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Endothelial Dysfunction and Decreased Exercise Tolerance in Interferon-Alpha Therapy in Chronic Hepatitis C: Relation between Exercise Hyperemia and Endothelial Function

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Summary

Background: We previously reported that reversible endothelial dysfunction is caused by interferon-alpha therapy (IFN) in patients with chronic hepatitis C. In experimental studies, limb blood flow during exercise is reported to be dependent on endothelium-derived nitric oxide.

Hypothesis: The purpose of this study was to confirm the effect of IFN on endothelial function and to investigate whether exercise hyperemia is dependent on endothelial function in humans.

Methods: We performed symptom-limited exercise treadmill testing and measured flow-mediated vasodilation (FMD, endothelium-dependent vasodilation) and sublingual glyceryl-trinitrate-induced dilation (GTN-D, 0.3 mg, endothelium-independent vasodilation) in the brachial artery by using high-resolution ultrasound in 10 patients with chronic active hepatitis C (age 53 ± 11 years, 2 men, 8 women) before and immediately after administration of recombinant interferon 2b (10 million U/day) for 4 weeks.

Results: There were no significant abnormal findings in any patients in routine studies of 24-h ambulatory electrocardiogram monitoring, two-dimensional echocardiography, and exercise treadmill testing both before and after treatment. Leg fatigue and exhaustion were the reasons for termination of exercise treadmill testing in each patient. Pressure rate product was calculated at rest and peak exercise. Interferon-alpha therapy significantly ($p<0.05$) decreased FMD (6.8 ± 3.1 vs. 1.9 ± 2.6%), exercise treadmill testing tolerance time (437 ± 89 vs. 395 ± 62 s) and peak pressure rate product (283 ± 41 vs. 241 ± 47 mmHg · beats/min · 10^{-2}), but not GTN-D (13.4 ± 5.4 vs. 17.0 ± 5.5%). The change of FMD due to IFN significantly and highly correlated with exercise treadmill testing tolerance time ($r = 0.86$, $p<0.001$), but not with change of peak pressure rate product, suggesting that FMD is more closely related to the condition of the peripheral circulation than is cardiac performance.

Conclusion: These results suggest that IFN in patients with chronic hepatitis C impairs endothelial function and exercise tolerance, and that endothelial function might be at least partly involved in exercise hyperemia in humans.

Key words: exercise physiology, endothelial function, interferon therapy, hepatitis C

Introduction

Several factors are reported to cause skeletal muscle vasodilation during exercise. However, the precise mechanism of exercise-induced vasodilation in skeletal muscle has long remained unclear. Recently, experimental study has shown that nitric oxide, which has a key role in endothelial function, is involved in exercise-induced vasodilation.[1]

Flow-mediated vasodilation (FMD) in a conduit artery, which can be detected by high-resolution ultrasound, has been used in many clinical settings.[2,3] This method is noninvasive and can be performed in outpatient clinics. In conduit arteries, the vasodilator response to an increase in blood flow is reportedly endothelium dependent. Such FMD has been investigat-

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ed in the brachial artery. This modality can reflect the condition of the endothelial function in the peripheral vasculature and the status of nitric oxide production.

Atherosclerosis is reported to be related to inflammation.[4] Since endothelial dysfunction is closely associated with atherosclerosis, it is possible that inflammation causes endothelial dysfunction. We previously reported that interferon-alpha therapy (IFN) in patients with chronic hepatitis C, which transiently aggravates the inflammatory reaction, reversibly impaired FMD in the brachial artery.[5] It is likely that IFN decreases exercise tolerance in the same patients. The purpose of this study is (1) to confirm the effect of IFN on FMD and to study its effect on exercise tolerance, and (2) to investigate whether exercise hyperemia is dependent on endothelial function in humans in our study model of IFN in patients with chronic hepatitis C.

Methods

Study Population

The study population consisted of 10 patients with histologically proven, chronic active hepatitis C (2 men and 8 women; average age 53 ± 11 years, range 41–67). They were referred to the Division of Gasteroenterology and Cardiology in the Self Defense Force Central Hospital and also agreed to both IFN and brachial ultrasound study. Each patient's diagnosis and indication for IFN was determined by a gasteroenterologist, as described previously.[6] Written informed consent was obtained from each patient before the start of the study. None of these patients was taking cardiovascular-acting agents during this study.

