the efficacy and safety of the new formulation TJM bimatoprost 0.01% (TJM-bimatoprost) and marketed bimatoprost 0.01% (BKC-bimatoprost) in healthy beagle dogs.

**Figure 1: Tight junction modulation technology.**

**METHODS**

**Animals**

Twelve beagle dogs (6 male and 6 female) were used in this study. On receipt of animals to the laboratory, veterinary health check-up was done to ensure all animals were healthy. Animals were acclimatized for one week before beginning of treatment. The animals had a mean age of 1-2 years and weight ranging between 9 and 15 kgs. They were kept in enclosures (2-3 animals per enclosure) with a temperature of 18-37°C and relative humidity of 30-70%. Alternating 12 hour light and dark cycles was followed and animals were fed with dog pellet feed (pedigree) ad libitum. All necessary permissions...
including institutional animal ethics approval were procured and the study conformed to all the guidelines laid down by the committee for the purpose of control and supervision of experiments on animals (CPCSEA).\textsuperscript{22}

**Study design**

This was a cross-over study and all animals in the study were assigned to one of two treatment arms to receive either TJM-bimatoprost ophthalmic solution (n=6) or BKC-bimatoprost (n=6). Baseline assessments were done on day 1 and 2 that included IOP and pupil diameter measurements, observation for blepharospasm and conjunctival hyperaemia. Dosing for period 1 was started on day 3 (8 am everyday) and it continued till day 12. Assessments were carried out every day at 8 am, 9 am, 2 pm and 8 pm throughout the study period till day 17. After end of period 1, there was a washout period of 10 days and the animals crossed over for treatment during period 2. Activities for the period 2 were undertaken in a similar manner i.e. assessments from day 1 to day 17 and dosing from day 3 to 12.

**Study procedures**

Previous treatment, on day 1 and day 2, IOP measurements were obtained from left eye of each dog at 8 am and 8 pm and the values were averaged out for computation of initial baseline reading. For measuring IOP, pneumatonometer model 30 classic\textsuperscript{TM} (Reichert, USA) was used and each dog was restrained manually without sedation in a head up position. The pneumatonometer probe was placed lightly covering the entire span of cornea in horizontal position and allowed to rest for 10-15 seconds. Five consecutive IOP readings were recorded, and values with a standard deviation of <1 were displayed on the screen. After each use, the probe filter was cleaned by using a to cotton swab that was immersed in normal saline. Care was taken to ensure that only gentle pressure was applied to carry out the cleaning procedure.

On day 3, each animal was weighed using a digital weighing balance and left eye of each animal was assigned to receive either test or reference solution. Immediately after reading at 8 am, 30 μl (3 mcg) equivalent to 0.01% of TJM-bimatoprost or BKC-bimatoprost ophthalmic solution was instilled once daily for the 10 consecutive days (day 3 to day 12). IOP readings were taken at 9 am (1 h), 2 pm (6 h), 8 pm (12 h) and 8 am (24 h). Although day 13-17 constituted the wash out period, IOP measurements were obtained once each day at 9 am.

As part of the safety assessment, pupillary diameter (pupil size), blepharospasm and conjunctival hyperaemia were assessed, during and after treatment for respective assigned eye. Pupillary diameter was defined as the size of the pupil that becomes constricted after administration of bimatoprost. Measurements were carried out in uniform illumination on the horizontal axis in the center of the pupil using a millimeter ruler (mm). Blepharospasm was defined as spasm of the muscles that control the eyelids. Conjunctival hyperaemia was defined as redness of the eye. Blepharospasm and conjunctival hyperaemia were evaluated using 4 points rating scale (0=none, 1=mild, 2=moderate and 3=severe).

Descriptive statistics were used to describe the variables mean ±SD (standard deviation). Equivalence margin was defined such that the lower and upper bound of the 95% confidence interval estimate had to be within ±1.5 mmHg of the primary variable i.e., mean IOP difference between the treatment groups. The percentage reduction in IOP and change in absolute values of IOP (AIOP) for both test and reference formulations were calculated with respect to the baseline using one-way repeated measures analysis of variance (ANOVA) followed by Bonferroni post-tests. P value less than 0.05 was declared as statistically significant. All statistical analysis was carried out with PRISM (GraphPad version 5.03, 10 December 2009).

