Exercise After Heparin Administration
—— New Therapeutic Program for Patients With Non-Option Arteriosclerosis Obliterans ——

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Background A prospective study examined whether a combination of an exercise program and heparin administration improves the clinical symptoms of patients with arteriosclerosis obliterans (ASO) without an indication for surgical revascularization because of the lack of distal target vessels or other reasons such as high surgical risk or lack of a vein conduit from previous coronary artery bypass surgery.

Methods and Results A total of 19 consecutive patients with symptomatic non-option ASO diagnosed by angiography were randomly assigned to 3 groups: heparin+exercise (walking for 60 min after heparin injection [3,000 units/day IV for 14 days], n=6), heparin administration only (n=6), and exercise only (n=7). Plasma levels of hepatocyte growth factor (HGF) were serially measured before and after intravenous administration of heparin. Ankle brachial pressure index was measured and treadmill exercise test (2.5 km/h, 12% slope) was performed before the 2-week treatment, just after finishing treatment, and 12 weeks after beginning the treatment. Ophthalmic examinations, including visual acuity test, ocular fundoscopy and fluorescein angiographic fundus photography, were performed before and 12 weeks after the treatment program. In all patients, HGF levels increased more than 4-fold of the basal level at 30 min after heparin injection. Maximum walking time was significantly higher in the heparin+exercise group than in the other 2 groups (p<0.05). There were no patients who showed pathological retinal angiogenesis.

Conclusion The combination of an exercise program and heparin administration improves the clinical symptoms of patients with non-option ASO. (Circ J 2005; 69: 1099 – 1104)

Key Words: Angiogenesis; Arteriosclerosis obliterans; Exercise; Heparin; Hepatocyte growth factor

The number of patients with arteriosclerosis obliterans (ASO), an atherosclerotic peripheral arterial occlusive disease, is estimated at 500–1,000 individuals per million per year! and the prevalence of intermittent claudication was higher than 1% in a free-living, elderly, North American population. Although bypass operation, stenting and atherectomy are effective therapies for patients with stenosis in the proximal large arteries such as the iliac arteries or femoral arteries, their effects are limited in ASO patients in whom stenotic lesions extend to peripheral small arteries (poor run-off). Enhancement of collateral vessel formation and angiogenesis is a promising therapeutic modality for such non-option ASO patients. Heparin administration reportedly improves the symptoms of ASO patients 9 months after the treatment and increases the exercise capacity of severe coronary heart disease (CHD) patients by enhancing collateral vessel growth. Recently, investigators have shown that systemic administration of heparin increases the concentration of plasma hepatocyte growth factor (HGF), which promotes neovascularization and other studies have shown the effectiveness in ASO and CHD patients of therapeutic angiogenesis using certain growth factors. The mechanism of the therapeutic effect of heparin in ASO patients may be strongly related to HGF. There have been no previous studies of whether the combination of an exercise program and heparin administration additionally improves the symptoms of ASO.

The aim of the present study was to determine whether a program combining heparin administration with exercise is a useful therapeutic strategy for stimulation of development of collateral circulation in the ischemic legs of patients with non-option ASO.

Methods

Study Patients
All subjects had severe chronic limb ischemia that lead to claudication within 400 m of walking. None of the subjects was a candidate for non-surgical or surgical revascularization because of the lack of distal target vessels or for other reasons, such as comorbidities that would increase
the surgical risk, or the lack of a vein conduit as a result of previous coronary artery bypass surgery (non-option ASO patients). Inclusion criteria were: stable intermittent claudication compatible with Fontaine stage IIb; inoperable peripheral arterial occlusive disease with poor run-off in the lower limbs confirmed by angiography; moderate to severe attenuation of the ankle–brachial pressure index (ABI <0.9 in one or both legs); no major change (>20%) in maximum walking time between 2 assessments within 4 weeks before beginning the study. Exclusion criteria were severe diabetic retinopathy (diabetic retinopathy severity scale ≥severe nonproliferative diabetic retinopathy),10 low likelihood of malignant tumor, and any condition of such severity that the patient was unable to walk. Inclusion and exclusion criteria of each patient were judged by the staff of both the cardiovascular division and the division of cardiovascular surgery in the weekly cardiovascular angiography meeting of Omiya Medical Center, Jichi Medical School. Consensus of all attending staff members was required for inclusion. The subjects selected were 19 symptomatic ASO patients admitted from May 2000 through March 2002 and their clinical characteristics are given in Table 1. All patients received cilostazol (200 mg/day) and/or aspirin (81 mg/day), and all medications were continued unchanged during the study. At the time of enrollment, maximum walking time was assessed on a walking treadmill (2.5 km/h, 12% slope). All subjects gave written informed consent to participate in this study.

