Eyelash Growth Induced by Topical Prostaglandin Analogues, Bimatoprost, Tafluprost, Travoprost, and Latanoprost in Rabbits

Amália Turner Giannico,1 Leandro Lima,1 Heloisa Helena Abil Russ,2 and Fabiano Montiani-Ferreira1

Abstract

Purpose: Prostaglandin analogues (PGA) are ocular hypotensive agents used for the treatment of glaucoma. Hypertrichosis of the eyelashes has been reported in humans as a side effect. Eyelash growth was investigated with clinical trials in people using bimatoprost. Scattered reports of eyelash growth during the treatment of glaucoma with other PGA are also found in the literature. We investigated the effect of 4 different topical PGA on eyelash length.

Methods: Forty New Zealand white rabbits were divided into 4 groups and received daily topical application of bimatoprost, tafluprost, travoprost, and latanoprost in the left eye for 4 weeks. The right eye received no treatment. Eyelash length was measured in both eyes before and after treatment using a stainless steel digital caliper.

Results: Bimatoprost and tafluprost groups had significant increases in eyelash length. We did not observe significant eyelash growth in rabbits receiving travoprost and latanoprost after 1 month of treatment.

Conclusions: Today, only bimatoprost is approved for growing eyelashes, and our research shows that tafluprost could be further explored by the cosmetic and pharmaceutical industry. Additional research using travoprost and latanoprost as agents for eyelash growth should be performed in the future using prolonged treatment periods to determine whether or not these PGA induce eyelash growth, and investigate other possible side effects.

Introduction

Eyelashes, besides providing a natural protective barrier against foreign bodies for the eyes, also possess an aesthetic function.1,2 In modern society, longer and fuller eyelashes are considered a desirable physical attribute on women and a sign of femininity and beauty.3

Since the beginning of the use of prostaglandin analogues (PGA) as an ocular hypotensive agent for the treatment of glaucoma, hypertrichosis of eyelashes has been reported as a side effect.1,4,5 Currently, a synthetic PGA, bimatoprost 0.03%, besides being used to lower intraocular pressure (IOP) is also approved for cosmetic purposes to increase eyelash length, thickness, and darkness in normal patients and in patients with palpebral hypotrichosis. For the latter use, the drug is applied topically to the skin of the eyelid margins.1,2,6

Bimatoprost, tafluprost, travoprost, and latanoprost share many similarities both structurally and pharmacologically1 and these PGA may have similar effects on eyelash growth. Several studies with human patients investigating these PGA drugs for glaucoma treatment qualitatively reported eyelash growth, merely as a side effect.7–17 However, only bimatoprost and latanoprost have been studied in clinical trials with human beings that assessed quantitatively and specifically the eyelash growth.1,2,18–23 Body hair growth, but not eyelash growth has been investigated in animal models.24–26 The effect of PGA specifically on eyelash growth has not been studied in animals.

To the author’s knowledge, this is the first study comparing eyelash growth secondary to the use of these topical PGA in a homogeneous group of animals. In this article, our goal was to evaluate and discuss the effects of 4 topical PGA on eyelash length in New Zealand white rabbits.

Methods

Animals

The investigation was carried out using 40 clinically healthy, 6-month-old New Zealand white rabbits (Oryctolagus cuniculus), 19 males and 21 females, weighing an average 2.66±0.03 kg...
(mean ± standard deviation). The animals were selected randomly from a commercial breeder collection. All procedures using live rabbits were conducted in accordance with the Federal University of Parana Animal Use Committee (Curitiba city, Parana state, Brazil) and with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Physical examinations were performed before ocular examinations to exclude animals with any indication of systemic disease. The anterior ocular structures of all animals were evaluated using a transilluminator and a slit lamp biomicroscope (Hawk Eye; Dioptrix, L’Union, France). Rabbits with any evidence of ocular or systemic diseases were excluded from this research. To avoid inter-investigator discrepancies, the same masked investigator performed all the ocular tests. Another investigator (not masked) instilled the eyedrops. The humidity (70%) and temperature (22.5°C) conditions were controlled.

