Retinal Circulatory Changes Associated with Interferon-Induced Retinopathy in Patients with Hepatitis C

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Purpose. To evaluate the effect of interferon (IFN) therapy on retinal microcirculation.

Methods. Thirty-six patients with chronic hepatitis C who were treated with high-dose IFN were included in this prospective study. The changes in vessel diameter and blood velocity were measured, and the retinal blood flow (RBF) and wall shear rate (WSR) were calculated in the retinal arteries before and 2, 4, 8, 16, and 24 weeks after IFN therapy by using laser Doppler velocimetry.

Results. Retinal blood velocity, RBF, and WSR significantly ($P < 0.0001$) increased in all patients, as early as 2 weeks after IFN therapy. The increase in RBF was independently correlated with a decrease in the red blood cell count. In 22 (61%) of the 36 patients asymptomatic retinopathy developed during treatment. In patients with retinopathy, the blood velocity and WSR increased, but the vessel diameter did not change, whereas the vessel diameter increased but the blood velocity and WSR did not change in patients without retinopathy 2 weeks after IFN therapy. Multiple logistic regression analysis showed that patient age and the change in WSR at week 2 were risk factors for the development of IFN-induced retinopathy.

Conclusions. RBF increases in association with IFN therapy in patients with chronic hepatitis C. In addition, the increased WSR in patients with retinopathy indicates that retinal vascular endothelial dysfunction may be associated with IFN-induced retinopathy, because wall shear stress should be constant under physiologic conditions. (*Invest Ophthalmol Vis Sci.* 2007; 48:368–375) DOI:10.1167/iovs.06-0182

Interferon (IFN) has been used clinically to treat numerous viral and malignant diseases in many organs including the eye.[1] However, IFN therapy often is associated with ocular side effects. Although most resolve while treatment continues and are asymptomatic,[2] severe ocular complications can occur, including branch vein occlusion,[3,4] retinal rubeosis,[5] and cystoid macular edema,[6,7] which cause severe visual loss. Although the exact mechanism of IFN-induced retinopathy is unknown, it may be related to a disrupted retinal microcirculation, as reported in studies using fluorescein angiography, that showed poorly perfused retinal areas in patients with IFN retinopathy.[8,9] It has also been reported that IFN-α increased leukocyte adherence to the vascular endothelium and subsequent leukocyte trapping in the rat retinal microcirculation, suggesting that the impaired retinal circulation may be associated with IFN-induced retinopathy.[10] In addition, one clinical study reported that flow-mediated vasodilation in the brachial artery decreased in patients with chronic hepatitis C treated with IFN, suggesting that IFN impairs endothelial function.[11] These findings suggest that the impaired vascular function in the retinal microcirculation may be associated with IFN-induced retinopathy. However, to our knowledge, the effect of IFN therapy on the retinal microcirculation in humans has not been studied.

We recently showed that a retinal laser Doppler velocimetry (LDV) system is a reliable, noninvasive, and useful tool to evaluate the retinal microcirculation in humans.[12] The system enables simultaneous measurement of vessel diameter and blood velocity and calculates the absolute retinal blood flow (RBF) and wall shear rate (WSR), which is an index of wall shear stress,[13,14] an important physiologic stress on the retinal vessels. We also reported that flow-induced vasodilation, which is an endothelium-dependent mechanism, is involved in increased RBF during hypoxia by measuring feline retinal WSR[13]; this study suggested that LDV is useful for evaluating the retinal circulation and endothelial function. In the present study, using LDV to test the hypothesis that the impaired blood flow and endothelial function in the retinal circulation are associated with the development of IFN-induced retinopathy, we examined the effect of IFN therapy on the retinal microcirculation in patients with hepatitis C. We also evaluated whether the changes in retinal microcirculation in the early phase of IFN treatment can predict the onset of IFN retinopathy.

Methods

Patients

The study included 36 consecutive patients (25 men, 11 women; age, mean ± SE 53.8 ± 3.7 years; range, 33–60) with chronic hepatitis C who began treatment with IFN between April 2002 and October 2003. All were inpatients at Asahikawa Medical College, Asahikawa, Japan. This study conformed to the guidelines approved by the ethics committee at our institution. Each subject provided written informed consent before enrollment in the study after receiving a thorough explanation of the study design and protocol. The procedure adhered to the tenets of the Declaration of Helsinki.

Patients were eligible for the study if they had had an elevated alanine aminotransferase level for at least 6 months, seropositivity for anti-hepatitis C virus (HCV) antibodies determined using a third-gen-

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Supported by Grant-in-Aid for Young Scientists (B)14770940 and (B)16791037 (TN) and Grant-in-Aid for Scientific Research (C)18591904 (AY) from the Ministry of Education, Science, and Culture, Tokyo, Japan; the Akiyama Foundation, Sapporo, Japan (TN); the Jamcon Award (TN) and the Uehara Memorial Foundation, Tokyo, Japan (TN).

Submitted for publication February 20, 2006; revised July 11, 2006; accepted November 28, 2006.

