Bimatoprost-Induced Periocular Skin Hyperpigmentation

Histopathological Study

Rashmi Kapur, MD; Smajo Osmanovic, MD, PhD; Sami Toyran, MD; Deepak P. Edward, MD

Objective: To investigate light microscopic and ultrastructural changes in bimatoprost-induced skin hyperpigmentation.

Methods: Eyelid biopsy specimens from bimatoprost-treated patients and matched controls were examined by light microscopy and transmission electron microscopy. Using an image analyzer, melanin granules were counted on Fontana-Masson-stained sections, and melanosomes were counted on electron micrographs. Immunohistochemical analysis was performed with antibodies against S100 and CD3. Positively labeled cells were counted.

Results: By light microscopy, a marked increase in the number of melanin granules was noted in the bimatoprost-treated specimens. Electron microscopy demonstrated dermal melanocytes with prominent rough endoplasmic reticulum and abundant normal-sized melanosomes in different stages of maturation as compared with control specimens. Furthermore, the keratinocytes of the bimatoprost-treated specimens showed abundant mature melanosomes when compared with controls. Also of note, atypical melanocytes were absent in both specimens. The S100-positive melanocytes were comparable in bimatoprost-treated and control specimens. Few CD3- and CD68-positive cells in the bimatoprost-treated specimens were noted in both groups.

Conclusion: Bimatoprost-induced periocular hyperpigmentation is caused by increased melanogenesis. There was no evidence of melanocyte proliferation or prostaglandin-induced inflammation in the specimens that were examined.


Prostaglandin analogues are currently the most commonly used class of medications in the treatment of glaucoma. Bimatoprost (Lumigan; Allergan Inc, Irvine, Calif) is a prostaglandin analogue in which the carboxylic acid group of prostaglandin F_2α has been substituted with a neutral ethylamide, creating a drug that is more effective than timolol in lowering intraocular pressure. Bimatoprost, like most topical prostaglandins, is associated with local adverse effects, 1 of which is hyperpigmentation of periocular skin.

METHODS

Following informed consent, biopsy specimens were taken from the lower eyelids of 2 different patients receiving bimatoprost therapy for primary open-angle glaucoma. Patient 1 was a 62-year-old white woman receiving bimatoprost in the right eye for 13 months, with hyperpigmentation clinically graded as moderate. Patient 2 was a 67-year-old white woman receiving bimatoprost in the right eye for 12 months, with hyperpigmentation clinically graded as mild. The biopsy specimens were taken from the right lower eyelids of both patients during excision of an epidermoid inclu-
sion cyst in both patients. The studied areas included a strip of hyperpigmented skin about 5 mm from the lesion. Archived eyelid biopsy specimens from age-, race-, and sex-matched controls who underwent blepharoplasty (upper eyelid in 1 patient and lower eyelid in the second patient) were selected for comparison. A deparaffinized block previously fixed in 10% buffered formalin was selected for 1 of the controls. This specimen was postfixed in 2% glutaraldehyde and embedded in epoxy resin for transmission electron microscopy. For the second control, archived formalin-fixed tissue was also postfixed in 2% glutaraldehyde and embedded in epoxy resin for transmission electron microscopy. Institutional review board approval was obtained for the analysis of these specimens.

Sections from both the patients and the controls were evaluated by light microscopy using hematoxylin-eosin staining. The presence of melanin granules was not obvious in the hematoxylin-eosin–stained sections; therefore, slides were stained with Fontana-Masson.

The number of melanin granules per high-power field (magnification ×100) was counted on Fontana-Masson–stained sections using an image analyzer (MetaMorph; Universal Imaging Corp, Westchester, Pa). Since the distribution of melanin granules was diffuse, they were counted in 4 consecutive high-power fields of the epidermis and of the dermis, starting at 1 edge of each section.

The skin specimens from the bimatoprost-treated patients and control patients were also examined by transmission electron microscopy, and areas of the epidermis and dermis were imaged. Melanocytes were identified by their dendritic processes, ovoid nuclei, and the presence of melanosomes. Melanosomes were counted in keratinocytes and dermal melanocytes using an image analyzer (MetaMorph) in 5 high-power fields in the epidermis and dermis of each patient and each control. Also in the same imaged fields, the average area of melanosomes in a single cell (both keratinocytes and dermal melanocytes) was calculated by the image analyzer. Maturation and distribution of melanosomes were also noted by electron microscopy.

