

Comparison Between Histopathologic Findings and Indocyanine Green Angiographic Findings in Lewis Rats with Experimental Autoimmune Uveoretinitis

Norio Masaoka, Ayako Takahashi and Atsuki Fukushima

Department of Ophthalmology, Kochi Medical School, Nankoku, Japan

Purpose: Recently, indocyanine green angiography (IA) was used to examine chorioretinal circulation in posterior uveitis in humans, and abnormal IA findings were reported. However, choroidal pathologic examination in conjunction with these abnormal IA findings has rarely been done. Experimental autoimmune uveoretinitis (EAU) is a model for posterior uveitis in humans. The purpose of this study was to correlate abnormal IA findings in Lewis rats with EAU with pathologic findings.

Methods: Eleven male Lewis rats were immunized with bovine S-antigen in complete Freund's adjuvant with *Bordetella pertussis*. After immunization, IA was performed by using a scanning laser ophthalmoscope. Eyes with abnormal IA findings were enucleated and examined histopathologically.

Results: Demonstrated leakage from retinal vessels at the initial stage of disease; a decrease of background fluorescence and of the number of moderate and large vessels of the choroid, and leakage from choroidal vessels at the progressive stage; and hypofluorescent areas at the late stage. Histopathologic examination at the initial, progressive, and late stages revealed inflammation of the inner layers of the retina, a large number of inflammatory cells in the thickened retina and choroid, and impaired choroidal capillaries, respectively.

Conclusion: Since a correlation between pathologic findings and IA findings was demonstrated in Lewis rats with EAU, IA may be useful for evaluating the severity of uveitis in humans. **Jpn J Ophthalmol 2000;44:620–626** © 2000 Japanese Ophthalmological Society

Key Words: Experimental autoimmune uveoretinitis, indocyanine green angiography, Lewis rats, S-antigen.

Introduction

In patients with posterior uveitis, fluorescein angiography (FAG) has been an important method for showing blood-retinal barrier breakdown. However, it is difficult to obtain similar information from the choroid. In recent years, indocyanine green angiography (IA) has been used to observe choroidal circulation in various choroidal disorders,^{1,2} and some abnormal IA findings were reported in posterior uveitis diseases, such as Behçet's disease, Vogt-KoyanagiHarada disease, and sympathetic ophthalmia. However, pathologic examination has rarely been done in conjunction with these abnormal IA findings. Therefore, we decided to correlate abnormal IA findings with pathologic findings in Lewis rats with experimental autoimmune uveoretinitis (EAU).

Experimental autoimmune uveoretinitis is a model for uveitic conditions in humans, such as Behçet's disease, Vogt-Koyanagi-Harada disease, sympathetic ophthalmia, and birdshot retinochoroidopathy. It is induced by immunization with interphotoreceptor retinoid-binding protein (IRBP)³ or S-antigen (S-Ag).⁴ Interphotoreceptor retinoid-binding protein is found at high concentrations in the interphotoreceptor matrix, and S-Ag exists in the photoreceptor outer segments in the retina.

Received: December 9, 1999

Correspondence and reprint requests to: Norio MASAOKA, MD, Department of Ophthalmology, Kochi Medical School, Kohasu, Oko-cho, Nankoku-shi, Kochi-Ken 783-8505, Japan

In this study, we confirmed the usefulness of IA to investigate chorioretinal circulatory disturbances in Lewis rats. In addition, we tried to assess the severity of disease through the use of IA findings.

Materials and Methods

Animals

Male Lewis rats between 6 and 8 weeks of age were used. They were obtained from Seac Yoshitomi (Fukuoka) and were maintained in a pathogen-free animal facility at Kochi Medical School. The maintenance, care, and experimental use of these animals were in compliance with institutional guidelines and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Immunization

Bovine S-Ag (kindly donated by Dr. Igal Gery, NEI, Bethesda, MD, USA) was emulsified (1:1) in complete Freund's adjuvant (Yatron, Tokyo). The emulsion was injected intravenously in the left hind footpad. A total volume of 100 μ L containing 50 μ g of S-Ag and toxoid from 5 × 10⁹ *Bordetella pertussis* (Wako, Osaka) was used for each immunized animal.

Fluorescein and Indocyanine Green Angiography

The Lewis rats were anesthetized with pentobarbital sodium (30 mg/kg) before angiography, and pupils were dilated with 1% tropicamide hydrochloride. Fluorescein angiography and IA were used to examine the 22 eyes of the 11 rats between 10 and 15 days after immunization; the 10 eyes of 5 rats that were not immunized were tested as controls.

