Histological Effects in the Iris After 3 Months of Latanoprost Therapy

The Mainz 1 Study

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Objective: To determine whether 3 months of topical latanoprost treatment caused proliferative or degenerative effects on the peripheral iris of patients with glaucoma.

Methods: Seventeen patients requiring filtering surgery for primary open-angle glaucoma or pseudoexfoliation glaucoma were randomized to receive topical latanoprost for 3 months (n=8) or alternative medication (n=9) before surgery. A trabeculectomy and a peripheral iridectomy specimen was obtained from each patient during surgery. The tissue was subjected to histological and immunohistochemical evaluation using 2 cell cycle markers: proliferating cell nuclear antigen and nuclear-associated protein (Ki-67).

Results: No degenerative or pathological changes were seen in the latanoprost-treated irides, including the one specimen in this series in which there was an eye color change. Proliferating cell nuclear antigen and nuclear-associated protein markers were negative for changes in all the test specimens.

Conclusion: Short-term treatment with latanoprost does not produce morphological changes or cellular proliferation changes in the iris.


Latanoprost is an analogue of the prostaglandin F₂α and, because it is an effective intraocular pressure (IOP)–lowering agent,¹⁻³ it is being used increasingly in the management of glaucoma. The one major ocular adverse effect of latanoprost is that it can cause darkening of the iris. During 1 year of treatment, about 20% of eyes treated with latanoprost darken to a noticeable extent. Uniformly brown and blue irides darken rarely, but hazel eyes darken frequently.²⁻⁴ The mechanism by which the color change is brought about is obscure. There is, to our knowledge, only one published article⁵ on the morphological features of a human iris treated with latanoprost, and this is a case report of a patient with drug-induced iris darkening. Monkeys undergoing long-term treatment with topical naturally occurring prostaglandins also show iris color change, so it does seem to be the case that iris darkening is a more general effect of some prostaglandins rather than being a specific property of the analogue latanoprost.⁶ Among the possible mechanisms by which color change could be brought about include hyperplasia of stromal melanocytes or stimulation of melanogenesis. Overproduction of melanin granules could result in their release into the stroma with consequent iritis⁷ and tissue degeneration or, if they accumulated in the chamber angle, glaucoma might ensue.⁸ Proliferation of stromal melanocytes, if it occurred, would be a particularly worrying adverse effect.

The present single-masked study was undertaken to compare peripheral iridectomy specimens taken from patients who had vs those who had not been treated with latanoprost for a 3-month period before glaucoma surgery. The histological features of the iris were evaluated, looking for degenerative changes in the tissue. However, the main thrust of the investigation was an attempt to identify cells by immunohistochemistry, using antibodies against proliferating cell nuclear antigen (PCNA) and nuclear-associated protein (Ki-67), which were in or had been in the cell cycle. In this way, we wanted to find evidence to support or refute the option that latanoprost might provoke cell proliferation in iris tissue.
PATIENTS AND METHODS

We performed a randomized, single-masked study and recruited 20 patients aged 40 years or older scheduled for routine trabeculectomy in one eye because of uncontrolled IOP caused by primary open-angle glaucoma or pseudoexfoliation glaucoma. Exclusion criteria included laser trabeculoplasty within 3 months before the study, signs of ocular infection, inflammation, and use of contact lenses. Eye color was classified as brown, blue, green, gray, or hazel. The first 4 were uniform coloring, while the hazel was brown mixed with 1 of the other 3.

Patients gave written informed consent. Then the patients were allocated to receive, in addition to their medical regimen, either approximately 20 µL of topical latanoprost, 30 µg/mL, once each evening or an alternative IOP-lowering agent (acetazolamide or α₂-agonist clonidine). The allocation of patients to treatment was at random, but an attempt was made to proportion between the 2 groups based on the color classification (Table 1). The treatment was restricted to a 3-month period, which is the usual waiting period for filtering surgery at Mainz University, Mainz, Germany. The patients and the consulting ophthalmologists (N.P., D.H., and A.W.-B.) were not masked to their treatment during the waiting period. All patients underwent measurements of visual acuity, biomicroscopy, ophthalmoscopy, and Goldmann applanation tonometry at baseline, at 2 weeks and 3 months before the day of surgery, and at 6 weeks after surgery. Iris and gonioscopic photography was done at baseline and at 3 months. The study was evaluated and approved by the Ethical Review Committee of Rheinland Pfalz.

