Choroidal Concentration of Interferon After Retrobulbar Injection

Harvey Lincoff,* Paulo Stanga,* Alex Movshovich,* Alicia Palleroni,† Bojidar Madjarov,* Rafael Rivera,* and Ronald Silverman*

Purpose. To determine whether recombinant human interferon alpha-2a (IFNa-2a) would diffuse into the choroid in significant amounts from a retrobulbar depot.

Methods. One million international units of IFNa-2a were injected into the retrobulbar space of the eyes of 17 rabbits, and choroidal and serum concentrations were measured at 1, 2, 4, 8, 12, and 24 hours. The same dose of IFNa-2a was injected subcutaneously into 10 rabbits, and choroidal and serum concentrations were measured at the same intervals for comparison. Parabulbar and ocular tissues were studied by light microscopy for evidence of local toxicity.

Results. Peak concentration IFNa-2a in the choroid after retrobulbar injection occurred at 2 hours and averaged 32,000 IU/mg. Peak concentration in the serum occurred at 4 hours and averaged 227 IU/ml. Concentrations in choroid and serum fell rapidly, and IFNa-2a was not detectable at 24 hours. No IFNa-2a was detected in the choroid of the paired eye, and only a trace (<50 IU/mg) was found in either eye after subcutaneous injection. Light microscopy revealed some lymphocytes in the fat adjacent to the retrobulbar depot after six daily injections of IFNa-2a or saline. Sclera, choroid, and retina appeared unaffected.

Conclusions. IFNa-2a diffuses into the choroid from a retrobulbar depot in significant amounts. The serum concentration from a retrobulbar injection is <1% of the choroidal concentration. The retrobulbar route to the choroid may be optimal for testing the effect of IFNa-2a on choroidal neovascularization. Invest Ophthalmol Vis Sci. 1996;37:2768-2771.

Since the letter of Fung1 in 1991 describing the regression of subretinal neovascularization in response to subcutaneously administered interferon, six reports have testified to its effect or lack of effect. All tell of systemic complications resulting from the subcutaneous injection of 3,000,000 or 6,000,000 IU of interferon.2-8

We sought to reduce the systemic complications of interferon by sequentially injecting a lesser dose into a retrobulbar depot. We had earlier demonstrated that sequential retrobulbar injections of hydrocortisone and clindamycin could maintain significant choriorretinal concentrations with relatively low serum levels in the rabbit. Interferon is a large protein molecule, much larger than hydrocortisone or clindamycin, and the ability of the molecule to diffuse across the sclera in significant amounts was in doubt.

We recognized that the use of the rabbit as an experimental model for interferon has limitations. Recombinant human interferon (IFNa-2a) is species specific and cannot be expected to yield a therapeutic effect in an animal, except possibly the monkey. The number of animals (28) projected for a valid result made the use of monkeys unfeasible. Our experiments did not attempt to test for therapeutic effect but only to determine whether IFNa-2a would diffuse into the choroid from a retrobulbar depot in measurable amounts and to compare it with the amount obtained in the choroid after subcutaneous injection. To our knowledge, no other measure of interferon in choroidal tissue of any species has been made.

MATERIALS AND METHODS. Twenty-eight New Zealand white rabbits (males and females, 3 to 3.5 kg) and five Dutch Belted pigmented rabbits (males, 2.5 to 3.5 kg) were approved for the experiments by the Institutional Review Board of Cornell University Medical College. All investigations conformed to the ARVO statement for the Use of Animals in Ophthalmic and Vision Research.

Rabbits were given intramuscular injections of ketamine hydrochloride (35 mg/kg, Ketaset; Fort Dodge Laboratories, Fort Dodge, IA), and xylazine hydrochloride (5 mg/kg, Rompun; Mobay, Shawnee, KS). Supplemental topical anesthesia was administered by applying proparacaine hydrochloride 0.5% (Alcaine; Alcon, Hamacoa, PR) to the conjunctiva. The pupils were dilated with topical cyclopentolate hydrochloride 1% (Cyclogyl; Alcon, Fort Worth, TX).

For the retrobulbar injection, a 2 mm incision was made through the conjunctiva and the superior rectus tendon, 3 mm posterior to the limbus. A 25-gauge curved, blunt needle was inserted through the incision into Tenon’s space. The scleral indentation of the tip of the needle was monitored ophthalmoscopically,
and the tip was advanced to a disc diameter from the edge of the optic disc.

Recombinant human interferon (Roferon-A) prepared by affinity chromatography³ and >98% pure was obtained from Hoffman LaRoche Research Laboratories (Nutley, NJ). It was reconstituted with sterile water to 10,000,000 U activity/ml. One million units (0.1 ml) was injected at the posterior sclera of the left eye of 10 albino rabbits and to both eyes of 7 albino rabbits and 5 pigmented rabbits.

To determine the peak concentration and decay of IFNα-2a in the serum, blood samples were drawn from the central artery of the ear at 1, 2, 4, 8, 12, and 24 hours from six albino animals in which one orbit had been injected and from 11 animals (six albinos, five pigmented) in which both orbits had been injected before the animals were killed with injections of sodium pentothal (5 mg/kg).