A scanning laser ophthalmoscope (Rodenstock Instrument, Munich, Germany) was used for both FAG and IA, and the fundus was investigated with a 60° field with an attachment. The images were recorded on S-VHS tapes. Fluorescein angiography was conducted after IA. Fluorescein and indocyanine green (ICG) angiograms were obtained by using, respectively, 1 mL/kg of a 10% sodium fluorescein solution and 25 mg/kg of ICG (Diagnogreen Injection, Daiichi Pharmaceutical, Tokyo). Both types of angiograms were taken more than 30 minutes after intravenous dye injection.

Preparation of Eyes for Light Microscopy

Eyes were enucleated immediately after angiography, fixed for 15 minutes in 2.5% glutaraldehyde, and then fixed in a 10% formaldehyde solution. All fixed tissues were embedded in paraffin, and sections were stained with hematoxylin-eosin.

Results

Ophthalmoscopic Examination

In the initial stage of EAU, abnormalities of fundi were not revealed by ophthalmoscopy. As EAU progressed, disc swelling, dilated and tortuous retinal veins, and hazy media were detected. However, retinal exudates, retinal pigment epithelial changes and choroidal changes were not apparent. Iris hyperemia and ciliary injection were observed beginning 10 days after immunization. Anterior chamber exudates and thick fibrin membrane around the pupil had formed by 15 days after immunization, and these pathologic changes made fundus examination impossible. Therefore, FAG and IA were performed between 10 and 15 days after immunization.

Indocyanine Green Angiography and Histopathologic Findings

Histopathologic stages in the active phase of EAU were divided into three stages: initial, progressive, and late. In the initial stage, there was inflammation of the inner retinal layers (Figure 1); in the progressive stage, inflammation progressed to the outer lay-



Figure 1. Pathologic changes at initial stage of experimental autoimmune uveoretinitis. Histopathologic findings showed lymphocytes (arrows) infiltrated around several vessels in nerve fiber layer 20(hematoxylin-eosin). Bar = 50μ m. Photoreceptor outer segments (POS) were intact at this time. Retinal pigment epithelium and choroid were also intact.



Figure 2. Pathologic changes at progressive stage of experimental autoimmune uveoretinitis. (A) Histopathologic findings showed destruction of photoreceptor cell layer. Retina and choroid were markedly thickened (hematoxy-lin-eosin). Bar = 50 μ m. (B) There were areas of dilated choroidal capillaries (large arrows) and markedly narrow or obstructed ones (small arrows) (hematoxylin-eosin). Bar = 50 μ m.

ers of the retina and the choroid (Figure 2); and in the late stage there was complete destruction of the photoreceptor layer and subsidence of inflammation (Figure 3).

In control rats, IA angiograms were grossly divided into early, middle and late phases similar to IA phases in humans (Figure 4). Early-phase angiograms clearly showed choroidal and retinal vessels. Middle-phase angiograms showed homogeneous choroidal fluorescence, and the fluorescence from retinal vessels gradually attenuated. Late-phase angiograms showed choroidal vessels as hypofluorescent channels, and retinal vessels were no longer visible.

Several IA findings were observed in each histopathologic stage. In the initial stage, characteristic IA findings were leakage from retinal vessels, and no abnormal choroidal findings. Several hyperfluorescent spots were observed in small retinal vessels in the early phase of IA and were enlarged in the late



Figure 3. Pathologic changes at late stage of experimental autoimmune uveoretinitis. Histopathologic findings showed loss of photoreceptor cell layer. Inflammatory infiltrates around retinal vessels, especially veins (arrow), were noteworthy. Layer of choroidal capillaries was unclear. Choroid was locally thickened (*) (hematoxylin-eosin). Bar = $50 \mu m$.

phase of IA (Figure 5). Histopathologic examination revealed that lymphocytes infiltrated around retinal vessels in the nerve fiber layer. The outer photoreceptor segments, the major sites of S-Ag concentration, were intact (Figure 1).