A fornix-based trabeculectomy without antimetabolites was performed by one of us (N.P.) at the 12-o’clock position, rendering a 1.5-mm square trabeculectomy and a peripheral iridectomy specimen.9 The specimens were placed in a coded bottle on a support of filter paper and fixed with 10% buffered formaldehyde solution.

LABORATORY PROCESSING AND HISTOLOGICAL FEATURES

Paraffin sections (3-4 µm thick) placed on 3-amino propyl triethoxysilane–coated slides were stained with hematoxylin-eosin, Masson trichrome, and periodic acid–Schiff. The histological sections were examined with the light microscope each of the sections available for the following features: (1) posterior epithelial degeneration, (2) stromal inflammation, (3) vascular alterations, (4) clump cell frequency, (5) presence of free stromal melanin, (6) stromal degeneration, (7) any atypical cellular features in stromal melanocytes, and (8) thickness of the anterior border layer. These were features highlighted as being of some interest based on the findings of a previous case report.6 The following grading system was used throughout the study: − indicates absent, +/−, marginal; +, minimal; +++, moderate; and ++++, marked. The grading was found to be consistent from section to section for a given specimen, reproducible on reanalysis, and comparable on interobserver checks. Throughout the study, specimens were coded so that it was not known by the sectioner or the evaluators (I.G., H.G., and P.A.) which were specimens from latanoprost-treated eyes.

IMMUNOHISTOCHEMISTRY

Wax sections were deparaffinized in xylene and rehydrated in graded ethanol. The sections available for each specimen were microwaved under citrate buffer at pH 6.0. Positive controls (tissue with cells known to be in the cell cycle) were human tonsil and developmental human eye tissue. The negative control was adult retina. The ocular material consisted of eyes ranging from 10 weeks of development to 9 months postnatal (n=11) and adult human eyes of various eye color (n=10). Following antigen retrieval, endogenous peroxidase was blocked with a 0.3% solution of hydrogen peroxide for 20 minutes. Background staining was eliminated by exposure to normal goat serum for a further 20 minutes. The primary antibodies used for this study were against antigens connected with the cell cycle, PCNA and Ki-67. In the study, the antibody against PCNA was an IgG2a mouse monoclonal antibody (DAKO A/S, Glostrup, Denmark) and that against Ki-67 was MIB-1, an IgG1 mouse monoclonal antibody (DAKO A/S). Both were applied at dilutions of 1:100 to sections for 16 hours at 4°C. After washing, the secondary biotinylated antibody was applied for 2 hours; then the sections were exposed to peroxidase-conjugated streptavidin (both from DAKO A/S). Positive reactive sites were revealed using on occasion 3,3′-diaminobenzidine, which stains brown, or 3-amino-9-ethylcarbazole, which produces a red color (Sigma-Aldrich Corp, St Louis, Mo).10,11

MICROSCOPY

At least 3 different levels of tissue of each specimen were examined by conventional light microscopy and differential interference contrast optics (Polyvar, Reichert, Vienna, Austria).

RESULTS

PATIENTS

We recruited 20 patients for the study. Twelve were women, and all were white and had either primary open-angle glaucoma or pseudoexfoliation glaucoma (Table 1). Seventeen patients completed the study according to protocol. Of the 3 who withdrew from the study, 1 (from the nonlatanoprost treatment group) underwent the surgery at his home hospital after 2 months. Of the 2 other latanoprost-treated patients, 1 declined surgery and the other had well-controlled IOPs with latanoprost treatment, so surgery was no longer considered necessary.

At baseline, the mean IOP in the latanoprost-treated eyes was 23.4±5.2 mm Hg; it was 24.5±4.4 mm Hg in the nonlatanoprost-treated eyes. At 3 months, the mean IOPs were 18.2±3.9 mm Hg in the latanoprost group and 23.2±5.2 mm Hg in the controls. (Data are given as mean±SD.)

