Intraoperative Floppy Iris Syndrome

Report of a Case and Histopathologic Analysis

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Objective: To understand the role of the $\alpha_{1A}$-adrenoceptors (ARs) in the pathophysiologic mechanism of intraoperative floppy iris syndrome (IFIS).

Methods: Iris specimens from a patient with tamsulosin hydrochloride–induced IFIS were obtained during trabeculectomy. Specimens underwent histological analysis and immunohistochemical analysis with antibodies specific for actin, myoglobin, $\alpha_{1A}$-ARs, and myosin. Iris specimens from a patient without IFIS were used for comparison. Samples were processed for transmission electron microscopy.

Results: Histological examination showed normal dilator muscle, arterioles, stroma, and pigment epithelium. Actin, myosin, and myoglobin distribution and intensities were similar between IFIS and non-IFIS tissue. The staining pattern and colocalization with myosin suggested that $\alpha_{1A}$-ARs are present in iris arteriolar muscularis in addition to the dilator muscle in both IFIS and control irides. Significantly less staining of IFIS tissue was found compared with the non-IFIS iris. Ultrastructures of melanocytes and stroma appeared to be normal. Iris arterioles possessed thick endothelial basement membranes, semilongitudinally oriented muscularis, and abundant perivascular collagen coats.

Conclusions: We confirm the presence of $\alpha_{1A}$-ARs in human iris by results of immunohistochemical analysis. The $\alpha_{1A}$-ARs localize to iris arteriolar muscularis in addition to the iris dilator muscle. This localization suggests that IFIS may develop because of iris vascular dysfunction and that iris vasculature may have structural in addition to nutritive functions.


INTRAOPERATIVE FLOPPY IRIS SYNDROME (IFIS) is a well-known risk factor for complications during cataract extraction. It was first described in 2005 by Chang and Campbell and is characterized by the triad of iris billowing, iris prolapse through the surgical wound, and progressive miosis. Its association with tamsulosin hydrochloride, a selective $\alpha_{1A}$-adrenoreceptor (AR) antagonist approved in the United States for the treatment of benign prostatic hyperplasia, has been widely reported. $^{1-4}$ Other $\alpha_{1}$-AR blockers have also been implicated in the pathogenesis of IFIS, albeit to a lesser extent. $^{5-8}$ There are at least 3 human subtypes of $\alpha_{1}$-AR: $\alpha_{1A}$, $\alpha_{1B}$, and $\alpha_{1D}$. $^{9}$ Of these, 2 are present in the iris. Molecular, protein, and functional assays have confirmed the presence of $\alpha_{1A}$-ARs in the iris of most species, including humans, and $\alpha_{1B}$-ARs in the iris of rats. $^{10}$ It is believed that $\alpha_{1A}$-ARs are primarily (but not exclusively) present in the iris dilator muscle, and $\alpha_{1B}$-ARs are present in the iris arterioles. $^{10}$ Because tamsulosin is a selective $\alpha_{1A}$-AR antagonist and because of the localization of the $\alpha_{1A}$-ARs, they have been specifically implicated in the mechanism of IFIS. $^{1}$ Although most of the ocular surgical complications associated with IFIS have been described during cataract extraction, in which the presence of a well-dilated pupil is important, IFIS has also recently been reported during trabeculectomy. $^{11}$ We herein report another case of IFIS that occurred during trabeculectomy. The iris specimen obtained during the procedure was used to perform histopathologic studies in an effort to understand the pathophysiology of this condition.

REPORT OF A CASE

An 83-year-old Hispanic man with very advanced open-angle glaucoma and mild cataracts demonstrated progressive visual field changes in the left eye. Intraocular pressure ranged from 13 to 22 mm Hg while he received topical therapy with timolol maleate, 0.5%, twice daily and latanoprost once daily at bedtime. The patient had previously developed an allergic reaction to treatment with topical carbonic anhydrase inhibitors and $\beta_{2}$-adrenergic agonists. His medical history was significant for chronic heart failure that resulted in aortic valve replacement and automatic implantable car-

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dioverter/defibrillator placement, type 2 diabetes mellitus, and hypothyroidism. He also had benign prostatic hypertrophy that had been treated with tamsulosin hydrochloride, 0.4 mg/d, for at least 1 year. Other systemic medications included amiodarone hydrochloride, hydrochlorothiazide, allopurinol sodium, glipizide, lisinopril, and metoprolol tartrate. To better control intraocular pressure and prevent further deterioration of the visual field, surgical intervention was elected.

Trabeculectomy with a fornix-based conjunctival flap and adjunctive mitomycin administration was performed in the superonasal quadrant. A square 3 × 3-mm scleral flap was raised at the limbus. A paracentesis was performed temporally, and a viscoelastic solution (hyaluronic acid) was injected in the superior part of the anterior chamber. The anterior chamber was then entered at the base of the scleral flap with a 15° supersharpe blade. The Kelly punch was used to remove a block of trabecular meshwork. Immediately after, the iris was noted to prolapse through the sclerotomy. A broad iridectomy was performed using scissors, and the specimen was placed in 10% formalin. The iris was noted to be floppy and continued to prolapse through the sclerotomy despite repeated attempts to reposition it into the anterior chamber through the sclerotomy using viscoelastic solution. Repositioning of the iris in the anterior chamber required the use of an iris spatula through the side port incision and injection of viscoelastic solution immediately above the iridotomy.

