Cone Damage in Patients Receiving High-Dose Irofulven Treatment

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Objectives: To describe the clinical, perimetric, and electroretinographic (ERG) results of 4 patients with cone dysfunction following irofulven treatment including the histopathologic and immunocytochemical features of one patient’s retinas.

Design: Observational case series.

Methods: The patients were examined clinically, including perimetric and ERG evaluations. Eyes from patient 1 and healthy postmortem eyes were processed for histopathologic and immunocytochemistry studies with antibodies specific for cones, rods, and reactive Müller cells.

Main Outcome Measures: Clinical signs and symptoms, perimetry, ERG, retinal histopathologic and immunocytochemistry study results.

Results: All 4 patients had ERG changes consistent with abnormal cone responses and relatively normal rod responses. Compared with control eyes, the retina of patient 1 had approximately half the normal numbers of macular cones and fewer peripheral cones. The number of rods were normal but all rod and cone outer segments were shortened.

Conclusion: High-dose irofulven treatment causes cone-specific damage with relative sparing of rods.

Arch Ophthalmol. 2005;123:29-34

A RETINAL TOXIC REACTION may result during treatment of cancer. Cisplatin treatment can cause abnormal cone or cone and rod ERG responses, transient cortical blindness, papilledema, and retrobulbar neuritis. High-dose carboplatin therapy can produce pigmentary maculopathy, optic neuropathy, chorioretinitis, and transient cortical blindness. Paclitaxel, often combined with carboplatin therapy, can cause photopsias and transient scotomas. Better therapies are needed for recurrent ovarian cancer. The goal is to maximize benefit and simultaneously minimize toxic effects, since therapy for recurrent disease is mainly palliative. New drugs require particular scrutiny for new or unusual toxic reactions such as retinal damage.

Irofulven (6-hydroxymethylacylfulvene, MGI 114; MGI Pharma, Inc, Bloomington, Minn), an illudin S derivative from the jack-o-lantern mushroom (Omphalotus illudens), is an investigational anticancer agent. Irofulven rapidly inhibits DNA synthesis and blocks mitosis. It causes DNA breaks and cell death by caspase-mediated apoptosis. Irofulven has reversible cytostatic activity in normal cells but cytotoxic activity in tumor cells.

Abnormal color vision and contrast were reported after biweekly irofulven treatment in phase 1 through 3 trials. Patients were initially dosed at 24 mg/m² every 2 weeks. Visual abnormalities occurred most frequently in individuals treated with doses exceeding 0.55 mg/kg, leading to a dosing modification by body weight to less than 0.55 mg/kg biweekly (total-dose limit, 50 mg).

Before this regimen change, our patients (4 of 18 enrolled at Massachusetts General Hospital, Boston) were enrolled in a phase 2 multicenter trial of irofulven for treatment of advanced epithelial ovarian cancer. All of our patients had progressive disease despite prior chemotherapy including carboplatin. After receiving high doses of irofulven treatment, clinical signs and symptoms of retinal cone dysfunction developed in these patients. Histopathologic and immunocytochemistry studies of one patient’s postmortem retinas revealed marked cone photoreceptor pathologic features.
REPORT OF CASES

CASE 1

A 79-year-old woman had stage III peritoneal papillary serous adenocarcinoma unamenable to surgery that continued to progress despite multiple regimens of chemotherapy including paclitaxel and carboplatin, oral etoposide, and intravenous topotecan, oral anastrozole, and liposomal doxorubicin. On July 13, 2001, she enrolled in a phase 2 irofulven trial and received a single dose of this drug (0.6 mg/kg). Four days later, she noted significant glare and "misty" vision. Two weeks later, she reported intermittent photopsias. Ocular history included best-corrected visual acuity (VA) of 20/30 OU and cataracts. On August 4, 2001, VA was 20/30 OU. With both eyes she identified 3 of 10 Ishihara color plates. Goldmann visual fields (GVFs) revealed dense paracentral and midperipheral scotomas (Figure 1A). Fundus examination showed peripapillary pigmentary changes but the macula and optic nerve appeared normal in both eyes. Dim scotopic electroretinographic (ERG) b-wave amplitudes were within the normal range for our laboratory (Figure 2). Bright scotopic b-wave amplitudes were in the low normal range. Bright photopic b-wave amplitudes were nonrecordable to a single flash and 30-Hz flicker amplitudes were low with prolonged implicit times. Her serum sample lacked antirecoverin antibodies (Charles Thirkill, PhD, written communication, August 2001).