Disclosure: **T. Nagaoka**, None; **E. Sato**, None; **A. Takahashi**, None; **S. Yokohama**, None; **A. Yoshida**, None

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eration enzyme-linked immunosorbent assay (Ortho Diagnostic System, Raritan, NJ), tested positive for HCV RNA in serum, and had a liver biopsy within 2 months of study enrollment with a histopathologic evaluation of chronic hepatitis by an experienced hepatologist (SY). Fifteen patients previously had received IFN monotherapy without a sustained virologic response. The patients who relapsed after IFN monotherapy were enrolled in the present study after a washout period of more than 1 year after the cessation of the previous IFN monotherapy. Patients with evidence of a decompensated liver, hepatitis B surface antigen, hepatitis B core antibody, as determined by radioimmunoassay (Abbott Laboratories, North Chicago, IL), were excluded. Patients with cardiovascular disease and renal failure were also excluded. Renal function was evaluated by a serum creatinine concentration (<1.5 mg/dL) and a 24-hour estimated creatinine clearance (<30 mL/min). In addition, the diagnosis of cardiovascular disease included coronary heart disease (coronary insufficiency, recognized myocardial infarction), congestive heart failure, and ischemic stroke. In addition, we have already screened patients for cryoglobulinemia, which is reported to be associated with HCF infection,[15] by measuring ANA and ENA before interferon treatment. These diagnoses were established by a well-trained specialist (SY) in our hospital who was masked to the information from the ocular examination.

Clinical Characteristics

Patients were considered to have hypertension if systolic/diastolic blood pressure exceeded 140/90 mm Hg or the patient was using antihypertensive drugs. Patients were considered to have diabetes if they were being treated with insulin or oral hypoglycemic agents or if fasting blood glucose exceeded 140 mg/dL. Patients with a plasma total cholesterol level greater than 220 mg/dL, a plasma low-density lipoprotein cholesterol level over 130 mg/dL, or both or those who were receiving cholesterol-lowering medication were classified as having hypercholesterolemia.

The baseline clinical characteristics included cigarette smoking in 13 (36%) patients, hypertension in 11 (31%), diabetes mellitus in 11 (31%), and both hypertension and diabetes in 4 (11%; Table 1). All patients with diabetes had type 2 diabetes mellitus. Patients with poorly controlled diabetes (hemoglobin A1c >7.0%) and hypertension (systolic/diastolic blood pressure >160/100 mm Hg) were excluded. In 11 patients with hypertension, six patients used a calcium channel blocker, two used an angiotensin converting enzyme inhibitor, and three used an AT1 receptor blocker. In 11 patients with diabetes, six used hypoglycemic agents and five did not require medication for glycemic control. These patients had not changed any medications at least 6 months before and during IFN treatment. In two patients with hyperlipidemia, their plasma cholesterol level was well controlled without medication.

All patients underwent a baseline ophthalmic evaluation before they started IFN treatment. All patients had good visual acuity (>20/20) and normal intraocular pressure (IOP; <20 mm Hg). Patients with a dense cataract, visual field abnormality, glaucoma, or any other ocular abnormalities were excluded.

Treatment Protocol

All patients received 6 million units of intramuscular IFN-α therapy six times weekly for 2 weeks, followed by three times weekly for 22 weeks. Patients were categorized as having a low viral load ($<10^5$ copies/mL) or a high viral load (10^5 copies/mL). Patients with a low viral load were treated with IFN monotherapy by intramuscular injection of 6 million units of natural IFN-α. Patients with a high viral load were treated with intramuscular injections of recombinant IFN α-2b, at a dose of 6 million units, in combination with oral ribavirin, which was administered twice daily. The total daily dose was 600 mg for patients whose weight was less than 60 kg and 800 mg for those whose weight was more than 60 kg.[16] The dose of ribavirin was reduced based on hemoglobin concentrations and patient symptoms: ribavirin was reduced to 600 or 400 mg/d when the hemoglobin concentration fell below 10 g/dL and was discontinued when the hemoglobin levels declined below 8.5 g/dL.[16]

RBF Measurement

A retinal LDV system (Laser Blood Flowmeter, model CLBF 100; Canon, Tokyo, Japan) was used to estimate the blood flow in the superior branch of the first-order major temporal retinal artery. The methodology of this system has been described in detail elsewhere.[12,14,17] Briefly, the retinal LDV system allows noninvasive measurement of the absolute values of the red blood cells (RBCs) flowing in the centerline of the vessel, based on the bidirectional LDV.[18] The Doppler-shifted light scattered from the RBCs flowing in the retinal vessel is detected simultaneously in two directions separated by a fixed angle. The signals from the two photomultiplier tube detectors undergo computer-controlled spectrum analysis, and sequential measurement of the maximum speed (V_{max}) at the center of the vessel. The retinal blood velocity was defined as the averaged V_{max} during one cardiac cycle. In addition, this device also contains a vessel diameter measurement system and a vessel tracking system.[12] The diameter of the retinal artery is determined automatically by computer analysis of the signal produced by the arterial image on the array sensor using the half height of the transmittance profile to define the vessel edge. All steps throughout the observation of a patient's fundus were virtually the same. To evaluate the reproducibility of the LDV system in our patient, we calculated the average coefficient of variation (CV) (100[SD/mean]; mean $\pm$ SD) during the first visit to our hospital. We obtained five readings from the same vessel every minute for 5 minutes and averaged them in each subject and calculated the CV in each vessel for each subject.