In the postoperative period, the iris remained atonic but with no further evidence of prolapse (Figure 1). The patient formed a diffuse bleb, and intraocular pressure remained well controlled, ranging from 6 to 10 mm Hg without any topical medications.

### METHODS

**HISTOLOGICAL ANALYSIS**

The formalin-fixed tissue was dehydrated through graded alcohols and embedded in paraffin. Sections 4 µm thick were obtained and stained with hematoxylin-eosin, trichrome, and periodic acid–Schiff. Sections from an iris specimen obtained during trabeculectomy from a patient without IFIS were also stained for comparison purposes.

**IMMUNOHISTOCHEMICAL ANALYSIS**

To detect differences in staining of iris tissue in the presence and absence of IFIS, sections from the patient with IFIS were compared with those from a patient who had undergone trabeculectomy 1 week earlier. That patient had no clinical IFIS and had never been treated with any α1-AR antagonists. Sections from both specimens were processed together. They were initially deparaffinized and digested with proteinase K (0.03% in distilled water for 20 minutes). They were then treated with blocking solution for 1 hour (SuperBlock; Thermo Scientific, Rockford, Illinois) and incubated overnight at 4°C in a humid chamber with the primary antibodies. Primary antibodies used included anti-α1A-AR (goat antihuman, 1:100 dilution; Santa Cruz Biotechnology, Santa Cruz, California), anti-smooth muscle actin (1:80 dilution; Dako, Carpinteria, California), anti-smooth muscle myosin (1:50 di-
lution; Biogenex Laboratories, San Ramon, California), and antimony globulin (1:4000 dilution; Dako). After incubation with the primary antibody, the sections were washed with Tris-buffered saline (pH 7.4) and incubated with the appropriate Alexa Fluor–labeled secondary antibody for 20 minutes (1:400 dilution; Invitrogen, Carlsbad, California). Sections were counterstained with 4′,6-diamidino-2-phenylindole, placed under a coverslip, and observed with an epifluorescent microscope (Axioskop; Carl Zeiss Meditec, Inc, Dublin, California) with the appropriate excitation filters. Some of the sections were subjected to double immunolabeling for \( ^{1}A\)-ARs and myosin using a similar protocol to the one described.

TRANSMISSION ELECTRON MICROSCOPY

Transmission electron microscopy was performed as described elsewhere. Briefly, after fixation with glutaraldehyde and osmication, the specimen was dehydrated through a series of graded ethanol to propylene oxide and embedded in epoxy resin (Epon; Miller-Stephenson Chemical Company, Inc, SYlmars, California). Thin sections were stained with uranyl acetate and lead citrate and observed with a transmission electron microscope.

RESULTS

Light microscopy of hematoxylin-eosin–stained peripheral iris showed normal anatomy with normal-appearing dilator muscle fibers, iris arterioles, stroma, and pigment epithelium. Periodic acid–Schiff and trichrome staining revealed normal anatomy. Actin, myosin, and myoglobin distribution and intensities as detected by immunohistochemistry were normal (Figure 2A–C, respectively).

Immunoreactivity of \( ^{1}A\)-AR was detected in IFIS (Figure 3A) and control (Figure 3B) irides. Strong immunoreactivity was detected around the arteriolar lumen of non-IFIS tissue (Figure 3B). In comparison, the intensity of staining around the iris vasculature was markedly decreased in iris tissue from the patient with IFIS (Figure 3A). The staining pattern suggested that \( ^{1}A\)-ARs are present on the iris arteriolar smooth muscle cells. Immunoreactivity of \( ^{1}A\)-ARs was also present in the dilator muscle, but no obvious difference could be seen between staining in IFIS-affected and -nonaffected tissue.

Colocalization of myosin (Figure 4A) with \( ^{1}A\)-AR staining (Figure 4B) confirmed the presence of these receptors on smooth muscle cells of iris arterioles (Figure 4C).

Intraoperative floppy iris syndrome occurs in approximately 2% of patients undergoing cataract extraction and can make this surgery difficult. Herein we report a case of IFIS in which the floppy iris made glaucoma surgery challenging. This case also provided us with a unique opportunity to examine the iris of a human subject histopathologically in an attempt to understand how IFIS develops. The rarity with which such tissue samples become available makes this specimen very valuable. We thus used different, complementary methods (histology, immunohistochemistry, immunofluorescence, and transmission electron microscopy) in an effort to elucidate the pathophysiologic mechanism of IFIS. Although IFIS has been previously reported as a result of medications other than tamsulosin and of conditions not asso-

Figure 4. Immunofluorescence staining in the iris of the patient with intraoperative floppy iris syndrome. Images of staining for myosin (A) and \( ^{1}A\)-adrenoreceptors (ARs) (B) are then merged (C). Colocalization of myosin and \( ^{1}A\)-ARs on the iris arteriolar wall is evident (arrows). Scale bar represents 50 µm.

