Original Research

Choroidal changes after intravitreal injection of interferon alpha-2b

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Impact statement

Type I interferons are proteins that are naturally secreted by cells in response to certain stimuli. They are approved for medical use to regulate immune responses in many disease states. Our findings of increased choroidal circulation after treatment of the eye with interferon alpha-2b indicate a possible effect of this cytokine on blood circulation. This is a novel finding that needs further investigation to elucidate mechanisms and applications.

Abstract

Intravitreal injection of interferon alpha-2b (IFN-α2b) is used to treat uveitis but its effect on the choroid is unknown. We histologically evaluated the choroidal changes after intravitreal injection of IFN-α2b. We compared histological samples of IFN-α2b-injected eyes to eyes with lipopolysaccharide (LPS)-induced uveitis and eyes injected with balanced salt solution (BSS) as controls. Rabbit eyes received intravitreal injections of BSS, IFN-α2b, or LPS, and enucleation was done seven days later. Choroidal changes were evaluated on histological cut sections. The thickness of the choroid was measured in micrometer and the severity of inflammation was scored. The mean maximum choroidal thickness was significantly greater in the IFN-α2b group in comparison to the LPS and BSS groups ($P < 0.001$). The mean minimum choroidal thickness was also significantly greater in the IFN group compared to the BSS group ($P = 0.009$). The observed changes were mainly due to vasodilation rather than interstitial fluid retention or inflammation. Mild inflammatory cell infiltration was observed in the choroid of four eyes in the IFN group and of three eyes in the LPS group. No inflammation was seen in the control group. The difference in inflammatory cell infiltration between the LPS and IFN groups was not statistically significant. Significant choroidal hyperemia was present after injection of IFN-α2b. This fact may suggest for some pharmacological applications of IFN-α2b when increased choroidal circulation is needed. However, further studies are required to elucidate the mechanisms involved.

Keywords: Interferon alpha-2b, uveitis, choroid, hyperemia

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Introduction

With the emergence of new imaging techniques like swept source or spectral domain optical coherence tomography, evaluation of the choroid in various chorioretinal diseases has received more attention.[1] Many studies focus on disease states in human subjects before and after treatment to highlight the difference in choroidal thickness between active and quiescent states of the disease. Most of the studies were conducted on uveitis patients in whom immunomodulatory agents are used as adjuncts to steroids[2] or even alone in steroid-sparing regimens.[3,4]

Few studies have investigated changes in choroidal thickness during the acute and chronic phases of uveitis or changes after intravitreal injection of anti-inflammatory drugs. Yan *et al.* showed that choroidal thickness was indeed decreased in inactive forms of uveitis.[5] Biological drugs such as anti-tumor necrosis factors or interferons (IFNs) are relatively new treatments that can break the inflammatory cycle and may be used in the future to treat some pathological conditions in the eye.[2,6,7] Interferons have effects on innate and adaptive immunity and can induce tolerance to autoimmune processes.[4,8] They are mainly administered systemically to treat refractory cases of uveitis;[4,9] however, intravitreal injection of interferon alpha-2b (IFN-α2b) has also been tried in order to avoid its systemic side effects and the results were promising.[3] In this study, we aimed to histologically evaluate the choroidal changes after intravitreal injection of IFN-α2b and then to compare the choroidal changes with two other groups including LPS-injected eyes (LPS is used to induce

acute uveitis in experimental studies) and BSS-injected eyes as control; we also aimed to see if we can directly point some of the findings to the effects of the drug itself, even in the absence of inflammation or disease in the eye. Most previous studies were conducted on diseased eyes; therefore, effects of the drug on normal state of the eye and specifically its circulation are not clear.

Our study can be the basis for further investigations on mechanisms involved in choroidal circulation and possible therapeutic applications.

Materials and methods

Tissue samples

Pathological samples of 31 eyes from 31 male New Zealand white rabbits weighing between 2 and 3 kg were included in this study. The procedures of intravitreal injections and animal care were compliant with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

In order to see the net effect of IFN, we planned to compare the findings between the eyes receiving the drug to two other groups. Those are the ones who received intraocular BSS (to consider any changes that induced by needling and injection of neutral fluid) and also the ones with induced uveitis (to see if there is any similarity in the findings and bringing the hypothesis of induction of inflammation by interferon injection).