Interferon Therapy

Patients were injected subcutaneously with 10×10^6 U of recombinant human alpha-interferon (rh-INF-a 2b, INTRON A, Schering-Plough Corporation, Kenilworth, N.J., USA, distributed by Yamanouchi Pharmaceutical Co., Tokyo, Japan) every day for 4 weeks on admission and 3 times a week for the next 20 weeks during attendance at the outpatient clinic.

Brachial Artery Endothelial Function Study

All studies were performed in a temperature-controlled room (25°C) with the subject in a fasting, resting, and supine state. An electrocardiogram (ECG) was monitored continuously. Blood pressure and heart rate were recorded from the left arm every 3 min with an automatic sphygmomanometer (Nihon Korin, BP-203, Tokyo, Japan). The subject's dominant arm (right) was immobilized comfortably in the extended position to allow consistent access to the brachial artery for imaging. The vasodilation responses of the brachial artery were obtained by a previously validated technique.[2] The brachial artery diameter was imaged using a 7.5-MHz linear-array transducer ultrasound system (Hewlett Packard, SONOS 1500, Andover, Mass., USA). For each subject, optimal brachial artery images were obtained between 2 and 10 cm above the antecubital fossa. First, baseline two-dimensional (2-D) images were obtained. After baseline measurements of the brachial artery diameter, a narrow-width blood pressure cuff (Hokanson SC-10, Seattle, Wash., USA) was inflated on the most proximal part of the forearm to occlusive pressure (200 mmHg) for 5 min to induce hyperemia. The ultrasound transducer position was carefully maintained throughout the procedure. The cuff was then deflated rapidly and 2-D images of the brachial artery were obtained for 60–120 s after cuff deflation. Using the same method, we calculated endothelium-independent vasodilation by administering 0.3 mg of sublingual glyceryl trinitrate (GTN-D). The brachial artery was imaged before (baseline) and 5 min after GTN-D administration. All images were recorded on videotape for later analysis. For the measurements of vasodilator responses in the brachial artery, a 10-20 mm segment of the brachial artery was identified for analysis using anatomic landmarks in each subject and playback of the videotape. To select images reproducibly at the same point in the cardiac cycle, images at peak systole (maximum dilation, close to the end of the T-wave on ECG) were identified and the diameter of the brachial artery was digitized using a quantitative coronary angiography analysis computer (Kontron Elektronik, Cardio 500, Munich, Germany). For each condition (baseline, reactive hyperemia at 60 s after cuff deflation, and before and after GTN-D), three separate images from three different cardiac cycles were digitized. The average segment diameter of the three images was determined. All measurements were performed in a blinded manner; brachial diameter was measured by a co-investigator (A.U.) who was blinded to the treatment phase. Also, at measurement of brachial diameter, images were scrambled so the investigator was blinded to image conditions such as baseline, hyperemia, or before or after GTN-D administration. The percentage diameter changes from baseline in responses to hyperemia and GTN-D were calculated.

The intra- and interobserver variability (coefficient of variance) for repeated diameter measurements at baseline and reactive hyperemia or GTN-D in the brachial artery were both $< 3\%$.[2]

Exercise Treadmill Testing

Maximum symptom-limited treadmill testing was performed using the standard Bruce protocol. Throughout the study, 12-lead ECGs were monitored and recorded at 1-min intervals continuously using a Marquette CASE 12 (Marquette Electronics, Milwaukee, Wisc., USA). An ST-segment depression was measured 80 ms after the J points. A horizontal or downsloping ST-segment depression of at least 1 mm was considered significant in exercise-induced ischemia. Blood pressure was measured by Korotkoff's method at 1-min intervals. A pressure rate product was obtained by multiplying systolic blood pressure by heart rate (beats/min·mmHg·10^{-2}). End points for the termination of exercise testing were typical angina, dyspnea, extreme fatigue, leg fatigue, or exercise-in-

duced ST depression exceeding 2 mm. The physicians who supervised the exercise testing in each phase were blinded to the phase of the IFN.