Only one 54-year-old patient (patient A6) with hazel eyes from the latanoprost group underwent an eye...
color change, appreciable in Figure 1A-B. The iridectomy specimen from this eye was also taken from the 12-o’clock position.

**HISTOLOGICAL FEATURES OF IRIDECTOMY SPECIMENS**

The specimens were examined for the presence of any histological abnormalities, and there was no evidence in any of our specimens of posterior epithelial degeneration (Figures 2, 3, and 4) other than traumatic disruption from handling (Figure 4B). The stroma of all specimens exhibited no signs of inflammation, vascular alterations, or any degeneration that could be appreciated by light microscopy (Figures 2-4). Free stromal melanin could not be seen easily by conventional optics; however, by differential interference contrast optics, free melanin could be appreciated more readily but was not abundant in any sample examined (Figures 3B and 4). Clump cells also were identified more readily using differential interference contrast optics. Thickness of the anterior border could be appreciated in this mode (Figures 2B, 3B, and 4A). The anterior border histological features of 1 specimen were difficult to assess because of the presence of an ephelis (Figure 2), which was noted as an anterior border area of marginally increased cellularity but clearly increased intracellular pigmentation.

**Table 2** shows the grading of the specimens (performed by the masked observer [I.G.]) for the various histological features. Posterior epithelial changes, inflammation, vascular alteration, and stromal degeneration were not recorded in any of the 17 iridectomy specimens in the series. Clump cells were just as common in the nonlatanoprost-treated irides as they were in latanoprost-treated specimens; the 2 specimens with the highest scores (specimens A7 and A9) were both untreated. Free melanin granules were found sparingly in the stroma of most irides. Melanocytes with nuclear features that were atypical (nuclear chromatin margination, prominent nucleoli, and invagination of the nucleus) were rare, and when identified they usually did not have all the atypical characteristics; these cells were called borderline atypical. Melanocytes with atypical or borderline atypical nuclei were present in 6 of the 9 untreated specimens compared with 5 of the 8 latanoprost-treated irides. The incidence would seem to be the same, but the only 2 specimens that were graded + were latanoprost treated. Anterior border thickness was above marginal in 5 of the 9 untreated specimens and in 5 of the 8 latanoprost-treated specimens. Only one iris had a moderate (grade ++) border; it was the one mentioned earlier that had an ephelis (Figure 2) and was identified as being in the nonlatanoprost group (specimen A16) (Table 2).

**IMMUNOHISTOCHEMISTRY OF CONTROLS AND IRIDECTOMY SPECIMENS**

Human tonsil as a positive control showed intense staining for PCNA and Ki-67. Adult human eye showed labeling for both antigens in some conjunctival epithelial nuclei and in some nuclei at the lens bow but nowhere else in the eye. In the developing eye, nuclei that labeled positively with Ki-67 and PCNA were identified in the immature stromal tissue. At 6 months following birth, and in all the control adult eye specimens, no labeling was ever found. In contrast, despite extensive examina-

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**Table 1. Patient Details**

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<th>Variable</th>
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<th>Patients Treated With an Agent Other Than Latanoprost</th>
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<td>Hazel</td>
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**Figure 1.** Pictures of one patient (patient A6) taking latanoprost, with iris darkening at baseline (A) and at 3 months (B).
tion of different levels in each specimen with the 2 antibodies, neither the latanoprost-treated nor the untreated irides of the test studies exhibited any positive staining (Figures 2B, 3B, and 4). In these staining runs, human tonsil and human fetal iris always exhibited numerous positively staining nuclei.

COMMENT

Latanoprost is one of the latest additions to the treatment of glaucoma. Since its introduction, it has received considerable attention and its use is widespread; it is an attractive antiglaucoma medication principally...
because of its relatively powerful IOP-lowering action, its ease of use (once-daily treatment), and the apparent absence of any systemic adverse effects. There are, however, local adverse effects, of which darkening of the iris is the most obvious and spectacular. The present study sought to (a) tackle the question of whether topical application of latanoprost had a deleterious effect on iris tissue and (b) gain some further insight into the mechanism by which prostaglandins bring about darkening of eye color. In this respect, we have valuable initial information on the former but provide little to resolve the problem associated with the latter.

