In Vivo Effects of Retrobulbar Bimatoprost Injection on Orbital Fat

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Purpose: Recent publications have reported the adverse effects of prostaglandin analogues on the periocular tissues. These medications may cause periorbital lipodystrophy, enophthalmos, and deepening of the superior sulcus deformity. While these effects may have adverse consequences for some patients, the atrophy of the periorbital fat may have a useful role in diseases that lead to orbital and periorbital fat hypertrophy such as thyroid eye disease. In this pilot study, the authors investigated the effects of retrobulbar bimatoprost injection on the intraocular pressure and orbital fat in a rat animal model.

Methods: Three rats were sedated and intraocular pressure was measured. A 0.1 ml aliquot of bimatoprost was injected into the right orbit of all rats. In the left orbit, 0.1 ml of phosphate-buffered saline was injected as a control. Three weeks later, all rats were sedated and intraocular pressure was measured before euthanizing. Routine histologic staining was performed and thin sections through the intraconal orbital fat were obtained. Density of intraconal adipocytes was measured and adipocyte heterogeneity was determined using a computer image analysis algorithm.

Results: The specimens injected with bimatoprost demonstrated atrophy of orbital fat with significantly increased adipocyte density ($p = 0.009$) and heterogeneity ($p = 0.008$) when compared with control. Intraocular pressure was not significantly decreased at 3 weeks after injection of retrobulbar bimatoprost.

Conclusions: In this pilot study, orbital injection of bimatoprost demonstrated atrophy of intraconal adipocytes when compared with control orbits injected with saline. The orbits injected with bimatoprost were noted to have smaller, more heterogeneous adipocytes that were densely packed in the intraconal space. The study limitations include the small sample size, which limited the ability for us to make conclusions about the effect on intraocular pressure. Nevertheless, the findings presented suggest that retrobulbar bimatoprost may present a nonsurgical alternative to induce atrophy of the orbital fat without inducing inflammation or hypotony.

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In the past decade, a newly recognized adverse effect of topical prostaglandin analogues (PGA) has been described in which patients develop periorbital fat atrophy. This periorbital lipodystrophy can cause noticeable aesthetic deformities including enophthalmos and deepening of the superior sulcus, and can make the routine applanation of these patients difficult without exerting pressure on the globe to lift the eyelids.[1] The effect appears to be reversible in some patients on cessation of PGA.[2] The exact pathophysiologic process by which these agents induce periorbital atrophy is unknown.

Studies have demonstrated increased density or crowding of adipocytes in the preaponeurotic upper eyelid fat of patients treated with PGA when compared with contralateral untreated eyes, suggesting atrophy of these adipocyte populations.[3] Other reports have shown that exposing orbital fibroblasts to prostaglandin $F_{2\alpha}$—the common base structure of the 3 major PGA bimatoprost, travoprost, and latanoprost—slowed adipocyte population growth in cell culture by decreasing adipogenesis when compared with cells not exposed to PGA.[4]

The current study examines the in vivo effects of exposure of rat orbital adipocytes to a retrobulbar depot injection of the PGA bimatoprost (Lumigan, Allergan Inc, Irvine, CA) as compared with a saline control. The authors assessed the effects of injection on the rat intraconal orbital fat and on rat intraocular pressure (IOP).

METHODS

Use of Animals. All experiments were conducted in accordance with the Association for Research in Vision and Ophthalmology Statement

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for the Use of Animals in Ophthalmic and Visual Research. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania.

IOP Assessment. Three adult male Lewis rats were sedated using inhalational isoflurane anesthesia. After sedation, IOP was measured in both eyes without topical anesthesia using an Icare TONOLAB (Icare Finland, Helsinki, Finland) tonometer designed specifically for rodent IOP measurement. The device takes 5 IOP measurements and reports the average IOP.

Retrobulbar Injections. The methods for orbital injection were adapted from a prior study.[5] A 30-gauge needle was used to inject 0.1 ml of 0.03% bimatoprost into the retrobulbar space of the right orbit of all 3 rats. In the left orbit, 0.1 ml of sterile phosphate-buffered saline was injected as a control. All rats awoke from anesthesia without complication.