Study Design

Brachial artery endothelial function study and exercise treadmill testing were performed before and during IFN (immediately after completion of initial 4 weeks of IFN). Both tests were performed during admission, and were carried out about 10 o'clock in the morning. All patients refrained from smoking tobacco and from either eating meals or drinking beverages containing caffeine for more than 12 h before the study.

To evaluate the cardiovascular side effects of IFN, a complete physical examination, a careful review of each patient's complaints, a routine 2-D echocardiograph, and an ambulatory ECG were performed by a cardiologist before and during IFN.

Statistical Analysis

Data are expressed as mean ±standard deviation. Paired Student's *t*-tests were used to compare data before and after IFN. The Pearson product-moment correlation coefficient was calculated to investigate the relationship between the results of the endothelial function study and the changes in exercise treadmill testing parameters. Differences were considered significant at $p \leq 0.05$.

Results

Cardiac risk factors and selected clinical characteristics for the 10 patients are as follows: cigarette smoking in 3 patients, hypertension in 1, hyperlipidemia (hypercholesterolemia) in 1, family history of premature atherosclerosis in 5, and no diabetics. Six of the eight women were post menopausal. In this study population, IFN had no cardiovascular side effects. During the first week of IFN, 9 of the 10 patients were febrile and 3 complained of general fatigue. However, these symptoms had disappeared at the end of the 4 weeks of IFN when the patients underwent the brachial artery endothelial function study and exercise treadmill testing. No abnormal findings were revealed by 2-D echocardiography and an ambulatory ECG in all patients.

As shown in Figure 1, IFN decreased FMD in 9 of 10 patients, and the mean value was significantly decreased from 6.8 ± 3.1 to 1.9 ± 2.6%. In contrast, IFN did not change GTN-D in any patient (from 13.5 ± 5.4 to 17.0 ± 5.5%, $p > 0.05$).

During exercise treadmill testing, no patient revealed exercise-induced ischemia both before and during IFN. All patients terminated the exercise treadmill testing because of both leg fatigue and general exhaustion. At least 85% of the predicted maximum heart rate was reached in all patients in both phases. The mean exercise tolerance time decreased significantly ($p \leq 0.05$) in 8 of the 10 patients from 438 ± 89 to 395 ± 62 s (Fig. 2). The mean value of the peak pressure rate product decreased significantly ($p \leq 0.05$) from 283 ± 41 to 241 ± 47 mmHg · beats/min · 10^{-2}.

Flow-mediated vasodilation does not correlate significantly ($p > 0.05$) with exercise tolerance time either before or after IFN (Figs. 3 and 4). The change of FMD caused by IFN correlates highly and significantly with exercise tolerance time ($r = 0.86$, $p \leq 0.001$), but not with the change of peak pressure rate product nor the change of GTN-D, as shown in Figure 5.

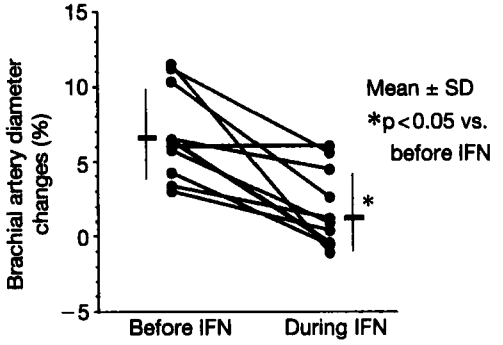

FIG. 1 Effect of interferon-alpha therapy (IFN) on endothelium dependent flow-mediated vasodilation. SD = standard deviation.

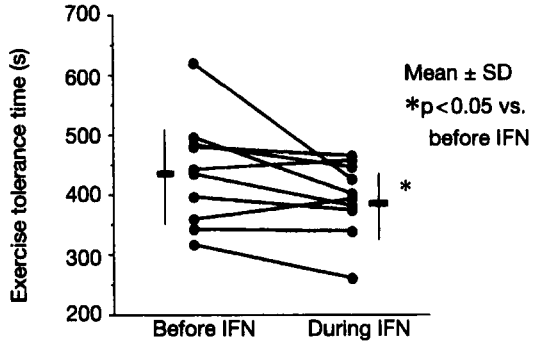

FIG. 2 Effect of interferon-alpha therapy (IFN) on exercise tolerance time. SD = standard deviation.