Eight weeks after receiving the single dose of irofulven treatment, the patient received 2 infusions of carboplatin. In September 2001, VA was 20/30 OD and 20/25 OS. Color vision was normal. The GVF's improved in size with fewer scotomas in the left eye (Figure 1B). The results of the remainder of her ophthalmologic examination were unchanged. Repeated ERGs in September and October showed improvement in dim scotopic re-

Figure 1. Patient 1. A, The Goldmann visual field reveals large midperipheral and paracentral scotomas. B, These later improved in both number and size in the left eye. In both figure parts, the left panels indicate the left eye; the right panels, the right eye.
responses but otherwise remained unchanged. Later that month, acute myeloid leukemia developed and the patient died 7 weeks later. Her globes were harvested postmortem for evaluation.

**CASE 2**

A 71-year-old woman with a history of ovarian cancer with abdominal metastases received 3 doses of biweekly irofulven treatment (0.61-0.63 mg/kg). Two days after the last dose she awoke with dim vision, glare, and photophobia. She denied positive visual phenomena. Her VA was 20/25 OD and 20/30 OS and with both eyes she identified 8 of 8 Ishihara color plates. Her GVF s revealed paracentral and midperipheral scotomas in each eye. An ERG showed markedly abnormal cone responses with normal rod responses. Findings from the remainder of her ophthalmologic examination were normal. She stopped irofulven treatment and at 10 weeks' follow-up, her symptoms were gone, VA was 20/20 OU, and GVFs showed resolution of the scotomas in the right eye and 1 midperipheral scotoma remaining in the left eye. Her serum sample lacked antirecoverin antibodies (Charles Thirkill, PhD, written communication, August 2001). She refused a second ERG.

**CASE 3**

A 44-year-old woman with ovarian cancer received 3 doses of biweekly irofulven treatment (0.55-0.57 mg/kg). The day after her last dose she noted a “dark film” in both eyes, photophobia, and photopsias. Her VA was 20/25 OD and 20/20 OS and she identified 13 of 16 Ishihara color plates with the right eye and 12 of 16 with the left eye. The GVFs revealed Bjerrum scotomas in each eye. The results of the remainder of her ophthalmologic examination were unremarkable. The ERG showed markedly abnormal cone responses and normal rod responses. She refused follow-up; however, by telephone 2 months later she related that her symptoms had resolved.

**CASE 4**

A 53-year-old woman with ovarian cancer received 4 doses of biweekly irofulven treatment (0.53 mg/kg) and noted photophobia and photopsias for 4 days following the last dose. She received a fifth dose of the drug and her photophobia persisted. Visual acuity was 20/25 OU and she identified 10 of 10 Ishihara plates with both eyes. Results of the GVFs showed paracentral scotomas in the right eye and arcuate defects in the left eye. The ERG showed moderately abnormal cone responses and normal rod responses. At 1-month follow-up, VA was 20/20 OU and her scotomas had decreased in size and severity. The ERG showed modest improvement but remained slightly abnormal.

**METHODS**

This was a multi-institutional phase 2 trial of a single agent—irofulven. Institutional review board approval and antemortem informed consent from patient 1 and her relatives were obtained. Scotopic flash, photopic flash, and 30-Hz flicker ERG results had been recorded according to the International Society for Clinical Electrophysiology of Vision standard.
HISTOPATHOLOGIC FINDINGS

Postmortem human eyes were obtained through Harvard Medical School, Boston, Mass., and the University of Washington, Seattle. Normal eyes (from a 76-year-old woman, 6 1/2 hours postmortem; 83-year-old woman, 6 hours postmortem; and 33-year-old man, enucleation) were processed as noted below. The eyes of patient 1 were fixed 8 hours postmortem for 4 days in a combination of 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1M phosphate buffer, pH 7.3, and stored in 2% paraformaldehyde. Retinal samples (from macula and mid and far periphery) were processed in glycol methacrylate, sectioned at 4 μm, and stained with methylene blue/azure II (Richardson stain).

IMMUNOCYTOCHEMISTRY

Retina samples from patient 1 and normal retinas (macula, mid and far periphery) were processed for immunofluorescence with mouse mAb 7G6 specific for cones (1:250); mouse mAb 4D2 antirhodopsin specific for rods (1:40); and rabbit antiglial fibrillary acidic protein (GFAP) specific for astrocytes and reactive Muller cells (1:750, DAKO Corp, Carpinteria, Calif). Secondary antibodies (goat antirabbit and antimouse IgG, 1:30) were labeled (red) with Cy-3 (Jackson ImmunoResearch Laboratories, West Grove, Pa). Cell nuclei were stained (blue) with 4′,6-diamidino-2-phenylindole (1 μg/mL; Molecular Probes, Eugene, Ore). Control sections had primary antibody omitted. Sections were imaged with an epifluorescence microscope (Leitz DMR-B 513810; Leica Inc, Deerfield, Ill) or an inverted laser scanning confocal microscope (Zeiss LSM 510; Carl Zeiss, Thornwood, NY).