Study Design

All patients underwent clinical and laboratory assessments, ophthalmic examination, and RBF measurement before and at weeks 2, 4, 8, 16, and 24 after the start of IFN treatment. RBC count, white blood cell (WBC) count, hematocrit, and hemoglobin were measured in our laboratory by routine methods. Systolic, diastolic, and mean arterial (MABP) blood pressures and heart rate were estimated by electronic

Table 1. Clinical and Laboratory Characteristics of Patients at Baseline

Parameter		SD
Sex (male:female)	25:11	
Mean age, y (range)	53 (30–69)	10.2
HCV RNA viral road: K IU/mL (mean, range)	666 (18–2130)	485.6
HDV genotype (1b:2a:2b)	32:2:2	
ALT level pretreatment: U/L (mean, range)	98 (22–534)	92.7
BMI (mean, range)	24.0 (17.3–36.3)	3.8
Diabetes, *n* (%)	11 (31)	
Hypertension, *n* (%)	11 (31)	
Hyperlipidemia, *n* (%)	2 (6)	
Current smoking, *n* (%)	13 (36)	

ALT, alanine aminotransferase; BMI, body mass index.

sphygmomanometer (EP-88Si; Colin, Tokyo, Japan). IOP was monitored by applanation tonometry (Haag Streit, Bern, Switzerland). After pupils were dilated with a combination of 0.5% tropicamide and 1% phenylephrine eye drops, the status of the retinopathy was assessed by indirect ophthalmoscopy and slit-lamp examination with a 90-D lens at every visit by a well-trained ophthalmologist, who was masked to the information about RBF measurement. Fundus photographs also were taken to document ocular complications. We defined IFN-induced retinopathy if patients had retinal hemorrhage, cotton-wool spots, or both during IFN treatment. No patient with diabetes had retinopathy before IFN treatment. In addition, we confirmed that all patients with hypertension had no retinopathy using a standard classification.[19,20] To exclude the possibility of the development of diabetic or hypertension retinopathy after IFN treatment, we deleted the data obtained from patients who had diabetes or hypertension and any retinopathy 1 month after the cessation of IFN treatment. After the ocular examination, the RBF was measured. The subjects abstained from drinking coffee and smoking cigarettes for at least 2 hours before the test.

Calculations

As previously described, the RBF was calculated as RBF = $V_{mean} \times$ area, where V_{mean} is the mean blood velocity calculated as $V_{mean} = V_{max}/2$, and area is the cross-sectional area of the retinal artery at the laser Doppler measurement site.[14,21] The factor of 2 in the formula for blood flow arises from the assumption of Poiseuille flow.[14,21] Ocular perfusion pressure (OPP) was determined by the formula OPP = 2/3(MABP) − IOP.[22] WSR was not measured directly in this model but was calculated using a Poiseuillean parabolic model of velocity distribution across the arterial lumen according to the formula[14,23,24]: $WSR = 8 \times V_{mean}/D$.

Data Analysis

All data are expressed as the mean ± SD. For statistical analysis, we used analysis of variance (ANOVA) for repeated measurements followed by post hoc comparison with the Dunnett procedure. The differences between the two groups were analyzed by Student's unpaired *t*-test. To evaluate the relation between the change in RBF and other factors, simple and multiple regression analyses were performed. Variables predictive of IFN-induced retinopathy, including retinal circulatory parameters, were analyzed by logistic regression analysis. $P < 0.05$ was considered statistically significant. All analyses were conducted with statistical software (StatView 5.0J; SAS Institute, Cary, NC).

Results

Twenty-two patients with a high viral load received combination therapy with IFN-α2b plus ribavirin; 14 patients with a low viral level received monotherapy with natural IFN-α. In all patients, the initial dose of IFN was maintained until the end of therapy. Five patients receiving the combination therapy could not continue on ribavirin during the treatment periods because of a drop in hemoglobin; in one of them, the hemoglobin decreased to <10 g/dL, and the ribavirin dose was decreased from 800 to 600 mg/d. Hemoglobin level did not go below 9 g/dL during the treatment and did not exceed 10 g/dL at the end of treatment in our patients.

Changes in Systemic Parameters

The mean hemoglobin values, WBC counts, RBC counts, and platelet counts decreased significantly ($P < 0.0001$) during IFN treatment (Table 2). There were no changes in systemic blood pressure, IOP, or OPP during treatment (Table 2).

Ocular Findings

All patients had good visual acuity (>20/20) and no visual symptoms during IFN treatment. Twenty-two (61%) of 36 patients developed an asymptomatic retinopathy (retinal hemorrhage [18%], cotton-wool spots [41%], or both [41%]) during treatment. The retinopathy was bilateral in 18 (82%) cases and unilateral in 4. The retinopathy was first diagnosed 2 to 16 weeks after the start of treatment and resolved at the end of treatment in 13 patients. A few cotton-wool spots, retinal hemorrhages, or both were observed in the remaining nine patients at the end of treatment but resolved within 1 month after treatment was stopped. Retinopathy did not recur in any patient after the IFN-induced retinopathy resolved. No other ocular complications (i.e., disc edema, cystoid macular edema, or subconjunctival hemorrhage) were observed.