Study Design
This study was designed as a prospective, randomized trial in a single center. One of the investigators supervised the study as the safety and data monitor.

Study Protocol
The present protocol was approved by the Ethics Committee of the Jichi Medical School. A random permuted blocks method was used to assign patients to 1 of 3 different treatment groups. The duration of therapy, 14 days, was based on the study protocol reported by Fujita et al.12 The first group (heparin+exercise group, n=6) received an intravenous injection of 3,000 units of heparin 10 min before each exercise session (every morning for 14 days), using a protocol described elsewhere, with some modifications.3 Okada et al reported that there was no difference in the degree of increase in circulating HGF levels between the patients who were administered 3,000 units of heparin and those who were administered 10,000 units. To minimize the risk of worsening of traumatic accidents during exercise, we used a dose of 3,000 units/day, which is the minimum recommended dose for angiogenesis.11 Vigorous walking exercise (>2.0 km/h, flat) was performed for at least 10 min until the patient noted claudication, under supervision by one of the investigators. In each session, the supervising physicians encouraged the patients to repeat the walking exercise several times after an interval, over a period of 60 min. Observers were unaware of the treatment groups. ECG monitoring was performed during the exercise if the subject had angina pectoris or critical arrhythmia. The second group (heparin group, n=6) received a daily injection of 3,000 units of heparin for 14 days, using the same protocol as the heparin+exercise group, but did not perform the walking exercise. Patients in the third group (exercise group, n=7) performed daily walking exercise for 14 days, using the same protocol as the first group, but did not receive heparin.

Just after finishing the treatment and 12 weeks after the treatment, ABI was measured and the treadmill exercise test was performed. Angiography and ophthalmic examinations were repeated 12 weeks after the treatment. Smoking was not permitted during the study period.

Measurement of ABI
Blood pressure, heart rate and ABI were measured using the Form PWV/ABI® non-invasive vascular screening device (Nihon Colin Inc, Tokyo, Japan) after the subject had rested supine for at least 20 min. ABI was calculated 2 or 3 times for both legs and averaged. Legs with an ABI <0.9 were considered diseased. Values given in this report are for the clinically predominantly diseased leg, which had the earlier onset of claudication. Blood pressure in the lower leg was measured manually if a pulse wave could not be clearly recorded using the Form PWV/ABI® device.

Biochemical Analysis
From each patient, disodium EDTA-treated plasma samples were serially collected before and after heparin infusion. All samples were frozen at –80°C until the assay. Concentrations of HGF and vascular endothelium growth factor (VEGF) were determined by commercially available monoclonal antibody-based ELISA assays (R&D Systems, Minneapolis, MN, USA; Chemicon International, Inc Temecula, CA, USA, respectively). All assays were performed in duplicate.
Angiographic Assessment

Digital subtraction angiography (DSA) was performed as the standard of reference. A 5Fr pigtail catheter was inserted via the right brachial artery using the Seldinger technique. The tip of the catheter was positioned in the lower abdominal aorta caudal to the bifurcation of the renal arteries. Aorto-iliac and peripheral DSA was performed using a Toshiba (Japan) angiography system. The injection speed of the power injector was 9 ml/s, for a total volume of 27 ml Iopamiron® (SCHERING, Berlin, Germany). Following image acquisition, the reconstructed series with optimal intra-arterial contrast density and absence of venous filling was selected for subtraction of the non-contrast study. We qualitatively assessed collateral vessel development by observing arterial filling in the distal leg. For comparison before and after treatment, arterial filling was assessed as “increased” or “no change”. Two cardiologists who were unaware of the treatment groups interpreted the DSA images.