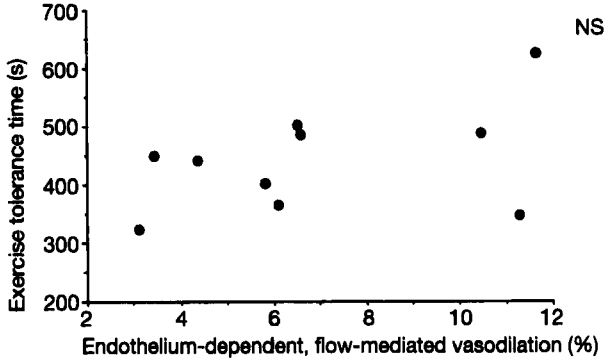

FIG. 3 Relation between flow-mediated vasodilation and exercise tolerance before interferon-alpha therapy (IFN). NS = not significant.

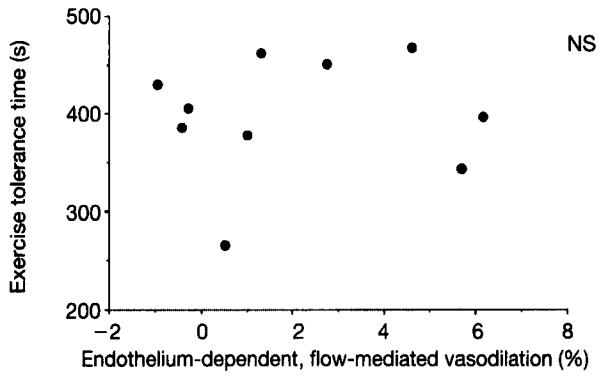

FIG. 4 Relation between flow-mediated vasodilation and exercise tolerance after interferon-alpha therapy (IFN). NS = not significant.

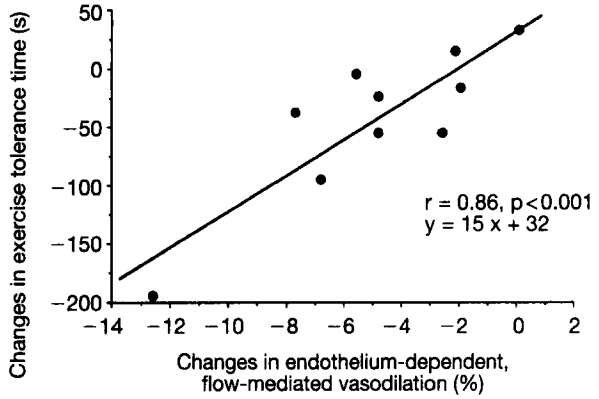

FIG. 5 Relation between changes in flow-mediated vasodilation and exercise tolerance by interferon-alpha therapy (IFN).

Discussion

Our results confirm that IFN decreases both FMD (endothelium-dependent vasodilation) and maximum exercise tolerance in patients with chronic hepatitis C. Interferon-alpha therapy also decreased peak pressure rate product in most of the patients (Figs. 1, 2). This observation is consistent with the previous report. There are few reports stating that IFN compromises endothelium. A search of the literature for the past 10 years shows that our previous report[5] might be the first observation of the effect of IFN on endothelial function in humans. However, in experimental studies, interferon inhibits endothelial cell proliferation,[7] and interferon-alpha suppresses iris neovascularization.[8] In theory, IFN can impair endothelial function.

Clinical IFN induces significant cardiovascular disorders in humans and this might be related to endothelial dysfunction. Interferon causes microangiopathy in patients.[9] Although IFN had no side effects on the heart in the present study, significant adverse effects such as arrhythmias, heart failure, myocardial ischemia, cardiogenic shock, and coronary spasm have been reported.[10–15] Since endothelial dysfunction is involved in the pathophysiology of vasospastic angina,[16] vasospasm could be attributable to interferon, possibly through damage to endothelium. Endothelial dysfunction is associated with various cardiovascular disorders. Interferon-alpha therapy might lead to significant cardiovascular side effects due to endothelial dysfunction in clinical settings if patients have a history of cardiovascular malfunction such as arrhythmogenesity, decreased left ventricular function, and atherosclerosis of the coronary artery.