Blood samples were allowed to clot at room temperature for 2 hours (at 20°C, IFNα-2a loses <5% of its bioactivity in 2 hours) and were refrigerated at 4°C for another 24 hours. Cold samples were centrifuged for 10 minutes at 3000 rpm. The supernatant was withdrawn and recentrifuged for another 10 minutes at 6000 rpm, and the resultant supernatant was frozen in preparation for shipping to LaRoche Laboratories for bioassay.

Both eyes of all the animals were enucleated immediately after death and dissected in a cold, sterile environment. Eyes were sprayed with 70% ethanol, rinsed with normal saline, and placed in sterile paraffin mold that supported the posterior two thirds of the globe. Under a dissecting microscope, the anterior segment was removed, and the vitreous and retina were aspirated. The entire choroid was scraped from the sclera with a spatula, placed in an Eppendorf tube, and weighed. A solution of 0.1 M phosphate buffer (pH 7.18) was added in a ratio of 500 mg tissue:1 ml buffer. The buffer contained 10 mg/ml of gentamicin. The tissue in buffer solution was homogenized with a Kontes pellet pestle (Fischer, Springfield, NJ) and then was centrifuged at 3500 rpm at 4°C for 15 minutes. The supernatant was recentrifuged at 15,000 rpm at 4°C for another 15 minutes. Choroidal tissue supernatant and serum samples were labeled with coded numbers, placed in an insulated container containing dry ice, and sent to LaRoche Research Laboratories for bioassay. The code was not disclosed until the project was completed.

At LaRoche, the choroidal extracts underwent further sequential centrifugation in a Beckman Airfuge (Beckman Instruments, Fullerton, CA) at 160,000g for 20 minutes. The antiviral activity of IFNα-2a in the choroidal extracts and in the serum samples was determined by measuring their ability to reduce the cytopathic effect of a vesicular stomatitis virus on Madin–Darby bovine kidney cells.¹⁰ Choroidal and serum samples were distributed in a plastic multiwell block. Each block contained bovine kidney cells and serial dilutions of cytopathic virus. The antiviral activity was manifested by a color change at 16 hours. All the samples were assayed in triplicate. Controls also were assayed to confirm that there was no degradation during sample processing. The bioassay has a sensitivity of 1 IU/ml and is specific for the measurement of IFNα and IFNβ, with a regression coefficient 0.87. It is linear in the range of 10 to 640.

To obtain the concentrations in serum and choroid from subcutaneous injection, 10 albino rabbits were injected with 1,000,000 IU of IFNα-2a. Blood samples were drawn at 1, 2, 4, 8, and 10 hours, and both eyes were enucleated from two animals at 1, 2, 4, and 6 hours, from one animal at 8 hours, and from another at 10 hours. The blood samples and eyes were processed in the same way as those from the animals that had been given retrobulbar injections and were sent for bioassay. Subsequently, to provide enough serum data to reach significance, three additional albinos were injected subcutaneously with 1,000,000 IU. Blood was drawn at 1, 2, 4, 8, 12, and 24 hours from each animal, processed, and sent for bioassay.

To examine for local toxicity of IFNα-2a, three albino rabbits were given daily retrobulbar injections of 1,000,000 IU of IFNα-2a to the left eye for 6 days. One animal also was given 0.1 ml of sterile saline to the right eye for 6 consecutive days. Eyes were monitored each day by indirect ophthalmoscopy for intraocular toxicity. On day 7, the left eyes of two animals and both eyes of the one given saline were enucleated together with a layer of parabulbar tissue. The vitreous was injected with 0.3 ml of 4% formalin, and the eyes were placed in 4% formalin for 16 hours. The poste-
The statistical significance of INFα-2a activity measurements was ascertained by use of a one-tailed, one-sample t-test with the hypothesis that the true INFα-2a activity was zero. P < 0.05 was considered statistically significant.

RESULTS. Peak levels of INFα-2a in the choroid after the injection of 1,000,000 IU occurred at 2 hours and averaged 32,000 IU/mg (t = 8.67; P < 0.0001). The concentration fell rapidly after peak, reaching zero at 24 hours. There was no significant difference in peak or decay between albino and pigmented animals. INFα-2a was not detectable in the paired eye of any animal in which only one eye had been injected (Fig. 1).

Peak serum concentration of animals injected in only one eye with 1,000,000 IU of INFα-2a, occurred at 4 hours and averaged 227 IU/ml (t = 19.1; P < 0.0001). At 2 hours, the average was 215 IU/ml (t = 10.5; P < 0.001). When both eyes were injected with 1,000,000 IU, the serum concentration reached a distinct peak at 2 hours that averaged 706 IU/ml (t = 5.0; P < 0.0005) (Fig. 2).

When 1,000,000 IU of INFα-2a was injected subcutaneously, the serum level peaked at 2 hours at an average of 275 IU/ml (t = 2.57; P < 0.0185). INFα-2a was undetectable in the choroid of either eye in 8 of the 10 animals after subcutaneous injection and was less than 50 IU/ml in the remaining two.