Retinal Microcirculatory Responses during IFN Treatment

The CVs from vessel diameter, blood velocity, and RBF were 2.4% ± 0.2%, 11.1% ± 1.0%, and 10.7% ± 0.9%, respectively, which is similar to those obtained from healthy volunteers in our previous study.[14] The retinal microcirculation was measured bilaterally. For analysis, we used the data from the right eye of patients without retinopathy. In patients with retinopathy, we used the data obtained from the worse eye, which

Table 2. Changes in Ocular and Systemic Parameters during IFN Therapy

	Baseline	2 Weeks	4 Weeks	8 Weeks	16 Weeks	24 Weeks
Retinal vessel diameter (μm)	107.8 ± 11.4	109.8 ± 11.4	109.2 ± 11.2	109.7 ± 11.4	108.0 ± 12.5	108.4 ± 10.0
Retinal blood velocity (mm/s)	38.1 ± 8.8	46.5 ± 12.5**	46.8 ± 10.6**	45.8 ± 9.9**	47.1 ± 13.6**	44.5 ± 9.5
RBF (μL/min)	10.5 ± 2.8	13.3 ± 4.1**	13.5 ± 4.8**	13.1 ± 3.7**	13.0 ± 4.3**	12.4 ± 3.2
Retinal WSR (/s)	1434 ± 384	1713 ± 505**	1719 ± 387**	1687 ± 430*	1771 ± 568**	1655 ± 410
IOP (mm Hg)	14.3 ± 2.4	14.1 ± 2.6	14.6 ± 2.6	14.5 ± 3.7	13.8 ± 2.8	15.1 ± 3.2
Systolic blood pressure (mm Hg)	128.9 ± 19.7	125.0 ± 20.4	120.8 ± 17.4	125.7 ± 14.9	125.0 ± 13.9	126.2 ± 18.3
Diastolic blood pressure (mm Hg)	75.1 ± 11.6	73.0 ± 12.3	72.1 ± 10.7	74.5 ± 10.9	74.5 ± 8.6	76.5 ± 11.3
Mean blood pressure (mm Hg)	93.0 ± 13.2	90.3 ± 14.1	88.3 ± 12.6	91.5 ± 11.3	91.3 ± 9.9	93.1 ± 13.0
Ocular perfusion pressure (mm Hg)	47.5 ± 1.5	46.1 ± 1.6	46.6 ± 1.4	47.4 ± 1.3	46.8 ± 1.1	47.6 ± 1.4
Heart rate (beats/min)	74.3 ± 2.9	70.5 ± 3.7	68.9 ± 2.7	74.4 ± 5.7	73.0 ± 3.2	68.9 ± 3.4
WBC (10^3/μL)	4.8 ± 1.6	3.6 ± 1.6**	3.5 ± 1.3**	3.3 ± 1.0**	3.5 ± 1.6**	4.1 ± 1.4*
RBC (10^6/μL)	4.4 ± 0.5	4.2 ± 0.7*	3.9 ± 0.7**	3.8 ± 0.6**	3.8 ± 0.7**	4.0 ± 0.6**
Hemoglobin (g/dL)	13.8 ± 1.6	12.8 ± 2.0**	12.1 ± 1.9**	11.8 ± 1.9**	11.6 ± 2.0**	12.4 ± 1.7**
Hematocrit (%)	41.1 ± 4.5	38.1 ± 5.9**	36.3 ± 5.2**	35.9 ± 5.2**	35.5 ± 5.2**	38.6 ± 4.5**
Platelet (×10^3/μL)	170.4 ± 60.2	123.1 ± 50.3**	146.7 ± 59.5	149.7 ± 52.6	149.4 ± 63.2	165.1 ± 65.6

Data are expressed as the mean ± SD.
* $P < 0.05$; ** $P < 0.01$ compared with the baseline.

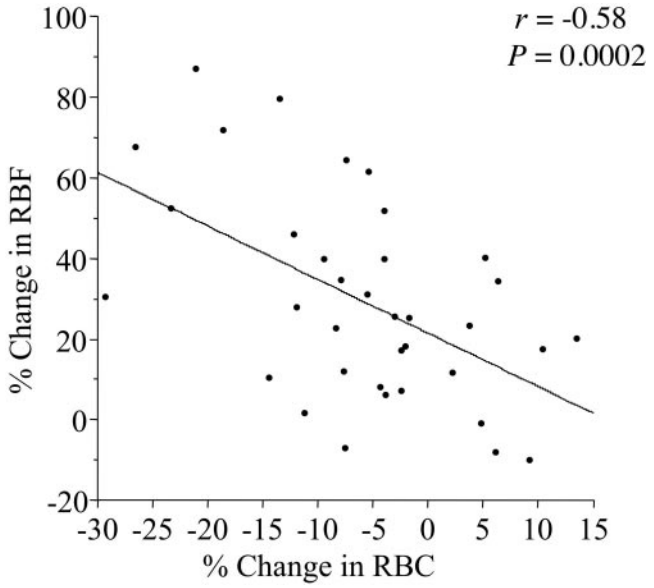

FIGURE 1. Correlations between the changes in RBF and RBC counts 2 weeks after IFN treatment.

was determined by the number of cotton-wool spots, retinal hemorrhages, or both, to evaluate the relationship between the changes in systemic condition and retinal circulatory parameters during IFN treatment.