In the progressive stage, IA revealed spot leaks all over the fundus. Spot leaks were identified not only in peripheral small retinal vessels, but in posterior large retinal vessels (mainly veins). A decrease of fluorescence from background continued from the early phase of IA to the late phase (Figure 6) Because very dense hypofluorescent areas lay scattered, the dark background fluorescence was mottled. The number of moderate and large choroidal vessels decreased markedly on the ICG angiogram. The vitreous became hazy according to the degree of vitritis. In the progressive stage, leakage from choroidal vessels was strongly suspected in 2 eyes (Figure 7). Spot leaks were sometimes detected at the ciliary process. Histopathologic examination showed destruction of the photoreceptor cell layer and infiltration by polymorphonuclear leukocytes and lymphocytes (Figures 2A,B). The retina and choroid were markedly thickened. Many choroidal capillaries were dilated, but a small number of choroidal capillaries were markedly narrow or obstructed (Figure 2B).

In the late stage, IA revealed choroidal hypofluorescent areas in the early phase of IA (Figure 8A) that became small and unclear in the late phase (Figure 8B). In the early phase of IA, choroidal vessels were clearly visible in the choroidal hypofluorescent areas (Figure 8C). Although leakage from retinal vessels was continued (Figures 8A,B), choroidal ves-

N. MASAOKA, A. TAKAHASHI AND A. FUKUSHIMA IA FINDINGS IN LEWIS RATS WITH EAU



Figure 4. Indocyanine green angiograms (IA) in control rats. The IA was grossly divided into early (A), middle (B), and late phases (C). In early phase, retinal and choroidal vessels were apparent. In middle phase, retinal and choroidal vessels were not seen and fluorescence from background became uniform. In late phase, middle-sized and large choroidal vessels became dark channels. Note accumulation of indocyanine green along choroidal vessels (arrows).

sels were clearly observed again in the early phase of IA (Figure 8C). Staining of retinal vessels was also seen (Figure 8B). Because it was impossible to determine the origin of every leakage, the existence of leakage through the retinal pigment epithelium



Figure 5. Indocyanine green angiogram at initial stage of experimental autoimmune uveoretinitis. Spot leaks in late phase of indocyanine green angiography. Retinal vessels were already unclear.

(RPE) could not be completely ruled out. The photoreceptor cell layer was completely destroyed. Choroidal capillaries were unclear (Figure 3).

Fluorescein Angiography

Fluorescein angiography, as well as IA, demonstrated leakage from retinal vessels throughout the disease (Figure 9). However, excessive fluorescence from the background prevented FAG from clearly showing the leakage. Moreover, FAG could not reveal the delay in choroidal filling apparently because sodium fluorescein rapidly spread into the areas where IA showed hypofluorescence.



Figure 6. Indocyanine green (ICG) angiogram showing dark background fluorescence at progressive stage of experimental autoimmune uveoretinitis. Background fluorescence was mottled due to scattered dense hypofluorescent areas (arrows). Number of visible choroidal vessels was few throughout ICG angiography except for large vessels.



Figure 7. Indocyanine green (ICG) angiograms showing leakage from choroidal vessels in progressive experimental autoimmune uveoretinitis rats. (A) Early-phase ICG angiogram showing hyperfluorescent spots in choroidal vessel (arrows). (B) ICG angiogram showing enlargement of hyperfluorescent spots (arrows).

Discussion

Indocyanine green, the dye used in IA, has a higher molecular weight than sodium fluorescein (775 vs. 376) and is highly bound to blood proteins.^{5,6} When ICG is bound to blood proteins, it becomes larger and is less able to escape through the fenestrations in choroidal capillaries. This property of ICG was useful for observing the disturbed retinal and choroidal vasculature in this study. Fluoroscein angiography has been known as the most useful method for clinically estimating the blood-retinal barrier breakdown. In pigmented eyes, RPE blocks background fluorescence from the choroid. Because the Lewis rats were albino, the nonpigmented RPE could not sufficiently block intense background fluorescence, making it difficult to use FAG to investigate leakage from sites of blood-retinal barrier breakdown (Figure 9). However, the slow progression of leaks of ICG in the retinal and choroidal ves-



Figure 8. Indocyanine green (ICG) angiograms of same eye as in Figure 3. (**A**) Spot leaks of ICG from many areas in retinal vessels over fundus (arrowheads) were detected in early phase of indocyanine green angiography (IA), which also revealed choroidal hypofluorescent areas (arrows). Asterisks in Figures 8 and 9 indicate same vein. (**B**) Spot leaks enlarged (arrowheads) and staining of retinal vessels (large arrow) became clear in late phase of IA. Choroidal hypofluorescent areas became small and unclear (small arrows). (C) In early phase of IA, choroidal vessels were clearly visible in choroidal hypofluorescent areas (arrow).

sels helped us to observe the sites of blood-retinal barrier breakdown even in albino rats.