Treatments

Rabbits were divided into 4 groups of 10 animals using the random function = RANDBETWEEN (1,40) on Microsoft Office Excel (Excel version in Microsoft Office 2007 for Windows) after all animals received a number from 1 to 40. The left eyes were treated daily for 4 weeks with one drop of topical PGA eye-drops applied to the conjunctival fornix. The ophthalmic drugs used in each group were, respectively: Group 1—bimatoprost 0.03% containing benzalkonium chloride (BAK) as preservative (Lumigan® colírio; Allergan Indústria Farmacêutica Ltda., Guarulhos, SP, Brazil); Group 2—tafluprost 0.0015% without preservative (Saflutan® colírio; Merck Sharp & Dohme Ltda., Guarulhos, SP, Brazil); Group 3—travoprost 0.004% with BAK (Travatan® colírio; Alcon Laboratórios do Brasil Ltda., São Paulo, SP, Brazil); Group 4—latanoprost 0.005% with BAK (Xalatan® colírio; Pfizer Indústria Farmacêutica, São Paulo, SP, Brazil). All groups are homogenous in the way that they have almost the same proportion of males and females (5 males and 5 females) each, with the exception of Group 2 (tafluprost) that presented 4 males and 6 females. This was the product of the random selection applied. The right eyes remained untreated throughout the study.

Eyelash evaluation

The left and right eyes were evaluated 1 day before the start of the treatment and 30 days after. The evaluation was performed with the rabbits manually restrained by the same investigator taking care to keep the animals comfortable during the measurements.

Eyelash lengths of both the left and right eyes were measured using a stainless steel digital caliper with an LCD display (PD-150, 0-150 mm, accuracy: 0.01 mm; Vonder, Curitiba, PR, Brazil) from the base of the eyelid margin to the end of the cilia. The central region of the upper eyelid was determined by measuring the entire horizontal length of the eyelid, dividing the resulting number by 2, thus locating the center of the lid. The central region was 6 mm in length with 3 mm from the center of the superior eyelid to each side, nasal, and temporal (Fig. 1). Eyelashes in this central area are naturally bigger than the other ones located at the nasal and temporal region. The 5 longer eyelashes were selected at the central palpebral region of the superior eyelid on each rabbit. Top 5 eyelash lengths were then measured pre- and post-treatment.

Statistical analysis

The Shapiro–Wilk normality test demonstrated that the data errors were normally distributed. Data were statistically analyzed using the computer software StatView (SAS Institute, Cary, NC) applying the 2-way analysis of variance and Tukey–Kramer post hoc test to compare pre- and post-treatment results. Values of P < 0.05 were considered significant.

Results

Mean, standard deviation, and standard error of the eyelash length values expressed in millimeters (mm) in pre- and post-treatment are demonstrated in Table 1.

Comparisons of the pretreatment eyelash length values of the left eye of all groups were not significantly different (P > 0.05), showing that the groups were homogeneous before treatment. After PGA treatment, all groups demonstrated a tendency to increase mean eyelash length values, however, only bimatoprost and tafluprost groups had a significant increase (P = 0.0001 and P = 0.0361, respectively). No significant differences were found on eyelash length on travoprost and latanoprost groups comparing pre- and post-treatment values (P = 0.1330 and P = 0.0581, respectively) (Table 1). Additionally, comparison between bimatoprost


Table 1. Mean, Standard Deviation, Standard Error, and Coefficient of Variation of the Superior Eyelid Central Region Eyelash Length Values Expressed in Millimeters with P value of the Comparisons Between Pre- and Post-Treatment (OS) with Prostaglandin Analogues and Untreated Eye (OD) in New Zealand White Rabbits