RESULTS

NORMAL RETINAS

All of the control eyes showed the same normal retinal histologic features. Cytoplasm of all cones was labeled with mAb 7G6 (Figure 3A). Cone cell bodies were numerous in the maculas and formed a monolayer elsewhere. Cone outer segments had normal length. The retinal pigment epithelium (RPE) was filled with autofluorescent lipofuscin. Rhodopsin was localized in long, thin rod outer segments (Figure 3B). The GFAP was limited to astrocytes in the nerve fiber and ganglion cell layers (Figure 3C). Control sections had only autofluorescent RPE lipofuscin (Figure 3D).

RETINA OF IROFULVEN-TREATED PATIENT

Gross Pathologic Features

Anterior segments were normal but lenses had mild cortical opacities. The vitreous was clear, but peripapillary pigment was present in both eyes. The maculas had no evidence of pigmentary degeneration.

Microscopic Pathologic Features

The foveal pit had reduced cone numbers. Parafoveal photoreceptors were reduced to 3 or 4 rows (normal, 6-8) (Figure 3E). Cones were lost gradually toward the edge of the macula (Figures 3F-H). All cone outer segments were shortened. No drusen or RPE abnormalities were evident. Inner nuclear and ganglion cell layers had normal neuron numbers (Figures 3E-H). Very few peripheral cones were retained: a 1-mm length of peripheral retina contained only 2 cones (Figure 3I).

Rods in the parafovea (Figure 3J) and periphery (Figure 3K) had shortened outer segments and rhodopsin was abnormally delocalized to their cell bodies. However, near-normal numbers of rods were present in all regions. In addition to GFAP-positive astrocytes in the nerve fiber and ganglion cell layers, Muller cells had hypertrophied GFAP-positive processes (Figure 3L). Control sections had only autofluorescent RPE lipofuscin.

Photophobia, dimmed vision, and positive visual phenomena consistent with cone dysfunction developed in these 4 patients. Three patients received doses of more than 0.55 mg/kg of irofulven while patient 4 received a 0.53-mg/kg dose. At the lower dose, patient 4 had less cone dysfunction with ERG testing. Visual symptoms developed in all 4 patients by the fourth biweekly dose. Color vision was variably affected but all had abnormal GVF's and prominent cone dysfunction on ERGs. Each had improved visual function after reduction or discontinuation of irofulven treatment. Abnormal cone ERG responses and normal rod ERG responses developed in 3 additional patients several days after the administration of irofulven.16

The ERGs and histopathologic features of the retinas of patient 1 demonstrated that the cones were more severely affected than the rods. Foveas and maculas had marked cone loss but normal numbers of rods as well as neurons in the inner nuclear and ganglion cell layers. Remaining cones and rods had shortened outer segments. The periphery retained few cones. Hypertrophied reactive Muller cells were filled with GFAPs, a sensitive index of retinal cell death.17 The microscopic findings correlate with the ERG abnormalities of cone cell death and outer segment shortening, most pronounced in the periphery but with significant cone cell loss in the maculas. The loss of peripheral cones may explain the midperipheral scotomas. Although the ERG remained unchanged, how did cone-mediated visual function improve in patient 1? Some viable macular cones remained but were abnormal with shortened outer segments. Following drug cessation they may have recovered some function. The ERG represents a summed response across the retina, and the flat cone response may reflect marked loss of cones throughout the periphery.