In all patients, the average vessel diameter tended to increase slightly but did not reach significance during IFN treatment. However, blood velocity, RBF, and WSR significantly ($P < 0.0001$) increased as early as 2 weeks after the IFN therapy (Table 2) and remained elevated until week 16. At 24 weeks of IFN therapy, these retinal circulatory parameters were still increased, but the increases did not reach significance. Within 1 month after the end of IFN therapy, all retinal circulatory parameters returned to baseline in all patients (data not shown). We examined the relationship between the changes in retinal circulatory parameters and some systemic parameters after 2 weeks of IFN treatment. Simple regression analysis showed significant negative correlations between the changes in RBF and RBCs ($r = -0.58$, $P = 0.0002$, Fig. 1), hematocrit ($r = -0.57$, $P = 0.0003$), and hemoglobin ($r = -0.51$, $P = 0.001$) 2 weeks after IFN therapy. To avoid multicolinearity on multiple regression analysis, we selected only the changes in RBCs, because the changes in RBCs, hematocrit, and hemoglobin were strongly ($r > 0.9$) correlated with each other. Table 3 shows the results of multiple regression analysis of the changes in RBF 2 weeks after IFN treatment in relation to RBCs, WBCs, platelet count, and mean blood pressure 2 weeks after IFN-treatment; age; sex; the presence of hypertension and diabetes; and the type of IFN. The changes in RBCs ($P = 0.0001$) and WBCs ($P = 0.04$) were correlated independently with the change in RBF at 2 weeks of IFN treatment (Table 3).

Differences in Retinal Microcirculation with and without Retinopathy

The patients were divided into two groups based on the presence of retinopathy during treatment, and the changes in the retinal circulatory parameters at 2 weeks in both groups (Fig. 2) were compared. In the patients with retinopathy ($n = 22$), the blood velocity, RBF, and WSR increased by 29.8% ± 5.6% ($P < 0.0001$), 30.5% ± 5.5% ($P < 0.0001$), and 30.5% ± 6.6% ($P = 0.0002$), respectively, compared with baseline, whereas the vessel diameter did not change significantly ($P = 0.88$). In the patients without retinopathy ($n = 14$), the vessel diameter, blood velocity, and RBF increased by 6.2% ± 1.2% ($P = 0.0003$), 12.0% ± 5.0% ($P = 0.04$), and 27.1% ± 7.3% ($P = 0.004$), respectively, compared with baseline, whereas the WSR did not change significantly ($P = 0.40$). Significant differences in the changes in vessel diameter ($P = 0.008$), blood velocity ($P = 0.04$), and WSR ($P = 0.007$) were seen between groups, whereas the degree of the increase in RBF was similar in both groups ($P = 0.63$).

In addition, we divided the patients with retinopathy into two groups based on the number of hemorrhages and soft exudates. Mild retinopathy was defined as fewer than four hemorrhages, soft exudates, or both during IFN treatment ($n = 16$, group-averaged number of hemorrhages and soft exudates, 2.2 ± 0.3 counts [mean ± SE]; range, 1–4) and severe retinopathy was defined as more than five hemorrhage and soft exudates ($n = 6$, group-averaged number of hemorrhages and soft exudates, 10.3 ± 1.9 counts [mean ± SE]; range, 6–18). This subgroup analysis revealed a significant difference in the change in WSR treatment between the mild and severe group 2 weeks after IFN (20.1% ± 6.7% in the mild group vs. 49.4% ± 8.8% in the severe group, $P = 0.02$). These results suggest that the increased WSR may be related to the severity of IFN-induced retinopathy.

Association between Changes in Retinal Microcirculation and Development of IFN Retinopathy

To determine the risk factors for the development of IFN-induced retinopathy, multivariate logistic regression analysis was performed. Our results indicated that the change in WSR at week 2 and patient age were significant risk factors for the onset of IFN-induced retinopathy during IFN therapy (Table 4).

Relationship between Systemic Disease and the Changes in Retinal Circulatory Parameters

To evaluate whether various systemic diseases, such as hypertension, diabetes, and hyperlipidemia, have any effect on the change in retinal circulatory parameters in patients with IFN treatment, we divided all patients into two groups (i.e., those with or without systemic disorders). There results showed no differences in any retinal circulatory parameters at baseline and during IFN treatment between the two groups (Table 5).

DISCUSSION

Many investigators have speculated that the mechanism of IFN-induced retinopathy may be the disruption of the retinal microcirculation, because IFN-induced retinopathy is characterized by capillary nonperfusion, cotton-wool spots, and reti-

TABLE 3. Multiple Regression Analysis of Independent Variables Affecting the Change in RBF 2 Weeks after IFN Therapy

Independent Variable	Standardized Coefficient	*P*
Change in RBC	−0.79	0.0001
Change in WBC	0.39	0.04
Presence of hypertension	−0.31	0.06
Change in platelets	−0.28	0.09
Change in MABP	−0.14	0.43
Presence of diabetes	−0.10	0.53
Age	0.07	0.69
Type of IFN	0.04	0.81
Sex	−0.01	0.94

$r^2 = 0.57$; $P = 0.007$.