<table>
<thead>
<tr>
<th>Group</th>
<th>Eye</th>
<th>Prostaglandin analogues drugs</th>
<th>Mean Pre</th>
<th>Mean Post</th>
<th>SD Pre</th>
<th>SD Post</th>
<th>SE Pre</th>
<th>SE Post</th>
<th>CV Pre</th>
<th>CV Post</th>
<th>P value Pre vs. Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OS</td>
<td>Bimatoprost 0.03%</td>
<td>9.50</td>
<td>11.99</td>
<td>1.09</td>
<td>1.02</td>
<td>0.35</td>
<td>0.32</td>
<td>0.11</td>
<td>0.09</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td></td>
<td>OD</td>
<td>Untreated</td>
<td>9.31</td>
<td>9.38</td>
<td>1.04</td>
<td>1.03</td>
<td>0.33</td>
<td>0.32</td>
<td>0.11</td>
<td>0.11</td>
<td>0.8850</td>
</tr>
<tr>
<td>2</td>
<td>OS</td>
<td>Tafluprost 0.0015%</td>
<td>9.97</td>
<td>11.03</td>
<td>1.01</td>
<td>1.08</td>
<td>0.32</td>
<td>0.34</td>
<td>0.10</td>
<td>0.10</td>
<td>0.0361*</td>
</tr>
<tr>
<td></td>
<td>OD</td>
<td>Untreated</td>
<td>10.08</td>
<td>10.11</td>
<td>1.04</td>
<td>0.87</td>
<td>0.33</td>
<td>0.28</td>
<td>0.10</td>
<td>0.09</td>
<td>0.9470</td>
</tr>
<tr>
<td>3</td>
<td>OS</td>
<td>Travaprost 0.004%</td>
<td>10.16</td>
<td>11.32</td>
<td>1.57</td>
<td>1.70</td>
<td>0.50</td>
<td>0.54</td>
<td>0.15</td>
<td>0.15</td>
<td>0.1330</td>
</tr>
<tr>
<td></td>
<td>OD</td>
<td>Untreated</td>
<td>9.90</td>
<td>9.92</td>
<td>1.63</td>
<td>1.73</td>
<td>0.51</td>
<td>0.55</td>
<td>0.16</td>
<td>0.17</td>
<td>0.9822</td>
</tr>
<tr>
<td>4</td>
<td>OS</td>
<td>Latanoprost 0.005%</td>
<td>9.70</td>
<td>10.51</td>
<td>0.97</td>
<td>0.81</td>
<td>0.31</td>
<td>0.26</td>
<td>0.10</td>
<td>0.08</td>
<td>0.0581</td>
</tr>
<tr>
<td></td>
<td>OD</td>
<td>Untreated</td>
<td>9.65</td>
<td>9.77</td>
<td>0.94</td>
<td>0.93</td>
<td>0.30</td>
<td>0.29</td>
<td>0.10</td>
<td>0.10</td>
<td>0.7807</td>
</tr>
</tbody>
</table>

*Significant difference.

SD, standard deviation; SE, standard error; CV, coefficient of variation; OS, oculus sinister (left eye); OD, oculus dexter (right eye).

and tafluprost post-treatment values were not statistically significant (P = 0.3820). There was no significant difference in eyelash length between males and females in pre- and post-treatment (P > 0.05). The mean eyelash length values from the right eye (that did not receive treatment) did not show significant difference between pre- and post-treatment for all groups (P > 0.05) (Table 1).

No clinical ophthalmic signs such as mucous secretion, blepharospasm, blepharoedema, conjunctival and palpebral congestion or pigmentation were observed after treatment in any group.

Discussion

There are several studies that aimed to evaluate the effect of PGA in human patients with glaucoma that mentioned as a curiosity or merely as a subjective observation some side effects on the eyelashes, such as increased growth, number, length, thickness, curvature, and pigmentation.12–14,17 Since in these articles, the main objective was the evaluation of the main action of these aforementioned PGA drugs, which is lowering the IOP, the experimental design and statistical analysis used to demonstrate eyelash growth were unclear or not mentioned at all. Previously, eyelash growth was described qualitatively and never quantitatively.9–11,16

No other study has detailed the method used to measure the eyelash growth in rabbits. We have chosen the 5 longer eyelashes of the central palpebral region for both, pre- and post-treatment because we have noticed that this region already has longer cilia. We assumed that most if not all the cilia chosen on both occasions were most likely the same. We believe that even if different cilia were measured in the post-treatment period, these would be the longest ones and it still would be a strong indicative of eyelash growth.