Ophthalmic Examinations

Ophthalmic examinations, including visual acuity test, funduscopy and fluorescein angiography, were performed before enrollment and at 12 weeks after the treatment program to evaluate whether the therapeutic programs had had a detrimental effects on retinal vessels.

Statistical Analysis

All values are reported as mean ± standard deviation. Differences were analyzed with one-way analysis of variance (ANOVA), and post-hoc analysis was performed using the Bonferroni/Dunn method. Probability values of p<0.05 were considered to indicate statistical significance (Statview 5.0, SAS Institute, Cary, NC, USA).

Results

There were no differences in age, gender, current smoking, diabetes, angina, ABI, maximum walking time or occlusion site of arteries before treatment among the 3 groups and there were no patients who suffered from either persistent leg pain at rest or skin ulcer. None of the patients had to undergo additional therapy for peripheral vascular disease until at least 12 weeks after the treatment. Bleeding complications were not observed. There were no signs of new retinal hemorrhage, exudates or neovascularization in any patients. None of the patients were withdrawn from the study.

Exercise Capacity

All patients had similar peak walking time at enrollment (Fig 1). Administration of heparin with exercise resulted in a continuous increase in maximum walking time. At 12 weeks after the treatment, the maximum walking time in
the heparin+exercise group was 9.4±5.2 min (p<0.05 vs baseline). In the other 2 groups, only a slight improvement in maximum walking time was observed.

**Measurement of ABI**

There was no significant difference in ABI among the 3 groups at baseline or just after the 2-week treatment. ABI tended to increase in the heparin+exercise group, but this was not statistically significant (Fig 2). In contrast, ABI remained unchanged in the heparin group and exercise group.

**Biochemical Analysis**

There was no significant difference in baseline plasma
HGF level among the 3 groups. In the heparin + exercise group and heparin group, intravenous injection of heparin induced a rapid, approximately 4-fold increase in circulating HGF levels after 30 min (Fig 3); after 120 min, the level of HGF decreased; after 240 min, it returned to near baseline. There was no significant difference in HGF levels at any time between the heparin + exercise group and the heparin group. In contrast, plasma levels of VEGF remained in the normal baseline range in all 3 groups.

Angiographic Assessment

In the heparin + exercise group, 1 patient showed a marked increase and the remaining 5 patients showed no change in collateral vessel flow. In contrast, none of the patients in the other 2 groups showed increased collateral vessel flow (Fig 4).

Discussion

The present results indicate that the combination of an exercise program and heparin administration is more effective than heparin alone or exercise alone for improving the clinical symptoms of ASO, such as maximum walking time, at 12 weeks after the treatment.

Therapeutic angiogenesis is a useful strategy in which growth factors, such as VEGF, fibroblast growth factor and HGF, delivered as proteins or via exogenous gene transfer, promote neovascularization to improve blood flow to ischemic organs. Although the effectiveness of these therapeutic protocols has been confirmed, there remain problems with safety and cost.

The present heparin – exercise therapeutic protocol is based on mobilization of endogenous HGF to ischemic lesions. HGF is synthesized in large amounts in the liver and secreted into the blood where it exerts multipotent actions via its receptor c-Met in various target organs, including the heart and vessels. HGF has been shown to be one of the most potent mitogens specific to endothelial cells and may contribute to vascular protection or repair. In the present study, heparin administration resulted in a dramatic increase in the plasma level of HGF, which is consistent with previous data. Endogenous HGF loosely bonds to extracellular matrices of the vessel wall, and is released by exposure to heparin. Expression of c-Met is reportedly enhanced by hypoxic conditions. Exercise training has been shown to promote increased HGF production in patients after acute myocardial infarction, as well as increased VEGF expression in ischemic skeletal muscle and enhanced angiogenesis. The present results indicate that our exercise protocol enhanced the effect of heparin administration in ASO patients. Induction of regional ischemia by exercise may be another key factor of this therapeutic protocol.