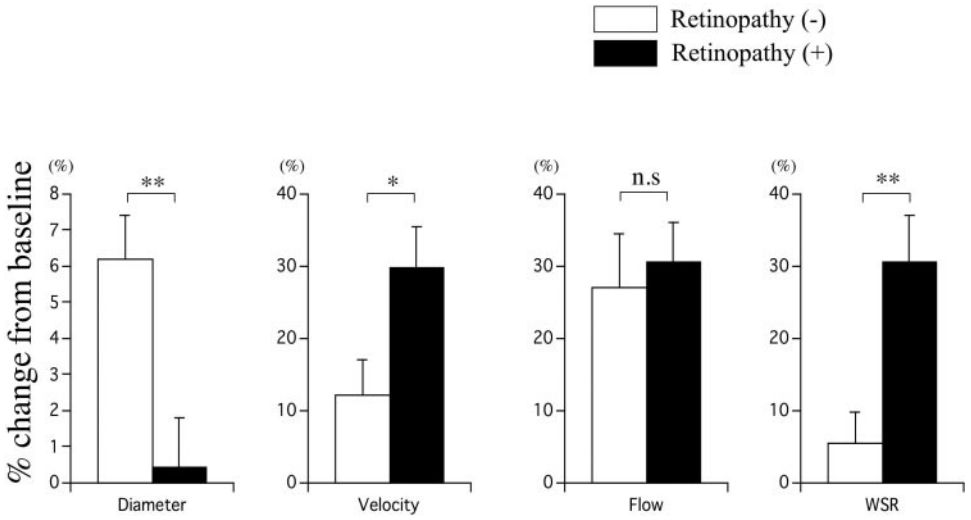

FIGURE 2. Percentage increase in retinal vessel diameter, blood velocity, RBF, and WSR 2 weeks after IFN treatment in patients with and without IFN-induced retinopathy. $^{*}P < 0.05$; $^{**}P < 0.01$; n.s., not significant.

nal hemorrhage, all indicators of retinal ischemic changes.[9,25] However, to the best of our knowledge, no study has examined the effect of IFN therapy on changes in the retinal circulation in humans. We showed for the first time that there was a significant change in RBF after IFN therapy in patients with chronic hepatitis C.

Anemia is a side effect of IFN therapy.[26–28] In the present study, there were significant decreases in RBCs, hemoglobin, and hematocrit values after the start of IFN treatment (Table 2). In addition, these parameters were significantly associated with increased RBF (Fig. 1). Anemia may lead to decreased tissue oxygen delivery, resulting in anemia-induced tissue hypoxia. Therefore, RBF, which is regulated to maintain the metabolic demands of the retinal tissue, may increase to compensate for IFN-induced anemia. Multiple regression analysis showed that the change in RBF was significantly and independently associated with the change in RBCs 2 weeks after the start of IFN therapy (Table 4). Therefore, the increase in RBF may be associated with the decrease in RBCs to maintain the metabolic demand in retinal tissues in response to anemia. Moreover, the current results that increased RBF was caused primarily by increased blood velocity without changes in vessel diameter indicate that downstream of the retinal microcirculation the retinal capillaries, which seem to be regulated mainly by a metabolic mechanism,[29] may dilate more than the large retinal artery at the measurement site. Indeed, our preliminary data showed increased retinal capillary blood flow in patients treated with IFN, by scanning laser Doppler flowmetry (Sato E et al. *IOVS* 2003;44:ARVO E-Abstract 361). Taken together, the increase in RBF seems to be associated with the anemia induced by IFN treatment, which may cause dilation of the retinal capillaries via a metabolic mechanism to compensate for the anemia.

Suspected risk factors in the development of IFN retinopathy include age,[30,31] diabetes mellitus,[30] systemic hypertension,[30,32] the initial dose of IFN,[25,31] and hemoglobin.[25] Multiple logistic regression analysis showed that changes in WSR and patient age were independent risk factors for the development of IFN-induced retinopathy (Table 4). A clinical study using visual-evoked responses and electroretinograms showed that patient age was associated with increased risk for subclinical neurovisual impairment.[33] These clinical data seem to support our findings.