DISCUSSION. These experiments demonstrated that recombinant human INFα-2a from a retrobulbar depot penetrates the sclera and enters the choroid of the rabbit in significant amounts. The peak concentration in the serum after 1,000,000 IU was injected into the retrobulbar space of one eye was <1% of the peak choroidal concentration and was similar to the peak serum concentration after 1,000,000 IU was injected subcutaneously (Fig. 2). The serum concentration approached 2% of the choroidal concentration when both eyes were injected.

The absence of detectable quantities of INFα-2a in the paired eye of animals injected monocularly and in the eyes of 8 of the 10 animals injected subcutaneously (trace amounts were present in two animals) was unexpected. We cannot explain why a serum concentration of 225 IU/ml at 2 hours did not manifest some significant presence in the choroid of the paired eye. A dose of 1,000,000 IU in a 3-kg rabbit is equivalent (by weight) to 23,000,000 IU in a 70-kg patient. It suggests that the clinical trials in which 3,000,000 or 6,000,000 IU were injected subcutaneously were dependent on small concentrations in the choroid for a therapeutic effect.

The ratio of total choroidal concentration of INFα-2a to the total amount injected in the retrobulbar space was 32:1000 at peak. The ratio would be less in humans because human sclera is thicker. Edelhauser,11 however, found that transfer of sulfonamides across the human sclera was only slightly less than across rabbit sclera.

We did not measure the concentration in the retina. Because the rabbit retinal vasculature is limited to the myelinated strip, we thought diffusion from the choroid would be small. Daily observation of the retina did not reveal any infarcts in the vasculature of the myelinated strip.

Key Words
- balloon, interferon, macular degeneration, retrobulbar catheter, subretinal neovascularization

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References
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Cone-Specific Measures of Human Color Vision

Jeff Rabin

Purpose. To describe a new test of color vision (cone-specific contrast sensitivity) and to evaluate its sensitivity in comparison to standard clinical tests.

Methods. Cone-specific colored letter charts were generated by computer and displayed on a color monitor. Each chart consists of colored letters that are most visible at the top but that gradually fade into a gray background. Cone contrast varies systematically on each chart so that letters are visible to only one cone type (L, M, or S cone). Cone-specific letter contrast sensitivity was measured in 50 color normals and 13 subjects with hereditary color deficiency. Values were compared to standard measures of color vision.

Results. In color normals, mean log contrast sensitivity was approximately the same on L-cone (1.84 ± 0.08 log contrast sensitivity) and M-cone (1.87 ± 0.08) tests but was reduced on the S-cone test (0.89 ± 0.15) because of the smaller number of S cones in the human retina. Subjects with red color deficiency showed some degree of red or green color deficiency. This condition is nonprogressive, hereditary (sex-linked recessive), and attributed to a decreased number of long wavelength- (L or red) or middle wavelength- (M or green) sensitive cone photoreceptors or to a shift in the peak absorption of L or M photopigments. Persons with color deficiency have difficulty judging differences in hue and confuse colors that appear distinct to color normal persons. Although blue (S-cone) hereditary defects are rare (0.001% to 0.007%), color deficiency can be acquired with ocular or systemic disease, and S-cone defects occur early in the course of these diseases. Despite numerous methods for detecting the presence of color anomalies, relatively few clinical tests readily identify type (L, M, or S) and severity of color deficiency. We describe a new approach, suitable for rapid clinical testing, based on the photopigments of normal color vision. Letters visible to only one cone type (L, M, or S) are presented in graded steps of cone contrast to determine the test. When standardized relative to variability, cone contrast sensitivity identified color deficiency unequivocally in all subjects, whereas FM 100 hue error scores detected 9 of 13 subjects with color deficiency.

Conclusions. Cone-specific contrast sensitivity provides a quantitative measure of normal color vision and indicates both type and severity of color deficiency. It is useful for diagnosing hereditary color deficiency and for monitoring early color vision loss in ocular and systemic disease. Invest Ophthalmol Vis Sci. 1996;37:2771-2774.

Most persons can discern small differences in color, but 8% of males and 0.5% of females are born with some degree of red or green color deficiency. This condition is nonprogressive, hereditary (sex-linked recessive), and attributed to a decreased number of long wavelength- (L or red) or middle wavelength- (M or green) sensitive cone photoreceptors or to a shift in the peak absorption of L or M photopigments. Persons with color deficiency have difficulty judging differences in hue and confuse colors that appear distinct to color normal persons. Although blue (S-cone) hereditary defects are rare (0.001% to 0.007%), color deficiency can be acquired with ocular or systemic disease, and S-cone defects occur early in the course of these diseases. Despite numerous methods for detecting the presence of color anomalies, relatively few clinical tests readily identify type (L, M, or S) and severity of color deficiency. We describe a new approach, suitable for rapid clinical testing, based on the photopigments of normal color vision. Letters visible to only one cone type (L, M, or S) are presented in graded steps of cone contrast to determine the