The first group (7 eyes) received 2 µg/0.1 mL *Salmonella typhimurium* LPS endotoxin (L6511; Sigma Chemical, St. Louis, MO, USA); the second group (12 eyes) received 200,000 IU/0.1 mL IFN-α2b (PDferon, Pooyeshdarou Pharmaceutical Co., Tehran, Iran), and the third group (12 eyes) received 0.1 mL of BSS. Intravitreal injections were performed under aseptic conditions through the pars plana (2.5 mm posterior to the limbus) using a 27-gauge needle with a 30-degree angulation toward the vitreous, avoiding trauma to the lens. To lessen the chance of an intraocular pressure rise, an anterior chamber tap (0.1 ml) was done prior to intravitreal injection. Topical ciprofloxacin and povidone iodine were applied before and after the injections to prevent iatrogenic endophthalmitis. Uncomplicated procedure was confirmed by slit lamp and binocular indirect ophthalmoscopic examination after the injections. Enucleation was performed one week after intravitreal injection in all three groups. The globes were fixed in 10% formaldehyde.

Procedures

Five-micrometer-thick pupilo-optic sections were prepared from the tissue blocks. The optic stalk and posterior eye wall on the nasal and temporal sides of the optic nerve in the horizontal meridian were present in each section. The sections were stained with hematoxylin and eosin and studied by light microscopy to assess the choroidal changes. Analysis Starter software was used along with a DP12 Olympus camera attached to an Olympus BX41 microscope to capture the images. Thickness measurement was performed on the captured images along the distance of 200 µm on both sides of the optic nerve head. The thickest and thinnest parts were measured from Bruch's membrane (outer border of the retinal pigmented epithelium) to the lamina fusca of the sclera. The measurements were repeated twice. Calibration was performed before each set of measurements. Inflammatory cell infiltration along the choroid was scored as mild, moderate, or severe and was recorded based on the number of extravasated cells in the stroma.

Statistical analysis

Statistical analysis was performed using the IBM SPSS Statistics V23.0 software. Data distribution was checked by the Shapiro-Wilk W test, which showed normally distributed data for minimum and maximum choroidal thickness. Descriptive results were presented as mean, standard deviation, and error bar charts. Choroidal thickness was compared among the three groups by analysis of variance (ANOVA) test. The Bonferroni post-hoc test was used for pairwise comparison. Significance was set at $P < 0.05$.

Results

The study involved samples from 31 rabbit eyes: LPS group ($n = 7$; mean weight 2.2 ± 0.87 kg, range: 2.1–2.5), IFN group ($n = 12$; mean weight 2.2 ± 0.90 kg, range: 2–2.4), and BSS group ($n = 12$; mean weight 2.3 ± 0.85 kg, range: 2.1–2.5). The mean weight of the three groups was not significantly different ($P = 0.65$, ANOVA).

Inflammation

Mild inflammatory cell infiltration was observed in the choroid of four eyes in the IFN group and of three eyes in the LPS group. No inflammation was seen in the BSS group. There was only one eye with severe choroidal inflammation in the LPS group (Figure 1). Moderate inflammation in the vitreous was present in all eyes of the LPS group, and no inflammation was observed in the vitreous of the IFN or BSS group. In the choroid, the difference in inflammatory

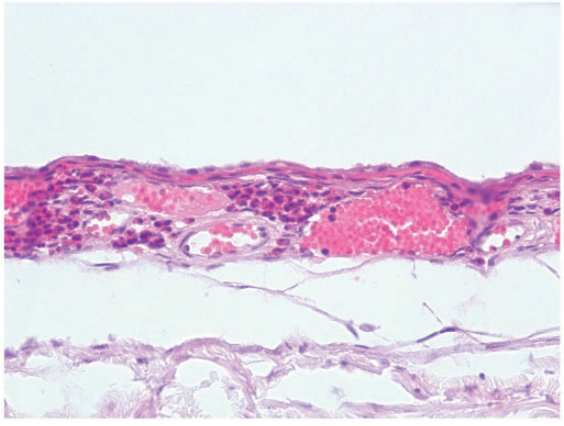

Figure 1. Severe inflammatory cell infiltration observed in the choroid of one eye from LPS group. Hematoxylin and Eosin ×400. (A color version of this figure is available in the online journal.)

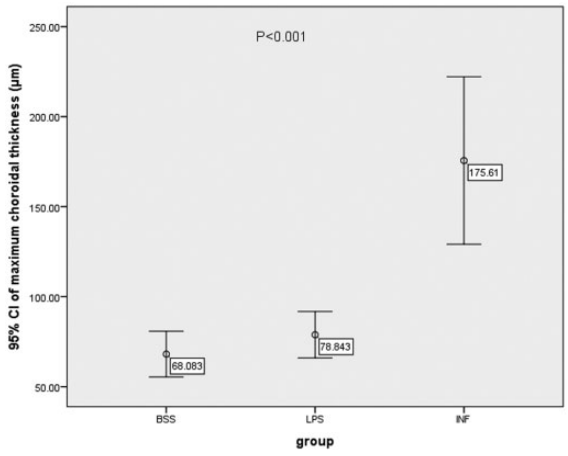

Figure 2. Choroidal thickness distribution among groups was compared by ANOVA test. BSS: balanced salt solution; LPS: lipopolysaccharide; IFN: interferon alpha-2b.