The data obtained from this study are summarized in Table 1. In posterior uveitis in humans, such as



Figure 9. Fluorescein angiogram (FA) of same eye as in Figures 3 and 8. The FA could not clearly reveal abnormalities of chorioretinal circulation because of excessive fluorescence from background. Asterisk shows same vein as in Figure 8A.

Vogt-Koyanagi-Harada disease, we^{7,8} and others^{1,9,10} by using IA have demonstrated leakage in the choroid, a marked decrease of background fluorescence, a decrease in the number of moderate and large choroidal vessels, and hypofluorescent areas. The data in this study agree with these previous reports.

Leakage from choroidal vessels was strongly suspected in 2 eyes in the progressive stage in this study. Hyperfluorescence areas were seen around large choroidal vessels. Because these areas gradually enlarged and moved as the choroidal vessels moved, we concluded that the leakage was from large choroidal vessels. However, even in the normal rats, hyperfluorescent areas along choroidal vessels were seen in the late phase of IA when choroidal vessels became dark channels (Figure 4C). We were able to distinguish leakage from choroidal vessels from the hyperfluorescent areas seen in normal rats because leakage from choroidal vessels was seen in the early phase of IA and increased in the late phase of IA. In our study, histopathologic examination of progressive cases revealed infiltration of inflammatory cells in the choroid. We concluded that choroidal vessels were occasionally impaired in these cases.

In the eyes that showed a marked decrease of background fluorescence and of the number of moderate and large choroidal vessels on the ICG angiograms, histopathologic examination revealed thickened retina and choroid, many dilated choroidal capillaries, and no apparent abnormal large choroidal vessels. From these findings, we hypothesized that the decrease in background fluorescence and in the number of moderate and large choroidal vessels detected by IA was caused by blockage of fluorescence from the background, resulting from inflammatory products. Indocyanine angiography also revealed very dense hypofluorescent areas. Because histopathologic examination revealed that many choroidal capillaries were dilated and a small number of choroidal capillaries were markedly narrow (Figure 2B), we concluded that the dense hypofluorescent areas corresponded to the areas of markedly narrow choroidal capillaries.

In the eyes that showed hypofluorescent areas in the late stage of disease, histopathologic examination only barely revealed small choroidal vessels in the hypofluorescent areas. Inflammatory cells in the

 Table 1. Indocyanine Green Angiography (IA) and Histopathologic Findings at Each Stage of Experimental Autoimmune Uveoretinitis

Disease Stage (Examined Eyes)	Days After Immunization (mean ± SD)	IA Findings*	Histopathologic Findings*
Initial stage (5 eyes)	14.0 ± 1.2	Leakage from small retinal vessels in periphery.	Lymphocytic infiltration around retinal vessel in nerve fiber layer, intact photoreceptor cell layer.
Progressive stage (5 eyes)	12.4 ± 0.5	Findings seen in initial stage, leakage from large retinal vessels, tortuosity of retinal veins, dark background fluorescence, leakage from choroidal vessels (rare), decreased number of choroidal vessels, hazy media.	Destroyed photoreceptor layer, thickening of each layer of retina and chorid; marked infiltration by inflammatory cells in each layer of retina, choroid, and vitreous.
Late stage (4 eyes)	11.0 ± 1.0	Subsidence of choroidal findings seen in progressive stage, choroidal filling delay.	Photoreceptor cell layer completely destroyed, impaired choroidal capillaries, locally thickened choroid, mild infiltration by inflammatory cells.

*Abnormal IA and histopathological findings were not detected in 8 of the 22 eyes.

retina and choroid were few and edema of the retina and choroid almost disappeared. These findings suggest that hypofluorescent areas were caused by a delay in choroidal filling. The delay in choroidal filling differed from blockage of background fluorescence to the extent that the choroidal vessels were clearly revealed in the hypofluorescent areas by IA.

To summarize, the data presented here suggest that (1) clearly visible choroidal vessels in the early phase of IA and light background fluorescence meant intact choroid, (2) a marked decrease in background fluorescence and in the number of moderate and large choroidal vessels was due to marked edema of the retina and choroid, and (3) dense choroidal hypofluorescence was caused by the delay in choroidal filling.

Indocyanine green angiography facilitates examination of disorders of the posterior segment and makes follow-up possible. Indocyanine green angiography findings may be helpful for evaluating the severity of uveitis in the same eye at different times.