Figure 5. Transmission electron microscopy image of the iris with intraoperative floppy iris syndrome shows morphologically normal arterioles (arrow) and surrounding stroma (original magnification ×1500).

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associated with any medication, its strong association with tamsulosin\textsuperscript{14} and the particular use of tamsulosin make it the most likely cause of IFIS in this patient.

It has been previously suggested that IFIS is caused by blockade of the $\alpha_{1A}$-ARs at the level of the iris dilator muscle.\textsuperscript{1,5} However, even in the original report on IFIS,\textsuperscript{1} it was pointed out that discontinuation of the $\alpha_{1A}$-AR antagonist therapy preoperatively (sometimes for periods of $\leq 1$ year) does not necessarily eliminate its occurrence but solely decreases intraoperative miosis. Although disuse atrophy caused by tamsulosin has been reported in IFIS\textsuperscript{5} and could explain the occurrence of IFIS even years after discontinuation of therapy, the presence of such extensive atrophy has not been confirmed. In fact, in the case reported herein, the morphological features of the iris appear to be normal at the microscopic and ultramicroscopic levels.

We confirmed the presence of $\alpha_{1A}$-ARs in the human iris immunohistochemically. However, unlike previous reports,\textsuperscript{16-18} $\alpha_{1A}$-ARs were localized in the muscularis of iris arterioles in addition to their presence in the iris dilator muscle. The antibody we used for immunolocalization of the $\alpha_{1A}$-AR is specific for the human $\alpha_{1A}$-ARs and, although the specificity of many antibodies for the mouse $\alpha_{1A}$-ARs has been challenged,\textsuperscript{18} it is unlikely that in our study the antibody is staining other subtypes of $\alpha_{1A}$-ARs because these are not affected in tamsulosin-caused IFIS. The difference in localization from previous studies may be accounted for by species difference because previous studies have been performed on pigs, rabbits,\textsuperscript{18} and rats.\textsuperscript{16} The presence of $\alpha_{1A}$-ARs on iris vessels may thus provide an alternative explanation for IFIS development.

We propose the hypothesis that treatment with tamsulosin leads to iris vascular dysfunction. It is our contention that the iris vasculature serves a structural function for the iris. Iris vessels form the web on which the iris stroma is supported and have a tremendous ability to coil and uncoil to allow for iris dilation. Once they lose this ability and become flaccid, iris dilation would not only be decreased but the iris arterioles cannot coil. Although the idea that vascular dysfunction may contribute to the development of IFIS has been raised in the past because of the poor dilation often seen in patients with diabetes,\textsuperscript{26} our finding of $\alpha_{1A}$-ARs in the iris arteriolar muscularis provides significant support for this hypothesis. It may also explain the observation that intraocular epinephrine partially reverses IFIS in certain cases.\textsuperscript{15,21,22} Presumably by increasing the contraction of the dilator muscle and the muscularis to allow for arteriolar coiling. The presence of reduced AR staining in IFIS is highly suggestive and can explain acute as well as prolonged IFIS formation after tamsulosin treatment. Iris arterioles also have $\alpha_{1A}$-ARs, the role of which in arteriolar contraction has been previously investigated.\textsuperscript{23} In contrast, the role of $\alpha_{1A}$-ARs has not been well studied outside of their effect on dilation despite their documented presence in the iris.\textsuperscript{10}

Atrophy and vacuolation of the iris dilator muscle and elimination of the associated nerve endings, along with differences in the distribution and size of pigment granules and the presence of lipofuscin-like granules, have also been reported in IFIS.\textsuperscript{11} Based on these findings, a mechanism for the development of IFIS has been proposed that involves the atrophy of nerve terminals to the dilator smooth muscle and the absorption and rerelease of $\alpha_{1A}$-AR agonists by melanin-containing granules. However, some of these changes may represent changes induced by fixation or tissue processing and need to be viewed as potential artifacts because they are noted in tissue from patients with and without IFIS. In addition, it is unlikely that concentration of $\alpha_{1A}$-AR blockers by melanin (which has not been shown for any AR blocker) could prolong their effect more than a few days.\textsuperscript{24} In the case reported herein, no discernible differences in size or distribution of pigment granules or any smooth muscle vacuolation were detected between IFIS and non-IFIS tissue.

Independent works by Prata and Ritch\textsuperscript{23} and Santella\textsuperscript{26} suggest that thinning of the iris stroma and dilator muscle is a mechanism that contributes to IFIS. The hypothesis put forth previously of vascular dysfunction is not necessarily contradictory to such a mechanism because the malfunctioning vasculature could lead to atrophy of these tissues. Moreover, the pharmacological blockade of both the arterial wall musculature and the iris dilator muscle could be required for IFIS to develop. More research is therefore needed before the mechanism for IFIS development is completely understood. Given the lack of a good understanding of the pathophysiologic process of this condition, we believe that any data pointing to new directions should be thoroughly examined.

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