To determine whether there was an increase in the number of melanocytes and/or inflammatory cells in the biopsies from bimatoprost-treated specimens as compared with controls, immunohistochemical analysis was performed using an indirect immunoperoxidase technique with antibodies against S100 (melanocyte marker), CD3 (pan–T-cell marker), and CD68 (macrophage marker) (DakoCytomation California Inc, Carpinteria, Calif). Aminoethylcarbazole was used as the chromogen. We chose to identify T lymphocytes and macrophages, as these are the predominant cells seen in the inflammatory response to UV exposure, and some of the changes noted in the bimatoprost-exposed skin showed findings similar to UV exposure. Positively stained cells were counted in 4 consecutive high-power fields at ×40 magnification that covered the entire section.

### RESULTS

By light microscopy, the epidermis and papillary dermis of the bimatoprost-treated specimens and control specimens were unremarkable. In the deep dermis, areas of loosely and densely arranged collagen were noted. There was no difference seen in the collagen arrangement in biopsies from the upper and lower eyelids in this study. No atypical melanocytes were noted in either the bimatoprost-treated specimens or the matched controls.

However, by Fontana-Masson staining, an almost 250-fold increase in melanin granule counts in the epidermis and a 6-fold increase in the dermis were noted in the bimatoprost-treated specimens as compared with the controls. There was some variability in the counts of both specimens, and no correlation could be made to clinical grading of pigmentation (Table, Figure 1A and B). The melanin granules appeared to be mainly in the basal keratinocytes of the epidermis and in the melanocytes of the superficial and deep dermis.

Transmission electron microscopy confirmed the increased number of melanosomes in the keratinocytes and dermal melanocytes seen by light microscopy. We did not observe any melanosomes in the keratinocytes of the control specimens in the grids that were examined. However, numerous melanosomes were noted in the keratinocytes of the bimatoprost-treated specimens (Table, Figure 2A and B). There was also an almost 4-fold increase in the number of melanosomes per dermal melanocyte (Table, Figure 3A and B). The keratinocytes of both the bimatoprost-treated specimens and the control specimens appeared to be of normal morphology with intact hemidesmosomes. Although melanin granules were seen by light microscopy, they were absent in the basal keratinocytes of the control specimens in the electron micrographs. A few intracytoplasmic electron-dense bodies, which were morphologically inconsistent with melanosomes, were noted in the basal keratinocytes of the control specimens (Figure 2A). The number of melanosomes in the basal keratinocytes of the bimatoprost-treated specimens was variable in density. Some keratinocytes showed abundant melanosomes whereas other keratinocytes showed scant melanosomes. The intracellular distribution patterns of the melanosomes in the keratinocytes of the bimatoprost-treated specimens varied...
from perinuclear to apical in position and from clusters of melanosomes to individual melanosomes (Figure 2B). Intralysosomal melanosomes were not seen in the keratinocytes of the specimens that were examined. Unlike the melanosomes of the basal keratinocytes that were fully melanized (Figure 2B), the melanosomes of the dermal melanocytes in the bimatoprost-treated specimens were in different stages of maturation (Figure 3B). In contrast, the dermal melanocytes of the control specimens contained fully melanized melanosomes (stage IV of melanogenesis) (Figure 3A). Also of note, the dermal melanocytes of the bimatoprost-treated specimens had prominent rough endoplasmic reticulum as compared with the controls (Figure 3A and B). The average size (cross-
sectional area in square micrometers) of melanosomes in the dermal melanocytes of the specimens from the patients was comparable to that of the controls (Table). Transmission electron microscopy confirmed the absence of atypical melanocytes in the grids that were examined. In addition, there was no change in the dendricity of the dermal melanocytes in the bimatoprost-treated specimens. A few melanocytes were seen at the dermoepidermal junction, with processes insinuating between the keratinocytes of the bimatoprost-treated specimens (Figure 3B).