Orbital Tissue Processing. Three weeks after injection, all rats were sedated using inhalational isoflurane anesthesia and IOP was measured using the same protocol. After measurement, rats were euthanized by cardiac perfusion with 4% paraformaldehyde. After perfusion, each rat was decapitated and the head was placed in 10% formalin solution for 2 weeks. Then, each orbit was carefully exenterated subperiosteally with a scalpel and placed back in 10% formalin solution for 2 weeks. Exenteration specimens were embedded in paraffin and cut into 5 μm sections. Sections centered around the intraconal fat and optic nerve were stained with hematoxylin–eosin for histologic examination by a trained ocular pathologist (V.L.) and a second observer (K.E.) in a masked fashion.

IOP and Orbital Fat Analysis. The IOP pre- and postinjection was compared using a 2-tailed Student *t* test. The intraconal fat of the orbital sections was examined under light microscopy and scanned into an online image database for further analysis. Using ImageJ software (Rasband W.S., US National Institutes of Health, Bethesda, MD), the areas of intraconal fat were cropped and the adipocytes were counted by a single investigator to determine adipocyte density using a previously described method.[3] The density of adipocytes per square micrometer area was calculated and compared between groups via a 2-tailed Student *t* test.

To further analyze the heterogeneity of adipocyte size in the intraconal orbital fat between groups, the microscopy images were analyzed using a maximally stable extremal regions-based segmentation algorithm implemented in MATLAB (Natick, MA). The previously cropped images of the intraconal fat were used to perform this analysis. A maximally stable extremal region algorithm entitled VL_FEAT was used to find extremal regions with parameters of delta = 1, maximum variation = 0.2, minimum diversity = 0.5. Maximally stable extremal region-determined regions with sizes less than 10 pixels or eccentricity larger than 0.9 were discarded as false positives. The mean segmented adipocyte size for each image was calculated and the 2 groups were compared using Student *t* test.

RESULTS

Baseline IOP was measured in both eyes of 3 adult male Lewis rats. As expected, the pressure between the 2 eyes in each rat was similar before injection, with the average IOP of 13.0 mm Hg in the bimatoprost group and 13.3 in the phosphate-buffered saline group ($p = 0.667$). Retrobulbar injection did not significantly raise IOP, with measurements made immediately following injection in representative orbits showing less than 4 mm Hg increase in IOP. Three weeks after injection, IOP was measured again at the same time of day to minimize the effects of diurnal variation. The mean IOP in the eyes of bimatoprost-injected orbits was 11.3 mm Hg, not significantly lower than the mean IOP preinjection of 13.0 mm Hg ($p = 0.496$). The mean IOP in the eyes of saline-injected orbits was 14.7 mm Hg at the 3-week time point ($p = 0.11$). Despite the rats receiving a depot dose of PGA in the right orbit, there was no demonstrable lasting effect on IOP after a single injection (Fig. 1).

Histopathologic evaluation of the tissue revealed no inflammatory cell infiltrate in either the bimatoprost-injected orbits or the saline controls. On qualitative light microscopy analysis, the intraconal fat appeared fragmented, with small, irregular-shaped atrophied adipocytes in the bimatoprost sections, whereas orbital fat appeared undisturbed in the saline specimens (Fig. 2).

Quantitative analysis of adipocyte density was performed between the 3 rats receiving bimatoprost in the right orbit and saline in the left orbit. The bimatoprost group demonstrated a mean density of 231 adipocytes per square micrometer, while the saline control showed 197 adipocytes per square micrometer, consistent with the observed atrophy of adipocytes in the former group ($p = 0.009$). Further analysis for adipocyte heterogeneity to calculate mean segmented adipocyte size (Fig. 3) demonstrated higher heterogeneity in the bimatoprost group compared with control ($p = 0.008$). This result was tested using different stringency criteria for the adipocytes to eliminate false positives.