**RESULTS**

Baseline IOP for period 1 did not significantly differ between groups (n=6 in each) (22.73±1.25) mean ±SD (standard deviation) vs 22.94±1.33 mmHg for TJM-bimatoprost and BKC-bimatoprost respectively. Mean IOP measurements, percentage change from baseline and absolute change from baseline in IOP values decreased steadily but were comparable across both treatment arms throughout study period 1. At the end of study period 1 (276 h from day 0), the mean IOP measurements were 14.42±1.91 vs 13.62±1.36 mmHg in TJM-bimatoprost and BKC-bimatoprost arms respectively. This value represented a statistically significant absolute IOP change from baseline of 8.32±1.95 and 9.32±2.10 mmHg; and percentage reduction of 36.53±7.98 and 40.38±7.74 for TJM-bimatoprost and BKC-bimatoprost treatment groups respectively. Maximum decrease in IOP measurements were recorded 6 hours post drug administration in both the groups.

During the washout period the, IOP values increased by approximately 4-6 mmHg. Expectedly, even after crossover the results were similar in both treatment arms. Table 1 illustrates the mean IOP, percentage reduction in IOP and absolute change from baseline in IOP on first day of treatment over a 24 hours period.

In order to overcome intra-individual variations, measurements were pooled and analyzed. In this analysis (n=12 in each group), baseline measurements were 21.27±1.82 and 21.35±2.04 mmHg in TJM-bimatoprost and BKC-bimatoprost treatment groups respectively. There was a significant decrease (p<0.001) in IOP 1 hour post administration as compared to the baseline and this trend continued all through the treatment period.
Table 1: Comparison of IOP reduction in TJM-Bimatoprost and BKC-bimatoprost in healthy beagle dogs.

| Treatment | No. of animals | IOP parameters | Period | Time (hours) | Baseline | 49 | 54 | 60 | 72 |
|-----------|----------------|----------------|--------|--------------|----------|----|----|----|----|
| Test item | n=6            | IOP (Mean±SD)  | PI     | 22.73±1.25   | 17.30±2.67 | 17.18±2.50 | 16.62±1.78 | 18.78±2.30 |
|           |                | % reduction    | PI     | -            | 23.93±10.62 | 24.63±7.83 | 26.89±6.77 | 17.39±8.65 |
|           |                |                | P2     | -            | 24.98±3.28  | 35.11±6.04  | 32.23±8.46 | 27.92±9.01 |
|           |                | Δ IOP (Mean±SD)| PI     | -            | 5.43±2.41   | 5.55±1.67  | 6.12±1.60  | 3.95±1.99  |
|           |                |                | P2     | -            | 2.57±1.26   | 4.90±1.53  | 6.00±0.47  | 3.08±1.87  |
| Reference Item | n=6          | IOP (Mean±SD)  | PI     | 22.94±1.33   | 17.72±2.57  | 16.63±1.35  | 17.25±1.80  | 18.28±1.58 |
|           |                | % reduction    | PI     | -            | 19.80±0.76  | 17.23±1.60  | 14.90±1.83  | 16.72±1.40 |
|           |                | Δ IOP (Mean±SD)| PI     | -            | 20.63±1.17  | 20.23±1.72  | 17.33±1.83  | 10.45±2.09 |
|           |                |                | P2     | -            | 19.93±6.40  | 34.21±5.91  | 32.23±8.46  | 27.92±9.01 |

P1=period 1, P2 period 2; IOP units in mmHg; *p<0.05 (from baseline).

Figure 2: Mean IOP difference between TJM-bimatoprost and BKC-bimatoprost ophthalmic solution.

Twenty fours after last dose, on day 12, IOP measurements were 14.20±1.59 mmHg for TJM-bimatoprost and 13.89±1.51 mmHg for BKC-bimatoprost group.

Importantly, the analysis of the primary end point revealed that 95% confidence interval for the between group differences in mean IOP values were well within the pre-defined equivalence margin of ±1.5 mmHg (Figure 2). This was true for all time points throughout the study period.