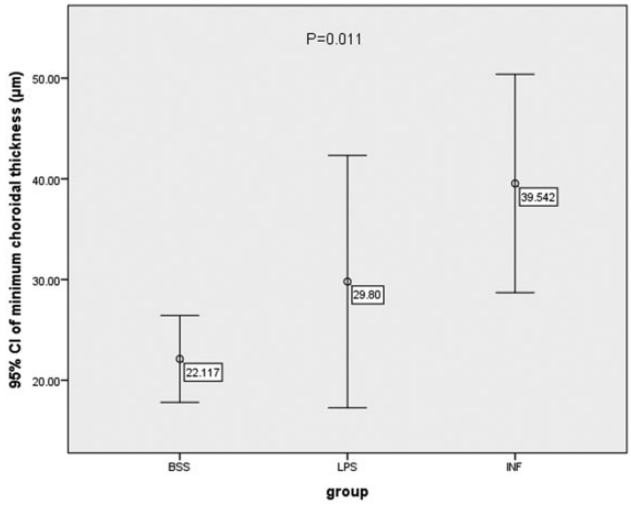

Figure 3. Minimum choroidal thickness among groups. ANOVA test used for comparison of three groups and Bonferroni test was performed for paired comparison. Significant difference was observed. BSS: balanced salt solution; LPS: lipopolysaccharide; IFN: interferon alpha-2b.

cell infiltration between the LPS and IFN groups was not statistically significant.

Choroidal thickness

Maximum choroidal thickness measured in the macular region was $78.84 \pm 13.94\,\mu m$ in the LPS group, $175.60 \pm 73.22\,\mu m$ in the IFN group, and $68.08 \pm 19.97\,\mu m$ in the control group. Maximum choroidal thickness was significantly different among the three groups ($P < 0.001$). Pairwise comparison by the Bonferroni test showed significantly greater maximum choroidal thickness in the IFN group compared to the LPS or the control (BSS) group ($P < 0.001$) (Figure 2).

Minimum choroidal thickness measured in the macular region was $20.80 \pm 13.54\,\mu m$ in the LPS group, $39.54 \pm 17.06\,\mu m$ in the IFN group, and $22.11 \pm 6.78\,\mu m$ in the control group. Minimum choroidal thickness was significantly different among the three groups ($P = 0.011$); however, pairwise comparison by the Bonferroni test showed a significant difference only between the IFN and the control (BSS) groups ($P = 0.009$) (Figure 3).

The mean and standard deviation of choroidal thickness in the three groups can be found in Table 1.

The observed changes were mainly due to dilatation of medium- and large-sized vessels rather than the presence of inflammation or accumulation of excess extracellular fluid (Figure 4). The mechanism of action of the drug is not investigated in this study and needs future exploration.

Discussion

The choroid contains a vascular bed consisting of various anastomosing vessels of variable sizes and capillaries. It is responsible for nutrition of the outer retina.[10] Inflammatory conditions can affect choroidal blood flow and thickness.[11] Increased choroidal thickness during the acute phase of uveitis has been observed in some studies and was attributed to the aggregation of inflammatory cells and dilation of choroidal capillaries.[12,13] In the chronic phase of uveitis, alterations in the choroidal capillaries and vessels of Haller's layer lead to ischemia, with a consequent decrease in choroidal thickness and atrophy of the outer retina.[14]

Table 1. Difference in the maximum and minimum mean choroidal thickness between groups.

	n	Mean	Standard deviation	Standard error	95% confidence interval for mean	
					Lower bound	Upper bound
Min						
BSS	12	22.1167	6.78673	1.95916	17.8046	26.4287
LPS	7	29.8000	13.54806	5.12069	17.2701	42.3299
INF	12	39.5417	17.06349	4.92580	28.7000	50.3833
Total	31	30.5968	14.87534	2.67169	25.1405	36.0531
Max						
BSS	12	68.0833	19.97916	5.76749	55.3892	80.7775
LPS	7	78.8429	13.94320	5.27003	65.9475	91.7382
INF	12	175.6083	73.22136	21.13719	129.0857	222.1310
Total	31	112.1355	69.26372	12.44013	86.7293	137.5416

BSS: balanced salt solution; LPS: lipopolysaccharide; IFN: interferon alpha-2b.