The present study also shows that exercise hyperemia is dependent on endothelial function in humans. Several conditions, including coronary risk factors, influence endothelial function.[17] Also, exercise tolerance is determined not only by cardiac function but also by several general conditions or peripheral factors.[18–20] Since these determinants of either endothelial function or exercise tolerance are quite variable, it is possible that absolute values of FMD and exercise time do not correlate well both before and after IFN (Figs. 3, 4). However, IFN significantly decreased both FMD and exercise time in most patients. As a result, the decrease of FMD, indicating impairment of endothelial function, correlate highly and significantly with decreased exercise time, which is a surrogate for exercise tolerance in this study, because all the patients terminated exercise because of leg fatigue and exhaustion. All patients are considered to have stopped the exercise treadmill testing at their maximum exercise capacity both before and after the IFN phase (Fig. 5). This observation suggests that endothelial function plays a significant role in exercise tolerance.

Our observation gives new insight into the relationship between endothelial function and exercise hyperemia. In earlier studies, the contribution of endothelium-independent vasodilation to exercise-induced hyperemia is inconsistent. Endo *et al.*[21] and Wilson and Kapoor[22] reported no contribution of endothelium-independent vasodilation to exercise-induced hyperemia, whereas Gillian *et al.*[23] observed a significant contribution. Dyke *et al.*[24] suggested that endothelium-independent vasodilation is partly involved in exercise-induced hyperemia. All these reports were conducted using a low-grade exercise protocol, and the study populations were all normal subjects. Recently, in an experimental study, Maxwell *et al.*[1] reported that hyperemic blood flow during maximum exercise is dependent on nitric oxide production. To the best our knowledge, no studies have been conducted using a maximum exercise protocol in humans; neither are there studies examining the role of endothelial function on maximum exercise in patients. Our results on IFN in patients with hepatitis C add useful information to the debate.

The present study has some limits. First, we only measured brachial arterial endothelium-dependent vasodilation and exercise tolerance. We did not measure the endothelial function in the legs, which are involved directly in treadmill exercise. To confirm these preliminary results, the study protocol used by Maxwell *et al.*[1] would be ideal, but such a protocol might be impossible in a human study. Also, we previously reported that brachial endothelial function correlates closely with coronary artery endothelial function in humans,[25,26] so it is likely that brachial artery endothelial function closely reflects lower-

limb endothelial function. Second, since many factors are reported to be involved in exercise-induced hyperemia such as prostaglandins, adenosine, lactate, phosphate, and potassium as mentioned above,[18–20] it is possible that endothelial function is not the primary cause of exercise hyperemia. Interferon influences many neurohumoral and/or immunohumoral factors including exercise-hyperemia-associated factors.[27, 28] However, these multifactorial measurements are again quite difficult in clinical study. Consequently, our results are preliminary but support the suggestion that nitric oxide production, at least partly but significantly, contributes to the physiology of exercise-induced hyperemia. The third limitation of the present study is that we did not measure the factors damaging endothelium such as plasma levels of endothelin, leukocyte adhesion molecules, angiotensin II, and so forth. This information is important to clarify a mechanism by which IFN decreases FMD. Finally, we did not study the dose-dependent response in FMD to IFN; these studies should be investigated in the future. However, only few studies on the effect of IFN on endothelial function in humans have been conducted. The observations in the present study are of potential significance in understanding the mechanisms of IFN-induced cardiovascular side effects. Similar studies should be duplicated in larger cohorts to confirm our findings since the number of patients studied was small.

Conclusions

Our preliminary observations suggest that IFN in patients with chronic hepatitis C impairs endothelial function in the brachial artery and exercise tolerance, and that endothelial function might be involved in maximum exercise-induced hyperemia in humans.

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