Differences in changes in retinal hemodynamics were seen between patients with and without retinopathy in the early phase of IFN treatment (Fig. 2). In patients with retinopathy ($n = 22$), the blood velocity and WSR significantly increased, but the vessel diameter did not change, whereas the vessel diameter increased but the WSR did not change in patients without retinopathy ($n = 14$) 2 weeks after IFN therapy. The current results also showed that the WSR increased in patients with severe retinopathy more than in those with mild retinopathy, suggesting that the increased WSR may be involved with the severity of IFN-induced retinopathy. Because there were no differences in systemic blood pressure, RBC counts, hemoglobin, and hematocrit between the groups, such distinctive re-

TABLE 4. Multiple Logistic Regression Analysis of Factors Affecting the Onset of IFN-Induced Retinopathy

Independent Variable*	RR	95% CI	*P*
Change in WSR at week 2	1.13	1.00–1.26	0.03
Age	1.26	1.01–1.57	0.04
Change in RBF at week 2	0.93	0.86–1.02	0.10
Change in RBC at week 2	2.28	0.18–15.30	0.20
Diabetes	0.08	0.01–4.61	0.22
Hypertension	3.40	0.14–11.61	0.26
Sex	2.93	0.14–7.29	0.35
Change in MABP at week 2	0.95	0.83–1.09	0.48
Smoking	3.00	0.10–11.10	0.55
Initial dose of IFN	1.00	1.00–1.01	0.58
Type of IFN	0.82	0.03–5.01	0.91

RR, relative risk.
* The variables were evaluated 2 weeks after IFN treatment.

TABLE 5. Retinal Circulatory Parameters at Baseline and 2 Weeks after IFN Treatment in Patients with and without Systemic Diseases

	With No Systemic Disease (n = 18)	With Systemic Disease (n = 18)	P
Retinal circulatory parameters at baseline			
Retinal vessel diameter (μm)	109.2 ± 11.7	106.9 ± 11.9	0.65
Retinal blood velocity (mm/s)	36.7 ± 8.2	39.9 ± 9.0	0.25
RBF (μL/min)	10.2 ± 2.1	10.5 ± 3.2	0.39
Retinal WSR (/s)	1373 ± 409	1483 ± 369	0.30
Changes in Retinal circulatory parameters at week 2 of IFN treatment			
Retinal vessel diameter (%)	3.5 ± 1.5	1.6 ± 1.7	0.34
Retinal blood velocity (%)	26.1 ± 7.0	24.8 ± 4.8	0.82
RBF (%)	34.7 ± 7.4	28.7 ± 4.6	0.43
Retinal WSR (%)	22.8 ± 7.8	24.0 ± 5.9	0.95

Data are expressed as the mean ± SD.

sponses of the retinal parameters may originate in the retinal microvasculature. Collectively, the results suggest that measuring the retinal circulatory parameters during the early phase of IFN treatment can predict or detect the development and severity of IFN-induced retinopathy.

The vascular endothelium plays an important role in the regulation of blood flow via a flow-induced mechanism, which maintains the wall shear stress on the vessel wall.[34] We could not evaluate the exact change in shear stress, which equals the viscosity times the WSR,[14] because we did not measure the blood viscosity in the present study. Moreover, anemia induced by IFN per se may decrease the blood viscosity. We roughly estimated that the change in blood viscosity was −7.4% of baseline by using the established relationship between hematocrit and blood viscosity.[35,36] Because the average increase in WSR in all patients was 16.3% over baseline after 2 weeks of IFN treatment (Table 2), the increase in shear rate was more than two times the estimated decrease in blood viscosity, suggesting that shear stress may increase as a result of the increased WSR, despite the estimated decrease in blood viscosity in our patients with IFN treatment.

The intact endothelium can sense shear stress and induce luminal diameter modifications to keep shear stress constant at a predetermined level[34,37,38] by the production of endothelium-derived nitric oxide.[37] The increased vessel diameter and the absence of change in the WSR observed in patients without IFN-induced retinopathy (Fig. 2) suggest that shear stress may be maintained by the dilation of the retinal arterioles via a flow-mediated mechanism in response to the increased RBF induced by IFN-associated anemia. In contrast, the increase in WSR with no change in vessel diameter observed in patients with IFN-induced retinopathy indicates that the endothelial function may be impaired by IFN therapy in patients with retinopathy. Nishiwaki et al.[10,39] reported that IFN-α increased leukocyte adherence to the vascular endothelium and subsequent leukocyte trapping in the rat retinal microcirculation, suggesting that IFN-α may activate leukocytes that may be involved in the pathogenesis of microinfarctions associated with IFN-induced retinopathy. Their data seem to be consistent with our results. In addition, Takase et al.[11] reported that the flow-mediated vasodilation in the brachial artery decreased in patients with chronic hepatitis C treated with IFN, suggesting that IFN impairs endothelial function. Although their result was obtained from other tissue vasculature, the current results seem to be supported by the previous finding. Taken together, it is reasonable that endothelial dysfunction may be involved in the pathogenesis of the IFN-induced retinopathy.

If impaired endothelial function were induced by IFN, this may be a new clue to the mechanism of IFN-induced retinopathy. The impaired endothelium function may in turn impair the production of nitric oxide from the endothelium, which protects blood vessels from endogenous injury by mediating molecular signals that prevent platelet aggregation[40,41] and leukocyte interaction with the vascular wall.[42] In addition, other studies have reported high circulating level of plasma-activated complement 5a (C5a), a potent intravascular aggregator of platelets, in patients with IFN-induced retinopathy.[43,44] Both impaired endothelial function and the increase in plasma C5 may cause abnormal platelet function, resulting in retinal capillary infarction, manifesting as capillary nonperfusion, cotton-wool spots, and retinal hemorrhages. Collectively, we speculate that IFN-induced retinopathy may be caused by endothelial dysfunction that can increase the aggregation of platelets and leukocytes adhering to the vascular endothelium, resulting in focal infarction of the retinal capillaries despite increased blood flow in the major retinal vessels.