We previously reported that HGF promotes vascular endothelial growth via an endogenous nitric oxide (NO)-dependent pathway and Böger et al demonstrated that administration of L-arginine, a precursor of NO, improves the symptoms of ASO patients. Increased shear stress as a result of exercise activates endothelial NO synthase and thus, NO may play an important role in heparin – exercise therapy in patients with ASO.

There was no difference in the degree of increase in circulating HGF level between patients who were administered 3,000 units of heparin and those administered 10,000 units, nor was there a significant increase in circulating HGF level in the patients who were administered 100 units of heparin. Salbach et al reported that administration of low-molecular-weight heparin (LMWH) resulted in significant increases in HGF serum values equal to the effect of heparin and therefore LMWH may be a useful therapeutic alternative for patients with ASO.

The combination of prostaglandin E1 (PGE1) and exercise training is also effective in patients with ASO. Although it is thought that the effectiveness of PGE1 for ASO patients mainly depends on its ability to increase peripheral blood flow via vasodilation and inhibition of platelet aggregation, some recent studies have shown that PGE1 is a potent stimulator of angiogenesis via up-regulation of VEGF expression. A randomized study of 1,560 patients with chronic critical leg ischemia (Fontaine III or IV) to treatment with intravenous PGE1 or no PGE1 for 28 days has shown that the combined endpoint (death, amputation, persistence of critical leg ischemia, acute myocardial infarction, or stroke) at hospital discharge was significantly lower in the PGE1-treated patients. However, at 6 months there was no significant difference between the 2 groups. Therefore there is no worldwide class 1 consensus on PGE1 therapy for ASO patients. LipPGE1 (incorporation of PGE1 into soybean oil microspheres with a diameter of 0.2 mm) has been developed to overcome the side-effects of PGE1, but has not been tested in a clinical mega-trial for ASO patients.

In some of the present patients, maximum walking time improved within a few days of commencing the therapy, which cannot be explained as a consequence of angiogenesis. HGF has been found to have a vasodilatory effect in conscious animals, and recent studies have demonstrated that heparin has an anti-inflammatory effect via attenuation of leukocyte adherence mechanisms. Therefore, the effect on the fibrinolysis-coagulation system of both heparin and HGF may be the cause of the acute effect of this therapy. Based on these previous findings and the present results, we speculate that heparin improves the symptoms of ASO in the acute phase by inhibiting constriction and inflammation at arterial stenotic lesions.

Clinical Implications

Compared with other therapeutic approaches for ASO, the present heparin – exercise therapeutic protocol is non-invasive and can be repeated. The available evidence indicates that it is remarkably safe, inexpensive and easy to perform. It has also been reported recently that heparin administration combined with exercise improves symptoms in young patients with occluded coronary arteries from Kawasaki disease. The present study is the first to show that heparin administration combined with exercise significantly increases maximum walking time of ASO patients in association with a significant increase of in the plasma HGF level after heparin administration.

Study Limitations

Patients who were unable to walk cannot perform this protocol. Also, the relevant duration of heparin administration has not yet been established and it is not known which method of administration, intravenous or subcutaneous, is more efficacious in combination with exercise. Furthermore, to evaluate angiogenesis of lower legs more precisely, we should have used laser Doppler imaging as well as angiography. Because of the relatively small number of non-option ASO patients in Japan, the present study was...
limited in size. Larger trials are needed to verify the implications of our findings.

Conclusions

The combination of an exercise program and heparin administration improved the clinical symptoms of patients with non-option ASO without adverse effects. The present findings indicate novel therapeutic implications of exercise with heparin for modulation of growth factors in ischemic vascular diseases.

Acknowledgments

We thank Geert W. Schmid-Schönbein, PhD, Yoshio Tsuaya, MD, Yoshitaka Sugawara, MD, Takamasa Iwasawa, MD, Takeshi Ishida, MD, Taishi Hirahara, MD, Nahoko Ikeda, MD, Yousuke Takagi, MD, Mikihisa Fujii, MD, and Masatoshi Kuroki, MD, for their advice and Kazuki Sekine, BS, and Norio Yufune for their technical assistance.

References