DISCUSSION

Prostaglandin analogue agents, specifically $PGF_{2\alpha}$, have been used for many years in the treatment of open angle glaucoma. Recently, numerous reports have described eyelid and orbital changes that occur with the use of this medication including hypertrichosis, hyperpigmentation, meibomian gland

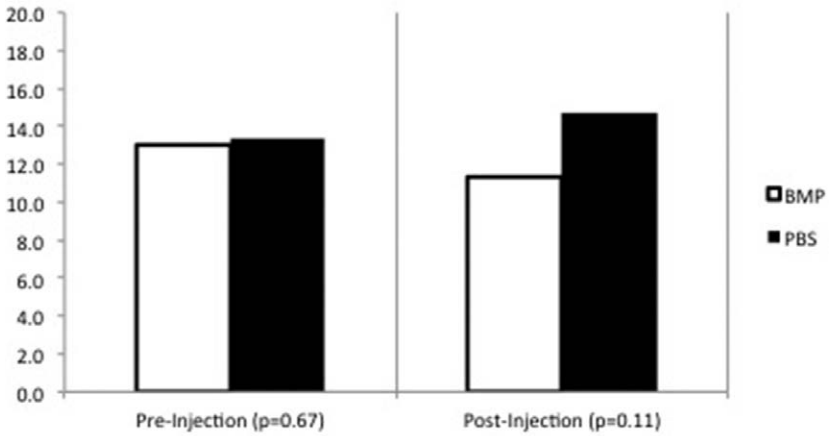

FIG. 1. Intraocular pressure (IOP) comparison after retrobulbar bimatoprost injection. Differences in IOP between the bimatoprost (BMP) and saline (phosphate-buffered saline) groups pre- and postinjection were not statistically significant.

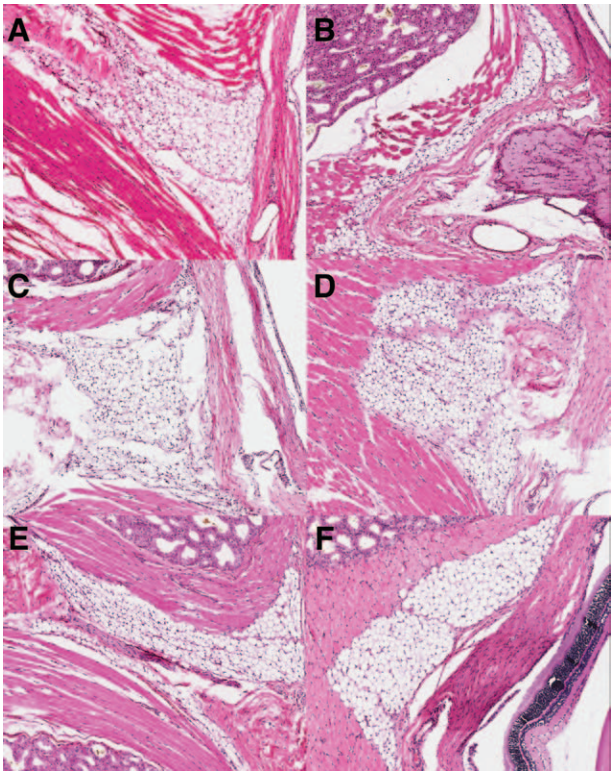

FIG. 2. Histopathologic comparison of retrobulbar fat compartment after bimatoprost and saline injection. Histopathologic evaluation of the orbital tissues following retrobulbar bimatoprost injection showed no inflammation, but did show small, irregularly-shaped, fragmented adipocytes with high adipocyte density per unit area suggesting atrophy of the intraconal fat (**A**, **C**, **E**). Examination of the contralateral orbit of each rat which had received retrobulbar saline revealed normal adipocytes without fragmentation (**B**, **D**, **F**).

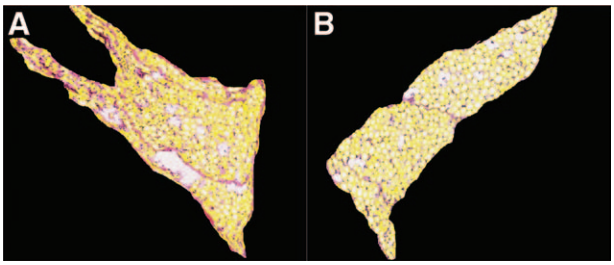

FIG. 3. Computer image analysis for adipocyte morphologic heterogeneity. Sample analysis frames from the maximally stable extremal regions segmentation-based algorithm showing cropped areas of orbital fat and individual selection of adipocytes highlighted in yellow. The heterogeneity in the morphology of adipocytes was compared between orbits injected with bimatoprost (**A**) and saline (**B**) and found to be significantly more heterogeneous in the bimatoprost group.

dysfunction, horizontal eyelid shortening, eyelid retraction, and lipodystrophy.[1,6,7] Other authors have shown that, in eyes exposed to $PGF_{2\alpha}$ analogues, there was increased adipocyte density in the preaponeurotic fat as compared with the untreated eye.[3] In addition, 1 group of investigators has shown that in a mouse model of thyroid eye disease (TED), the doubling time of orbital fibroblasts was increased when the cells were exposed in vitro to $PGF_{2\alpha}$ analogues.[4] This suggests that the $PGF_{2\alpha}$ analogues may decrease adipogenesis and thus induce fat atrophy in vivo.