Rods were retained in normal numbers throughout the retina, although their outer segments were shortened and rhodopsin was delocalized to their cell bodies. Delocalized rhodopsin is commonly found in rods that have shortened outer segments due to diseases such as retinitis pigmentosa.17 Altered cone metabolism may have contributed to abnormalities in the rods, but the rod damage might also be explained by the previous chemotherapy. Alternatively, irofulven may have contributed to rod damage as well.
Figure 3. Images A through D are of a healthy human retina (53-year-old man) and E through L represent the retina of patient 1 (a 79-year-old woman). Cell nuclei in immunofluorescent images are stained blue with 4',6'-diamidino-2-phenylindole. R indicates retinal pigment epithelium; O, outer nuclear layer; N, inner nuclear layer; G, ganglion cell layer; OSs, outer segments; GFAP, glial fibrillary acidic protein; F, astrocytes in the nerve fiber layer; and bars, 25 µm in Figure I and 50 µm in all of the other figures. A, Immunofluorescence image of normal parafovea. The cones are labeled (red) with mAb 7G6. Note the normal length of cone outer segments (arrowheads) and numbers of cone cell bodies in O in this region. The R contains yellow gold autofluorescent lipofuscin granules. B, Rod OSs in the parafovea are long and thin, as demonstrated by labeling with antirhodopsin (red). C, The GFAP (red) in healthy human retina is limited to F and G layers. D, Control section of healthy human parafovea treated with no primary antibody shows only autofluorescent lipofuscin granules in R. E, Parafovea of irofulven-treated patient labeled with mAb 7G6 (red). Note reduction of nuclei in the outer nuclear layer and short cone outer segments (arrowheads). F through H, Adjacent microscope fields from the parafovea to the edge of the macula of irofulven-treated patient. Note gradual loss of cone cell bodies and shortened OSs (arrowheads). I, Glycol methacrylate section of retinal periphery of irofulven-treated patient. Although rods are present in normal number with OSs, only 2 cones (arrowheads) were found in a section 1 mm long. Normally a monolayer of cone cell bodies exists in the outer nuclear layer of the retinal periphery. Cells in N and G layers are normal in number (methylene blue/azure II [Richardson’s] stain). J, Parafovea of the irofulven-treated patient retina labeled (red) with anti-rhodopsin. Note positive rod OSs (*) that are shortened and delocalized rhodopsin in the surface membranes of the rod cell bodies (arrowheads). K, Periphery of the irofulven-treated patient retina labeled (red) with anti-rhodopsin. The rod OSs (*) are shortened and rhodopsin is delocalized to the rod cell bodies (arrowhead). L, The GFAP (red) is localized in astrocytes in the inner retina and Müller cell processes in the outer part of the irofulven-treated patient retina. Asterisk indicates GFAP-positive Müller cell processes in Henle fiber layer of cone axons.
We acknowledge that our patients had undergone pretreatment with chemotherapy and may have had existing, subclinical damage that contributed to their visual disturbances. However, it is probable that high-dose irofulven treatment caused the cone-specific damage given the close temporal relationship of irofulven administration to the onset of symptoms and subsequent dramatic improvement of color vision and GVF's when irofulven treatment was discontinued. Accordingly, the manufacturer has modified the dosage because of the visual disturbances.

Rankin and Pitts described 2 patients with pigmentary maculopathy and optic neuropathy secondary to carboplatin treatment. Our patients received carboplatin treatment, but did not have optic neuropathy or pigmentary maculopathy by funduscopic, gross, or histopathologic examination. Katz et al described a patient that inadvertently received a 4-fold dose of cisplatin and developed significant antemortem vision loss in both eyes. That patient demonstrated nearly flat photopic and scotopic ERG responses. Histopathologic features revealed a split outer plexiform layer, but cones and rods were intact, suggesting that cisplatin therapy does not cause cone cell death.

Paraneoplastic retinopathy, including specific cone loss only, develops in some patients with cancer. However, the serum samples of patients 1 and 2 lacked antirecoverin antibodies and their retinas had relatively normal rod function, which is typically lost in cancer-associated retinopathy.

Prospective ophthalmologic, perimetric, and ERG testing are ongoing in patients treated with irofulven before and after dose reduction in the amended protocol. Results of our microscopic study of the retinas from a patient treated with irofulven demonstrated marked loss of cones with relative sparing of rods. High-dose irofulven treatment seems to be associated with a clinical picture consistent with cone damage, confirmed by ERG testing and histopathologic findings.

Submitted for Publication: March 8, 2004; final revision received August 17, 2004; accepted September 22, 2004.

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Funding/Support: This study was supported by funds from MGI Pharma, Inc, Bloomington, Minn, and The Foundation Fighting Blindness, Owings Mills, Md.

Previous Presentations: Presented in part at the North American Neuro-Ophthalmology Society Annual Meeting; Copper Mountain, Colo; February 13, 2002; and the Association for Research in Vision and Ophthalmology; Fort Lauderdale, Fla; May 4, 2003.

Acknowledgments: We thank Peter R. MacLeish, PhD, and Robert S. Molday, PhD, for providing the mouse monoclonal antibodies.

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