The number of S100-positive melanocytes in the bimatoprost-treated specimens was comparable to that of the controls (Figure 4A and B). The dermal melanocytes appeared normal in their distribution. Although there appeared to be an increase in the number of melanocytes lining the dermoeipidermal junction in focal areas on Fontana-Masson-stained sections, this increase was not evident when melanocytes were counted on S100-stained sections. A few CD3-positive T lymphocytes and CD68-positive macrophages were noted in both groups, with great variability in the counts (Table). Intracytoplasmic melanin granules were absent in the CD68-positive cells and were only seen in the S100-positive cells, suggesting that the melanin granules were present within the melanocytes and were not engulfed by tissue macrophages.
This histopathological study of biopsies from patients with bimatoprost-induced periocular skin hyperpigmentation highlights light microscopic and ultrastructural changes in the skin. It also alludes to the possible mechanisms related to increased periocular skin pigmentation in these patients. The striking findings in the bimatoprost-treated specimens were the marked increase in melanin granules, the lack of melanocyte proliferation and melanocyte atypia, and the absence of an inflammatory reaction. These results indicate that the periocular skin hyperpigmentation seen with bimatoprost therapy is a result of increased melanogenesis.

In normal melanogenesis, an exogenous or endogenous stimulus induces the synthesis and maturation of melanosomes within the dermal melanocyte. The melanocyte then interacts with a number of neighboring keratinocytes to transfer the melanin granules to the basal keratinocytes via the dendritic tips. The melanocyte then interacts with a number of neighboring keratinocytes. In our study, the dermal melanocytes in the bimatoprost-treated specimens were marked increase in melanin granules, the lack of melanocyte proliferation and melanocyte atypia, and the absence of an inflammatory reaction. These results indicate that the periocular skin hyperpigmentation seen with bimatoprost therapy is a result of increased melanogenesis.

In normal melanogenesis, an exogenous or endogenous stimulus induces the synthesis and maturation of melanosomes within the dermal melanocyte. The melanocyte then interacts with a number of neighboring keratinocytes to transfer the melanin granules to the basal keratinocytes via the dendritic tips. In our study, the dermal melanocytes of the bimatoprost-treated specimens demonstrated an increased number of melanosomes that were in different stages of maturation. Furthermore, abundant, fully melanized melanosomes were seen in the basal layers of the epidermis in these patients. These observations indicate increased melanogenesis in the dermal melanocytes, as well as an accompanying increase in transfer to the basal keratinocytes. Interestingly, we did not observe a shift of the dermal melanocytes to the dermal-epidermal junction, which is often seen with hyperpigmentation, in the bimatoprost-treated specimens. It is possible that because the treatment is long-term, the number of melanocytes lining this junction diminished over time.

In the bimatoprost-treated specimens, melanosomes were mainly localized to the basal epidermis without a marked presence in superficial layers. A similar localization of melanin granules to the basal epidermis is seen in physiological conditions and in pigmented lesions such as ephelides. The intracellular distribution patterns of the melanosomes in the keratinocytes of the bimatoprost-treated specimens varied from perinuclear to apical in position and from clusters of melanosomes to individual melanosomes. Supranuclear clusters of melanosomes tend to predominate in normal skin of white persons whereas apical individual melanosomes are seen in normal skin of African American persons. In our white patients who received bimatoprost, the distribution pattern points toward a pattern more typical of darkly pigmented individuals. In contrast, sparse numbers of melanosomes were noted in the basal epidermis of the control specimens by light microscopy but were absent on electron microscopy. The melanosome counts within keratinocytes of white patients (with normal skin) with a category of skin pigmentation comparable to that in our bimatoprost-treated patients can normally vary from 12 to 40 melanosomes per keratinocyte when evaluated by electron microscopy. It is likely that the absence of melanosomes in our specimens on electron microscopy was a result of tissue sampling and variation in distribution. Regardless, the melanosome counts in the keratinocytes of our bimatoprost-treated specimens were 5- to 16-fold greater than that seen normally.

It is well established that persons of races with more darkly pigmented skin have larger melanosomes as compared with skin of white persons. For this reason, we chose to investigate melanosome size in bimatoprost-treated specimens and in matched controls. The size of melanosomes within keratinocytes can be variable, and an average size of 0.08 µm² has been described, which is comparable to the melanosome size noted in our bimatoprost-treated specimens. Also, the melanosome size noted in the dermal melanocytes of the bimatoprost-treated specimens was comparable to that in the control specimens that were described in normal skin by Ghadially. Therefore, it is likely that melanosome size does not play a role in the periocular hyperpigmentation induced by bimatoprost.