Diurnal variation of mean IOP values across both the treatment arms are depicted in (Figure 3). Variations in both treatment arms were similar and once the drug administration was stopped the mean IOP began to steadily rise for the next 3 days.

Figure 3: Mean IOP (mmHg) after once daily instillation of TJM-bimatoprost and BKC-bimatoprost in healthy beagle dogs: 30 μl/day for 10 days for each period (n=12).

In terms of safety, there were no difference in baseline mean pupillary diameter (8.97±0.60 vs 8.82±0.57 mm for TJM-bimatoprost and BKC-bimatoprost respectively). Repeated measures analysis of variance between baseline and the subsequent measurements (1 h, 6 h and 12 h post dose) showed statistically significant (p<0.001) differences in both treatment arms. There was approximately 4 mm decrease in pupil size immediately 1 hour post drug administration, indicating severe miosis.
However, non-significant differences were observed at 24 hours post administration measurement in most days except for day 11 and 12 in for TJM-bimatoprost group and day 12 in BKC-bimatoprost group. Baseline conjunctival haemorrhage scores were 0.42±0.67 and 0.17±0.39 in the TJM-bimatoprost group and BKC-bimatoprost group. Throughout the study period, the conjunctival haemorrhage scores were only of mild grade (score 1 to 2) and 1.17±0.72 and 1.08±0.51 were the highest scores reported in TJM-bimatoprost group and BKC-bimatoprost group respectively. It is also noteworthy to mention that no incidence of blepharospasm was detected in both treatment arms.

**DISCUSSION**

PG analogs effectively decrease IOP by increasing the outflow of aqueous humor. Although this is the most plausible mechanism explaining the action of PG analogs, some reports point out to a possibility of a FP receptor independent action. Irrespective of the mechanism, quantification of IOP lowering is the barometer through which effectiveness of anti-glaucoma medication can be assessed. Apart from efficacy, reasonable safety profile is paramount. In this context, the newly developed TJM formulation from SPARC may not only increase efficacy by improving ocular penetration but may decrease the adverse events profile as well.

In our study, we tested the efficacy of IOP lowering effects of two formulations in healthy beagle dogs. This was a cross-over study and when the pooled results of both study periods were analyzed, TJM-bimatoprost 0.01% was adjudicated to be equivalent to BKC-bimatoprost 0.01% treatment arm, as the between group difference for IOP was within ±1.5 mmHg i.e., the equivalence margin.

Although the primary endpoint was proven, it is imperative to scrutinize the results to comprehensively conclude the equivalent efficacy of the new formulation. A closer look at the results revealed that there was no difference in mean IOP at baseline (21.27±1.82 vs 21.35±2.04 mmHg). However, IOP values were statistically significant ten days after treatment in comparison to the baseline, with a mean value of 14.20±1.59 vs 13.89±1.51 mmHg representing an absolute change of 7.07±2.20 vs 7.46±2.59; and percentage reduction of 37.64±7.74 vs 39.01±7.30 in the TJM-bimatoprost and BKC-bimatoprost treatment arms respectively. In the Lee et al study, where a sustained release intracameral preparation of bimatoprost 0.01% was used in beagle dogs the mean IOP at baseline was 19 mmHg and an absolute reduction up to 6-7 mmHg was seen after treatment. This meant an approximate 35% reduction in IOP from the baseline. These findings taken together are comparable to our study findings. Further, in a glaucomatous dog study, the absolute reduction in IOP was 20 mmHg at the end of 5 days of treatment with bimatoprost 0.03%. Additionally, the results of the Kato report also contrasted with our study as there was 17 mmHg decrease in mean IOP from the baseline after 1-2 weeks of treatment with another PG analog, latanoprost. These disparate results could be explained because the dogs had a higher baseline IOP of 40.5 mmHg, a known covariate effecting the reduction of IOP with anti-glaucoma medications.