There is also the possibility that the retinopathy observed in our patients was caused by systemic disorders including diabetes, hypertension, and anemia. We confirmed that the retinopathy did not exist before IFN treatment and resolved spontaneously and immediately on cessation of IFN treatment in all patients with retinopathy. In addition, it is unlikely that anemia per se caused retinopathy in our patients whose hemoglobin levels did not fall below 9 g/dL during IFN treatment, because anemia can cause retinopathy when the hemoglobin decreases to 6 to 7 g/dL.[45] We believe that the retinopathy observed in our patients developed as the result of the IFN treatment.

It is also necessary to consider whether these systemic disorders affected the retinal circulation in the present study because of the impaired retinal circulation in patients with diabetes mellitus[46,47] and hypertension.[20,48] To minimize this possibility, we included only patients with well-controlled glycemic and blood pressure levels and no retinopathy before the IFN treatment. In addition, almost all patients with hypertension had well-controlled blood pressure while taking medications, except for two patients with blood pressure that exceeded 140/90 mm Hg. We confirmed that our results were unchanged when these two patients were excluded. It is worth noting that there were no significant changes in retinal circulatory parameters at baseline or in the changes in retinal circulation, especially the change in WSR 2 weeks after IFN therapy between patients with and without systemic disorders (Table 5). In addition, diabetes and hypertension were not significant risk factors for the development of IFN-induced retinopathy by multiple regression analysis (Table 4). Therefore, we believe that diabetes mellitus and hypertension had little effect on our results.

In the present study, patients were treated with two types of IFN, natural IFN-α (n = 14) and IFN-α2b with ribavirin (n = 22), depending on their viral load. Although there were slight and significant differences in the decreases in RBCs and hemoglobin induced by IFN between the groups (P = 0.03 and P =

0.04, respectively, by two-way ANOVA; data not shown), there were no significant differences in the retinal circulatory parameters between the groups. In addition, the type of IFN was not an independent risk factor for the development of IFN-induced retinopathy and probably had little effect on our results. In addition, a new type of IFN (i.e., pegylated IFN), which has an extended serum half-life that provides constant viral suppression for several days, has enhanced clinical efficacy over standard IFN treatment.[49,50] However, a recent study reported that more than twice the number of cases of retinopathy occurred with pegylated α-IFN than with standard α-IFN.[51] Further study is needed to reveal the relationship between the retinal circulation and ocular complications in patients receiving pegylated α-IFN treatment.

Chronic infection with hepatitis C virus itself has been suggested to cause the retinopathy.[52] In the present study, no patients had retinopathy on the first visit to our clinic. In addition, the retinal circulatory parameters obtained before IFN treatment (baseline) were similar to those in age- and gender-matched healthy subjects (data not shown). Therefore, we believe that chronic hepatitis per se had little effect on our results.

The strengths of our study include its prospective nature, the quantitative measurement of the retinal circulation, and calculation of the WSR as an index of shear stress for the investigation of retinal endothelial function. This study also had some limitations. First, it is difficult to exclude the possibility that systemic disorders, especially hypertension, may have any effect on the current, results because recent population-based studies have shown that the retinal microvasculature undergoes a series of pathophysiologic changes in response to elevated blood pressure.[53–55] In addition, it was not suitable to perform multiple regression analysis by excluding patients with systemic disorders because of the small number of patients with no such systemic disorders in this pilot study. A further study including more patients is needed to elucidate whether systemic disease affects the changes in retinal circulation in patients receiving IFN treatment. Because new guidelines for hypertension have been recently published,[56] the new guidelines should be included in the study to evaluate the effect of hypertension. Second, we should consider the effect of medication on patients with these systemic diseases. Although it is hard to exclude these confounding factors completely, all patients who were taking some medications did not change their medications before, during, and after IFN treatment, suggesting that medication has little effect on the changes in retinal circulation in patients receiving IFN treatment. Third, the changes in the retinal circulation may have occurred not only downstream but also upstream in the retinal microvasculature, such as in an ophthalmic artery, a central retinal artery, or both. Because laser Doppler velocimetry allows measurement of only retinal arterioles,[14] another technique to evaluate ocular circulation (i.e., color Doppler imaging), is needed to examine the effect of IFN treatment on the ocular circulation upstream of the retinal microvasculature.

In conclusion, our results indicate for the first time that RBF increases in association with IFN therapy in patients with chronic hepatitis C. In addition, there were significant differences in the changes in diameter, velocity, and WSR between the patients with and without IFN-induced retinopathy. The increase in WSR in patients with retinopathy especially indicates that endothelial dysfunction may play an important role in IFN-induced retinopathy because shear stress should be constant under physiologic conditions. We believe that the observations in the present study are of potential importance in understanding the mechanism of IFN-associated complications and that evaluation of the retinal vascular function using LDV is useful for evaluating other forms of treatment for retinal vascular disorders. The current results suggest that both hepatologists and ophthalmologists should be aware of the importance of the evaluation of retinal circulation in patients with chronic hepatitis C who receive IFN treatment.

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