At the level of light microscopy, our findings show that a short period of 3 months of exposure to latanoprost did not produce either observable degeneration in the structure of the iris or inflammation. Melanin granules are thought to be cytotoxic, and ocular melanin, including that from the iris, is known to be uveitogenic. As a consequence, some inflammatory pathological features might have been expected if there had been an accumulation of melanin granules within the iris stromal melanocytes leading to rupture and scattering of the granules in the tissues, but there were no indications that melanin release was happening in latanoprost-treated irides. Only one of the latanoprost-treated eyes actually darkened in color. The morphological analysis did not show any remarkable change in melanocyte pigmentation in the iris specimen from this patient nor did it pick up any extensive release of free melanin into the stroma. To some extent, it would have been surprising if such findings had been evident. Monkeys that underwent iris color change after being treated with latanoprost for 1 year did show some accumulation of mature melanin granules within stromal melanocytes, but this could be detected only after careful and exacting image analysis. In addition, comparison of melanocytes from the extremes of blue to brown in normal irides showed that not much of a difference in melanin content was needed in the melanocytes to account for the color difference.

The clump cell frequency was assessed in this study because this local macrophage cell is associated with melanin granule incorporation. If there was or had been free melanin in the stroma, the clump cells might have been expected to be abundant. As it turned out, there was no difference between latanoprost-treated and untreated eyes, and the 2 specimens with the highest incidence of these cells turned out to be from untreated patients.

The most important observation from the present study was the finding that neither the latanoprost-treated (including the specimen from a patient with an eye color change) nor the untreated iridectomy specimens showed any positive staining nuclei by immunohistochemistry (melanocytes or other cells in the iris) using antibodies against the cell cycle antigens PCNA and Ki-67. These methods may also pick up a few cells that have recently left the cycle. However, for the present study, the advantage gained by high sensitivity with PCNA and Ki-67 offsets their poor specificity.

Our finding in the present study of an absence of cell division in latanoprost-treated human irides is in keeping with the findings in latanoprost-treated monkeys, which showed no increase in the numbers of iris stromal melanocytes after 1 year. Nuclear activation in the form of nuclear atypia was noted in a previously described patient who had darkening of the iris while enrolled in the latanoprost phase 3 trial. In addition, the patient had a thickened anterior border layer, although this was in association with a nevus. A pronounced anterior border layer was associated also with the untreated patient in this series who had an epheles or nevus in the iridectomy specimen. No relation with latanoprost treatment and thickness of the anterior border layer was found in our histological investigation.

Atypia was noted in the specimen with the epheles or nevus but was marginal. After close histological examination of many brown irides, including those that served as controls in this experiment, a few melanocytes could be recognized that have features, like margination of nuclear chromatin and nuclear invagination, in common with nuclear atypia. Some incidence of atypia was found in most specimens in the present series, but usually only at the marginal level, and it was just as common in untreated as it was in latanoprost-treated tissues. In primary acquired melanosis, atypia is associated with marked labeling for the PCNA and Ki-67 antigens. In these circumstances, it seems to be an entirely different entity than what we are seeing in the present study and what was seen in a previous case report. On the other hand, the only 2 specimens that recorded a grade of + were both latanoprost treated, and one of these was from the patient whose eye color darkened during the study. We are, however, dealing with small differences in minimal numbers, so we are a long way from demonstrating that atypia increases with latanoprost treatment; the finding may well be spurious, especially since the ratio of positives to negatives for the presence of atypia in the treated and untreated groups was much the same.

In conclusion, the present study shows that a 3-month treatment with latanoprost does not produce...
morphological changes in the iris and does not push iris cells into the cell cycle. The reservations should be emphasised that only 1 of our series of latanoprost-treated patients underwent a clinically noticeable eye color change and our morphological investigations were restricted to a small segment of peripheral iris.

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REFERENCES