Further, the mean IOP in our study 1-hour post administration of the drug decreased by 3-4 mmHg initially (day 3) and 5-6 mmHg towards the end of treatment period (day 12). In contrast, there was 4-5 mmHg decrease in IOP in the first 24 hours and 7-8 mmHg decrease 24 hours post administration of drug on day 12. The maximum decrease in IOP were generally seen around 6 hours post administration of the drug. Moreover, fluctuation due to diurnal variation in mean IOP was 3-4 mmHg on an average across ten days of treatment. These results were agreeable with the Lee et al study as the quantum of reduction achieved were similar.

Bimatoprost is known to induce miosis immediately after administration. In our study, the pupil size decreased from a baseline value of approximately 8mm to 4mm one-hour post administration of the drug. Not surprisingly, the Gelatt study also showed similar decrease in pupil size as the baseline reading of 5mm decreased to 1mm, implying severe miosis induced by bimatoprost. However, it is noteworthy to mention that higher concentration of bimatoprost 0.03% was used in this study. Conjunctival haemorrhage noticed in this study was also of mild to moderate grade which was noted in both arms. This can be attributed to the class action of bimatoprost.

Although the results are limited to animals’ models, nevertheless, the study suggest that the new TJM technology lower strength bimatoprost 0.01% could be an effective alternative to the marketed bimatoprost 0.01% which contains BKC 0.02% as a preservative. The reduced strength marketed BKC-bimatoprost has shown to be efficacious with a superior safety profile compared to bimatoprost 0.03% in a 12 months multi-centre, randomized clinical study. Notwithstanding the decrease in adverse events compared to the higher strength bimatoprost, the ocular surface adverse events attributable to BKC were still persistent in subsequent reports.

New formulation of SPARC with TMJ technology could overcome this limitation and further improve the safety outcomes. This study gives a preliminary insight into the expected benefit with the new technology. As with any drug development process, the next logical step would be to clinically test the TJM-bimatoprost 0.01% ophthalmic solution in large human population to concretely establish the safety and efficacy.

**CONCLUSION**

The results of the study show that TJM-bimatoprost 0.01% has equivalent IOP lowering activity in comparison to the marketed BKC-bimatoprost 0.01% in...
healthy beagle dogs. Safety results were also comparable and, thus, the study furthers our understanding of the proprietary TJM technology.