Howe et al¹¹ and Okada et al¹² reported that RPE lesions resembling Dalen-Fuchs nodules were observed by light or electron microscopy in the convalescent phase of EAU and that IA revealed hyperfluorescent or hypofluorescent spots in areas of these lesions. However, we could not detect such findings using both IA and histopathology. We conclude that the reason is the short observation period of ours.

In the intial stage, infiltration of lymphocytes around retinal vessels was detected. Because it is strongly suspected that sensitized T cells play an important role in the occurrence of the blood–retinal barrier breakdown,^{13,14} we think it necessary to study the movement of sensitized T cells in rats with EAU. Experimental autoimmune uveoretinitis can be induced by passive immunization by adoptive transfer of antigen-sensitized T cells as well as by active immunization.^{14,15} Therefore, visualization of adoptively transferred T cells is now under investigation in our laboratory to clarify the direct involvement of sensitized T cells in the blood–retinal barrier breakdown.

In conclusion, IA is a useful method for obtaining information about abnormal vascular changes in Lewis rats with EAU. Moreover, the data presented here suggest that IA may be a candidate for evaluating the efficacy of treatment as well as for evaluating the severity of uveitis. The authors gratefully acknowledge the advice of Dr. Hisayuki Ueno.

References

- Guyer DR, Yannuzzi LA, Slakter JS, Sorenson JA, Orlock DA. Diagnostic indocyanine green videoangiography. In: Schachat AP, Murphy RP, eds. Retina. Vol. 2. St. Louis, MO: Mosby-Year Book, 1994:985–996.
- Regillo CD, Maguire JI. Indocyanine green angiography. In: Benson WE, ed. Clinical ophthalmology. Vol. 3. Philadelphia, PA: Lippincott-Raven Press, 1994:1–13.
- Gery I, Wiggert B, Redmond TM, et al. Uveoretinitis and pinealitis induced by immunization with interphotoreceptor retinoidbinding protein. Invest Ophthalmol Vis Sci 1986;27:1296–300.
- 4. de Kozak Y, Sakai J, Thillaye B, Faure JP. S antigen-induced experimental autoimmune uveo-retinitis in rats. Curr Eye Res 1981;1:327–37.
- Cherrick GR, Stein SW, Leevy CM, Davidson CS. Indocyanine green: observation on its physical properties, plasma decay, and hepatic extraction. J Clin Invest 1960;39:592–600.
- Fox IJ, Wood EH. Indocyanine green: physical and physiologic properties. Staff Meet Mayo Clin 1960;35:732–44.
- Masaoka N, Yasuoka K, Ueno H. Indocyanine green angiography in Harada's disease. Rinsho Ganka (Jpn J Clin Ophthalmol) 1994;48:569–72.
- Nishino K, Shimodera Y, Fukushima A, Masaoka N, Ueno H, Wariishi S. A case of Harada disease with choroidal detachment. Ganka Rinsho Iho (Jpn Rev Clin Ophthalmol) 1998;92:920–3.
- Yuzawa M, Kawamura A, Matsui M. Indocyanine green video-angiographic findings in Harada's disease. Jpn J Ophthalmol 1993;37:456–66.
- Oshima Y, Harino S, Hara Y, Tano Y. Indocyanine green angiographic findings in Vogt-Koyanagi-Harada disease. Am J Ophthalmol 1996;122:58–66.
- Howe LJ, Stanford MR, Whiston R, Dewhirst R, Marshall J. Angiographic abnormalities of experimental autoimmune uveoretinitis. Curr Eye Res 1996;15:1149–55.
- Okada AA, Goto H, Mizusawa T, Morimoto K, Ebihara Y, Usui M. Angiography of experimental autoimmune uveoretinitis ultrastructural correlation. Graefes Arch Clin Exp Ophthalmol 1998;236:865–72.
- Lightman S, Greenwood J. Effect of lymphocytic infiltration on the blood-retinal barrier in experimental autoimmune uveoretinitis. Clin Exp Immunol 1992;88:473–7.
- 14. Prendergast RA, Iliff CE, Coskuncan NM, et al. T cell traffic and the inflammatory response in experimental autoimmune uveoretinitis. Invest Ophthalmol Vis Sci 1998;39:754–62.
- 15. Mochizuki M, Kuwabara T, McAllister C, Nussenblatt RB, Gery I. Adoptive transfer of experimental autoimmune uveoretinitis in rats. Invest Ophthalmol Vis Sci 1985;26:1–9.