Results of the current pilot study suggest that retrobulbar injection of bimatoprost, a $PGF_{2\alpha}$ analogue, can induce fat atrophy in an in vivo rat model without causing hypotony or orbital inflammation. Consistent with this, there was a statistically significant increase in the density of adipose cells in the orbits exposed to $PGF_{2\alpha}$ analogue as compared with control orbits injected with saline. Due to intraorbital variability, the total area of orbital fat could not be compared between rats, but the increased cell density within regions of orbital fat is consistent with an overall contraction of the fat and reduction in orbital volume.

The observed atrophy of orbital fat in the bimatoprost-injected orbits may represent the other end of the spectrum for the mechanism that leads to expansion of the orbital fat compartment in TED.[8,9] This is based on the link between prostaglandins and TED through the metabolic pathway involving peroxisome proliferator-activated receptors (PPARs).

The PPAR pathway has become relevant in the oculoplastic literature by the noted effect of diabetic medications in the class of oral thiazolidinedione agents, such as rosiglitazone and pioglitazone, to cause orbital fat enlargement and proptosis.[10–14] The thiazolidinedione agents activate PPARs through the gamma receptor subtype (PPARγ).[15] Interestingly, PPARγ also plays a central role in adipogenesis of orbital fibroblasts in TED.[16] Further evidence for the importance of PPARγ in TED is supported by the observation that a polymorphism that downregulates the PPARγ gene may be protective against developing the manifestations of TED.[17] This may explain why patients without TED who are exposed to a PPARγ agonist such as thiazolidinedione agents may experience fat-predominant proptosis.[10,13,14]

Prostaglandins are also known to be present in the inflammatory cascade involved in TED, with certain orbital fibroblasts subtypes producing prostaglandin E_2.[18] Different prostaglandins may activate or suppress adipogenesis depending on the effect they have on the PPARγ pathway.[19] Notably, in regards to the present study, prostaglandin $F_{2\alpha}$ downregulates the adipogenic effects of PPARγ.[20,21] Thus, prostaglandin $F_{2\alpha}$—the active agent in bimatoprost and used for lowering IOP in glaucoma patients—can downregulate adipogenesis via a central regulatory pathway in the inflammatory cascade involved in TED.

Our study is limited primarily by its small sample size. While the results were consistent within each group, the effect of bimatoprost on orbital fat atrophy in Lewis rats cannot be generalized to humans. In addition, the average IOP in the rats was slightly lower than that of a prior study using a Tonopen (Bio Rad, Santa Ana, CA), in which the average IOP was 17.30 in Lewis rats.[22] Our lower measurement may have been secondary to IOP decrease after inhalational isoflurane used for sedation. Thus, the lower baseline IOP measurements may have made it harder to detect a pressure-lowering effect with rats injected with retrobulbar bimatoprost. Moreover, the IOP was measured several weeks after the injection for comparison, and it is possible that any pressure-lowering effect may have since dissipated. Our pilot study was not powered to detect a small decrease in IOP.

A second limitation of our study was the smaller intraconal orbital fat compartment the authors noted in the rat specimens. Rat orbits have a large Harderian gland which consumes a large volume of the orbit, leaving less relative room for adipocytes. This smaller fat compartment may have limited the ability to detect differences in the samples. However, this should not bias the results given that a difference in adipocyte density was noted in the comparison between orbits injected with prostaglandin and with saline.

In conclusion, the observed atrophy of the intraconal orbital fat induced by bimatoprost in this study suggests a potential role for PGA as an adjunctive treatment for diseases that affect the orbital fat. A nonsurgical modality for reducing orbital fat would be a useful adjunct in certain patients, and the current pilot study suggests that prostaglandins may have a role. Future studies would evaluate the in vivo effect of PGA in an animal model of TED, as well as the effects of topical prostaglandins on human orbits.

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