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REFERENCES

1. Sambhara D, Aref AA. Glaucoma management: relative value and place in therapy of available drug treatments. Ther Adv Chronic Dis. 2014;5(1):30-43.
2. European Glaucoma Society. Terminology and guidelines for glaucoma. 3rd ed. Savona: Editrice DOGMA; 2008.
3. Weinreb RN, Toris CB, Gabelt BAT, Lindsey JD, Kaufman PL. Effects of prostaglandins on the aqueous humor outflow pathways. Surv Ophthalmol. 2002;47(1):53-64.
4. Valk VDR, Webers CA, Schouten JS, Zeegers MP, Hendrikse F, Prins MH. Intraocular pressure-lowering effects of all commonly used glaucoma drugs: a meta-analysis of randomized clinical trials. Ophthalmology. 2005;112(7):1177-85.
5. Novack GD, Donnell OMJ, Molloy DW. New glaucoma medications in the geriatric population: efficacy and safety. J Am Geriatr Soc. 2002;50(5):956-62.
6. Wirta D, Vandenburgh AM, Weng E, Whitcup SM, Kurstjens S, Beddingfield FC. Long-term safety evaluation of bimatoprost ophthalmic solution 0.03%: a pooled analysis of six double-masked, randomized, active-controlled clinical trials. Clin Ophthalmol. 2011;5:759-65.
7. Mccarey B, Edelhauser H. In vivo corneal epithelial permeability following treatment with prostaglandin analogs (correction of analogs) with or without benzalkonium chloride. J Ocul Pharmacol Ther. 2007;23(5):445-51.
8. Myers JS, Vold S, Zaman F, Williams JM, Hollander DA. Bimatoprost 0.01% or 0.03% in patients with glaucoma or ocular hypertension previously treated with latanoprost: two randomized 12 weeks trials. Clin Ophthalmol. 2014;8:643-52.
9. Chen YY, Wang TH, Liu C, Wu KY, Chiu SL, Simonyi S, et al. Tolerability and efficacy of bimatoprost 0.01% in patients with open-angle glaucoma or ocular hypertension evaluated in the Taiwanese clinical setting: the Asia Pacific Patterns from Early Access of Lumigan 0.01% (APEAL Taiwan) study. BMC Ophthalmol. 2016;16(1):162.
10. Guenoun JM, Baudouin C, Rat P, Pauly A, Warnet JM, Baudouin BF. In vitro study of inflammatory potential and toxicity profile of latanoprost, travoprost, and bimatoprost in conjunctiva-derived epithelial cells. Invest Ophthalmol Vis Sci. 2005;46(7):2444-50.
11. Steven DW, Alaghband P, Lim KS. Preservatives in glaucoma medication. Br J Ophthalmol. 2018;102(11):1497-503.
12. Jaenen N, Baudouin C, Pouliquen P, Manni G, Figueiredo A, Zeyen T. Ocular symptoms and signs with preserved and preservative-free glaucoma medications. Eur J Ophthalmol. 2007;17(3):341-9.
13. Pisella PJ, Pouliquen P, Baudouin C. Prevalence of ocular symptoms and signs with preserved and preservative-free glaucoma medication. Br J Ophthalmol. 2002;86(4):418-23.
14. Farshori P, Kachar B. Redistribution and phosphorylation of occlude in during opening and resealing of tight junctions in cultured epithelial cells. J Membr Biol. 1999;170(2):147-56.
15. Findley MK, Koval M. Regulation and roles for claudin-family tight junction proteins. IUBMB Life. 2009;61(4):431-7.
16. Ikeda T, Ledwith A, Bamford CH, Henn RA. Interaction of a polymeric biguanide biocide with phospholipid membranes. Biochim Biophys Acta. 1984;769(1):57-66.
17. Chadeau E, Dumas E, Adt I, Degraeve P, Noel C, Girodet C, et al. Assessment of the mode of action of polyhexamethylene biguanide against Listeria innocua by Fourier transformed infrared spectroscopy and fluorescence anisotropy analysis. Can J Microbiol. 2012;58(12):1353-61.
18. Mashat BH. Polyhexamethylene biguanide hydrochloride: features and applications. British Journal of Environmental Sciences. 2016;4(1):49-55.
19. Castillo Y, Ernest J. Inventor; Alcon Laboratories Inc., assignee. Sustained release and comfortable ophthalmic composition and method for ocular therapy. United States patent US 4,867,749; 2000.
20. Larkin DF, Kilvington S, Dart JK. Treatment of Acanthamoeba keratitis with polyhexamethylene biguanide. Ophthalmology. 1992;99(2):185-91.
21. Sutton SV, Porter D. Development of the antimicrobial effectiveness test as USP chapter <51>. PDA J Pharm Sci Technol. 2002;56(6):300-11.
22. Pereira S, Tettamanti M. Ahimsa and alternatives: The concept of the 4th R. The CPCSEA in India. ALTEX. 2005;22:3-6.
23. Woodward DF, Liang Y, Krauss AH. Prostamides (prostaglandin-ethanolamides) and their pharmacology. Br J Pharmacol. 2008;153(3):410-9.
24. Lee SS, Dibas M, Almazan A, Robinson MR. Dose-Response of Intracameral Bimatoprost Sustained-Release Implant and Topical Bimatoprost in
Lowering Intraocular Pressure. J Ocul Pharmacol Ther. 2019;35(3):138-44.

25. Gelatt KN, Mackay EO. Effect of different dose schedules of bimatoprost on intraocular pressure and pupil size in the glaucomatous Beagle. J Ocul Pharmacol Ther. 2002;18(6):525-34.

26. Kato K, Woerd VDA. Effect of Long-term Topical Application of 0.005% Latanoprost on Intraocular Pressure Uncontrolled by Multiple or Single Drug Therapy in Dogs with Secondary Glaucoma. 2017;6(1). Available at https://www.scitechnol.com/peer-review/effect-of-longterm-topical-application-of-0005-latanoprost-on-intraocular-pressure-uncontrolled-by-multiple-or-single-drug-therapy-3Mof.php?article_id=5856. Accessed on 19 March 2020.

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