The histopathological findings in the skin specimens from the 2 bimatoprost-treated patients have some similarities with the changes seen in hyperpigmentation induced by UV radiation and inflammation. With UV exposure and inflammation-induced hyperpigmentation, there is increased melanogenesis, melanocyte proliferation, melanocyte dendricity, and melanocyte migration to superficial layers. Some findings in the bimatoprost-treated specimens that are similar to these conditions include the increase in melanogenesis and the increase in melanosome transfer to the basal epidermis. Also, an additional component in postinflammatory hyperpigmentation is the destruction of the basal epidermis followed by macrophage accumulation at the dermoeipidermal junction, which was absent in our patients. Irritant contact dermatitis has been suggested as a plausible mechanism for bimatoprost-induced periocular skin hyperpigmentation. Unlike irritant contact dermatitis where dermal inflammation is present, we did not observe any signs of inflammation in the dermis of our bimatoprost-treated patients.

It is well established that inflammation and exposure to UV radiation induces the release of prostaglandins, such as prostaglandin E₂ and prostaglandin F₂α, from either keratinocytes or dermal fibroblasts. These prostaglandins may act on dermal melanocytes to stimulate melanogenesis. It is possible that topical application of prostaglandins may directly stimulate dermal melanocytes through interaction with prostaglandin receptors, such as FP, EP1, and EP3, to induce melanogenesis. Our findings in the bimatoprost-treated specimens support this hypothesis.

Increased melanogenesis in the dermal melanocytes of periocular skin in our bimatoprost-treated patients bears some resemblance to the increased melanogenesis in iridial melanocytes seen in patients receiving long-term prostaglandin therapy. However, iridial melanocytes are different from dermal melanocytes in that they are “continent” and, therefore, retain their melanosomes. On the other hand, dermal melanocytes are “incontinent,” and they discharge their melanosomes into the epidermis, which accounts for the differences in the anatomical distribution of pigment in the skin and the iris.

Although some studies have shown that prostaglandins may lead to an increase in melanosomes in mouse
dermis and human iris as well as an increase in atypia of human iris melanocytes.\textsuperscript{21-23} It was encouraging to note the lack of melanocyte proliferation or atypia in our biomatoproct-treated specimens. Similarly, a lack of proliferation of iris melanocytes has been noted following in vitro\textsuperscript{21,24} and in vivo\textsuperscript{25,26} exposure to latanoprost (Xalatan, Pfizer Inc, New York, NY).

Periocular skin hyperpigmentation is a recognized adverse effect of prostaglandin analogues.\textsuperscript{3-6} In the Physiciss' Desk Reference, periocular pigmentation was described in 3\% to 10\% of patients enrolled in clinical trials of bimatoprost therapy.\textsuperscript{3} Although the Physicians' Desk Reference mentions that periocular pigmentation is seen with travoprost (Travatan; Alcon Laboratories Inc, Fort Worth, Tex) and latanoprost, it does not include the incidence rates. It is likely that factors other than drug exposure may determine the degree of skin pigmentation. These factors include UV exposure, genetic factors, hormones,\textsuperscript{27} inflammation,\textsuperscript{15} chemical exposure,\textsuperscript{28} drugs,\textsuperscript{28} pathological diseases associated with hyperpigmentation,\textsuperscript{28} and the "chromatic tendency" of a person.\textsuperscript{28} Another factor that might contribute to periocular pigmentation is excessive or improper application techniques, leading to frequent contact of the topical prostaglandin analogues with periocular skin.

In summary, this histopathological study demonstrates that the bimatoprost-induced periocular skin hyperpigmentation occurs from increased melanogenesis and increased transfer of melanosomes to basal keratinocytes, with the absence of melanocyte proliferation and melanocyte atypia. Some of the histopathological findings indicate that the mechanisms involved in bimatprost-induced skin hyperpigmentation have some similarities to hyperpigmentation induced by UV exposure or inflammation.

Submitted for Publication: August 27, 2004; final revision received January 13, 2005; accepted January 23, 2005.

Correspondence: Deepak P. Edward, MD, Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago, 1855 W Taylor St, M/C 648, Chicago, IL 60612 (deepedwa@uic.edu).

Financial Disclosure: None.

Funding/Support: This study was supported in part by Core Grant for Vision Research EY01792 from the National Eye Institute, Bethesda, Md, an unrestricted grant from Research to Prevent Blindness Inc, New York, NY, and a gift from Mrs Doris Semler.

REFERENCES