The bimatoprost solution 0.03% (Latisse6) is the only prescription product approved by the US Food and Drug Administration for growing eyelashes.6 Our research shows that tafluprost 0.0015% has similar effects as bimatoprost 0.03% on eyelash growth of rabbits after 30 days of daily treatment.

In the conditions of the present investigation, travoprost and latanoprost did not significantly increased eyelash length even though higher values were found in the post-treatment period. Human patients affected by glaucoma and treated with latanoprost also showed a slight tendency toward an increase in eyelash length, however, with no significant differences.8,15 These references corroborate our result. Nevertheless, other studies with human patients showed that latanoprost significantly increased eyelash length.18,22 Perhaps, the differences in experimental conditions, species studied (human vs. animals), and duration of the treatment might be responsible for these discrepancies and should be taken into account.

Possibly, 1 month of treatment was time enough to demonstrate differences in eyelash growth in rabbits receiving bimatoprost and tafluprost groups, but not enough time to promote a difference in eyelash length in rabbits receiving travoprost and latanoprost. In some studies of PGA use in humans, treatments were longer than 3 months.7,9–12,14–16 Considering this, possibly one of the limitations of our investigation was a short duration of treatment.

Other side effects reportedly caused by PGA drugs in humans include conjunctival hyperemia, increase of iris pigmentation, eyelid pigmentation and, in rare cases, periorcular pigmentation.8,7,28 Hyperpigmentation can occur within 3 to 8 weeks after treatment initiation in human patients.7,29 Some researchers suggest that a faster onset of periorcular skin hyperpigmentation may occur with direct application of PGA to the eyelid.29 Conjunctival hyperemia, skin hyperpigmentation, and erythema of the eyelid are the most common adverse events caused by the bimatoprost solution 0.03% (Latisse)6 and these side effects were observed in another study with human patients diagnosed with ocular hypertension and treated with latanoprost, travoprost, tafluprost, and bimatoprost eye drops for more than 3 months.30 Probably, all PGA used in our study may cause similar effects if used for a long time, as eyedrops and as a solution applied to the upper eyelid margin. No other signs such as erythema of the eyelids or conjunctiva hyperemia were observed in our study. Changes in pigmentation could not be evaluated in this study because New Zealand white rabbits are albinos and do not have any pigmented cells. The next logical step for further studies would be developing new solutions to be applied to the upper eyelid margins containing tafluprost and observing possible side effects.

Based on the general desire for longer, thicker, and darker eyelashes as a sign of femininity and beauty in most cultures, we tested other PGA as alternatives for those seeking to
augment their lash appearance. Tafluprost 0.0015% eye drops can promote similar eyelash growth as bimatoprost 0.03% in rabbits when used once daily for 30 days. Travoprost 0.004% and latanoprost 0.005% did not affect eyelash length in this study.

Acknowledgments

The authors thank Dr. Gillian Shaw, Johns Hopkins University, Baltimore, MD, for her invaluable help in the preparation of this manuscript.

Author Disclosure Statement

No competing financial interests exist.

References

6. Allergan, Inc. LatisseTM (Bimatoprost Ophthalmic Solution) 0.03%. Prescribing Information. Irvine, CA: Allergan, Inc.; 2009.

Received: April 6, 2013
Accepted: July 25, 2013

Address correspondence to:
Fabiano Montiani-Ferreira, DVM, MSc, PhD
Department of Veterinary Medicine
Federal University of Paraná
Rua dos Funcionários, 1540
Juvevê
Curitiba City CEP 80035-050
Brazil
E-mail: montiani@ufpr.br