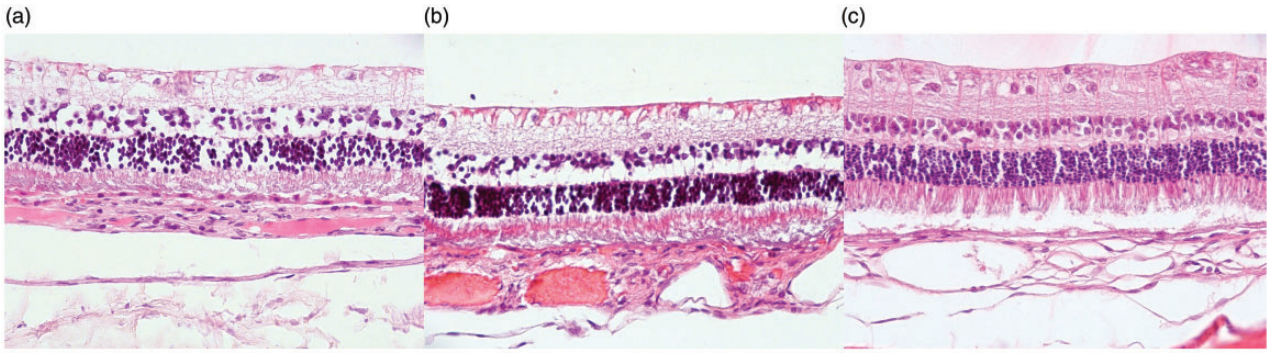

Figure 4. Histology of choroid in samples from (a) LPS-, (b) INF-, and (c) BSS-injected groups. Note the highly dilated vascular channels in INF-injected eye when compared with BSS-injected eye. Choroidal hyperemia is an expected finding in LPS-induced uveitis. Hematoxylin and Eosin ×400. (A color version of this figure is available in the online journal.)

Among inflammatory conditions, choroidal alterations have been described in Vogt-Koyanagi-Harada (VKH) disease,[15] and there are studies reporting the effect of anti-inflammatory agents on the diseased choroid in VKH disease.[16] Mehta *et al*. described the features of the choroid in sarcoidosis and tuberculosis uveitis and found a significant increase in the thickness of the middle layer (Sattler layer) in both types of uveitis.[17] It has been shown that the use of steroids in the treatment of certain forms of inflammation can reverse some changes and improve blood flow.[15] However, to the best of our knowledge, this is the first study to evaluate the effect of intravitreal injection of immunomodulatory agents (IFNs) on choroidal histology and thickness in normal eyes and to compare the findings with diseased and healthy states of this vascular tissue. In the eye, IFNs may exert their anti-inflammatory effects by changing the vitreous microenvironment or by improving the obstruction capacity of retinal capillaries.[12,13]

The exact mechanisms of action of IFNs in controlling inflammatory response have yet to be clarified. Gillies and Su showed that IFN-α2b improves the barrier function of the retinal endothelium and suggested its use as a therapeutic agent in the treatment of retinal diseases caused by vascular leakage.[18] A recent study found that the blood levels of some types of IFNs are correlated with retinal disorders like age-related macular degeneration. Furthermore, vascular changes in some parts of the choroid and retina have been detected in age-related macular degeneration. Interferons may play a role in these vascular changes; however, increased levels of IFNs can be secondary to these vascular changes.[19]

We have previously studied the role of IFNs in the treatment of uveitis at our center and found that intravitreal injection of these cytokines exerts a positive anti-inflammatory effect without significant side effects.[3] In addition, topical application and subtenon injection of IFNs have been investigated in patients with diabetic macular edema and were promising in terms of improving visual acuity and macular function.[20,21] Topical application of IFN-α2b in a patient with pseudophakic macular edema was also successful.[22] Increased blood supply to the ischemic retina and decreased secretion of vascular endothelial growth factor may be the possible mechanisms involved in the success of IFN therapy. The hypothesis of improvement of blood supply is supported by the results of our study, which showed increased vascular volume in the choroid after intravitreal injection of IFN. We observed these changes one week after injection; considering the wash out of this drug from systemic circulation after intramuscular administration[23] (less than 24 h), persistence of the effects in the eye in our study (as late as one week after injection) is a positive point to be considered later in the therapeutic applications and selection of roots of administration. In this study, we observed that IFN-α2b increased choroidal thickness mainly through vascular congestion in the middle and outer parts of the choroid (medium- and large-sized vessels). This finding was considerably more significant in the IFN group in comparison to the LPS and BSS groups. The observed changes may indeed be caused by increased resistance to outflow or simply, an increase in blood flow. It seems that various mechanisms are involved in different situations; for example, in tumor growth, it has been shown that type I interferons play roles in suppression of angiogenic properties of neutrophils.[24]

This study can be considered a starting point for further investigations focusing on its mechanisms of action in normal and disease states of the eye, hopefully, opening a new window for applications of this drug in the treatment of eye diseases.

Authors' contributions: All authors had active participation in design, interpretation, and analysis of data and preparation of the manuscript. ME and MA conducted the study based on ideas from previous observations by ME. HA contributed in data processing and statistical analysis, and MM worked on the preparation of the manuscript which was finally reviewed and approved by all authors.

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DECLARATION OF CONFLICTING INTERESTS

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