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Original Article

Protective effect of Ixeris sonchifolia hance injection against endothelial dysfunction in patients undergoing tourniquet ischemia

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Abstract: Objective: To investigate the effects of Ixeris sonchifolia Hance injection (ISHI), a traditional Chinese medicine, on endothelial dysfunction and skeletal muscle injury in patients undergoing tourniquet-induced ischemia reperfusion (IR). Methods: Sixty patients undergoing elective unilateral total knee arthroplasty under epidural anesthesia were randomly assigned into IR+ISHI group or IR group. The patients in IR+ISHI group received intravenous infusion of ISHI 0.6 ml/kg before tourniquet inflation, while same volume of normal saline was given in IR group. Flow-mediated dilation (FMD) value and perfusion index (PI) were measured, serum levels of malondialdehyde (MDA), von Willebrand factor (vWF), nitric oxide (NO), endothelin-1 (ET-1), superoxide dismutase (SOD), creative kinase (CK), CK-MB and lactate dehydrogenase (LDH) activities were also measured respectively prior to anesthesia and 60 min after reperfusion. Results: Compared with baselines, serum levels of MDA, vWF, ET-1, CK, CK-MB and LDH in both groups were significantly increased while SOD activity, NO level, FMD and PI values were decreased at 60 min after reperfusion ($P < 0.05$). The values of FMD and PI were increased in IR+ISHI group as compared to IR group ($P < 0.05$), and the changes in serum variables were also attenuated by pretreatment with ISHI. Conclusion: Pretreatment with ISHI could prevent against endothelial dysfunction and attenuate skeletal muscle injury in patients undergoing tourniquet-induced IR.

Keywords: Ixeris sonchifolia hance, reperfusion injury, muscle, skeletal, endothelial cells

Introduction

Restoration to an extremity exposed to ischemia may initiate an inflammatory cascade and induce muscle injury [1]. Tourniquet deflation may cause lower limb IR injury in orthopedic surgery, resulting in transient neutrophil and monocyte activation, enhanced neutrophil transendothelial migration, and endothelial dysfunction [2], where oxidative stress might play a role [3].

Ixeris sonchifolia Hance Injection (ISHI) is a kind of traditional Chinese medicine that has been widely used to activate blood circulation and dissolve stasis [4], and its major ingredients are flavonoids. Several studies on ISHI extracts have showed a variety of biological and pharmacological activities, including scavenging of oxygen radicals and anti-inflammatory property [5, 6], which make ISHI a probable alterative treatment for IR injury. It is unknown whether ISHI could provide protection against endothelial dysfunction and reduce skeletal muscle damage following tourniquet-induced IR.

Thus, this prospective, randomized, double-blind clinical study aims to investigate the effects of ISHI in attenuating endothelial dysfunction and muscle injury induced by tourniquet. Here, flow-mediated dilation (FMD) and serum von Willebrand factor (vWF), nitric oxide (NO) and endothelin-1 (ET-1) were measured to evaluate endothelial function, malondialdehyde (MDA) level and superoxide dismutase (SOD) activity were used to indicate oxidative stress, whereas systemic creative kinase (CK), CK-MB and lactate dehydrogenase (LDH) were employed as skeletal muscle injury biomarkers.

Material and methods

Patients

This study was approved by the Clinical Research Ethics Committee of our hospital and written informed consent was obtained. Patients undergoing elective unilateral total knee arthroplasty with American Society of Anesthesiologists physical status I or II, age 45-65 years, body mass index 18-28 kg/m^2, tourniquet duration 60-90min were enrolled in this study. Exclusion criteria include peripheral arterial disease, hypertension, any contraindications to ISHI usage or other severe systemic disorders (e.g., diabetes, liver and renal disease). Sixty patients were enrolled and randomly assigned into IR group or IR+ISHI group, 30 cases in each group. Intravenously administration of 0.6 mL/kg ISHI (product of Shuangding pharmaceutical co., LTD, Shenyang, China, dissolved in 250 mL normal saline) were given at 30 min before tourniquet inflation in IR+ISHI group, whereas the patients in IR group were treated with equal volume of normal saline instead.

Tourniquet application

Under standard monitoring, epidural anesthesia was performed at the level of L2-3 or L1-2 vertebral interspace using 0.5% ropivacaine. The lower extremity undergoing surgery was exsanguinated with an Esmarch bandage before tourniquet application. An automatic gas-filled tourniquet was then inflated to a pressure of 65 kPa (480 mm Hg) on the upper one-third of the thigh causing ischemia of the lower extremity, which was confirmed by the disappearance of pulse oxygen saturation waveform. At the end of the procedure, the tourniquet was released, pulse oxygen saturation waveform was restored and the reperfusion phase followed.

Detection of perfusion index (PI)

A reusable R2-25 Revision E sensor was attached to the tip of second toe of the operated extremity and was covered with a black, opaque protector to reduce the interference by the surrounding lights. The sensor was then connected to the Rad7 device equipped with a software ver. 7.8.0.1 (Masimo Corp., Irvine, CA, USA) to measure the PI. The PI was recorded prior to anesthesia and 60 min after reperfusion.

FMD assessment

Vascular endothelial function of the operated lower extremity was evaluated by the FMD of the homolateral arteria dorsalis pedis. The arteria dorsalis pedis was imaged using a 5- to 10-MHz multifrequency linear array transducer attached to a high resolution ultrasound machine (X-Porte, FUJIFILM SonoSite, Inc). The arteria dorsalis pedis was scanned in a longitudinal section and the baseline diastolic inner diameter (D_0) was measured in incidence with the R wave on a continuously recorded ECG. Once a satisfactory position was found, the transducer and cuff positions were marked to ensure consistent placement on subsequent measurements. Ischemia was subsequently induced by inflation of a pneumatic tourniquet placed around the ankle to a pressure of 280 mmHg and maintained for 4 min. The diastolic inner diameter was re-measured at 60 s after deflation of the cuff and recorded as D_1. Three cardiac cycles were analyzed to obtain average diameter for each time point prior to anesthesia and at 60 min after reperfusion. FMD was determined using the formula: FMD = $(D_1-D_0)/D_0*100\%$.

Detection of serum parameters

Venous blood sample was obtained from the homolateral dorsal venous arch prior to anesthesia and 60 min after reperfusion and centrifuged to separate the serum, which was then stored at -20°C for further detection. The levels of serum vWF and ET-1 were determined by enzyme-linked immunosorbent assay (Westang biotechnology Co. Ltd, Shanghai, China), and serum MDA, SOD, NO, CK, CK-MB and LDH were detected using commercially available assay kits (Jiancheng Bioengineering Institute, Nanjing, China) with a spectrophotometer.

Statistical analysis

All data was expressed as means ± SD and analyzed with software SPSS 13.0. Data between each group were compared with the pared samples *t* test, and independent two samples *t* test was used to compare data between two groups. A *P* value < 0.05 was considered statistically significant.

Results

Clinical characteristics of patients

All the participants completed the study. No significant differences between two groups were

Table 1. Clinical characteristics of patients

	IR group	IR+ISHI group
Number of patients	30	30
Gender (M/F)	16/14	18/12
Age (yr)	56 ± 16	60 ± 22
Body mass index (kg/m^2)	25 ± 8	27 ± 11
Tourniquet duration (min)	74 ± 12	79 ± 16
Total fluid volume (mL)	2410 ± 332	2252 ± 316
Crystalloid solution	1685 ± 196	1562 ± 189
Colloid solution	725 ± 85	690 ± 92

Table 2. Measurements of FMD and PI in two groups (mean ± SD, n=30)

	Group	T0	T1
FMD (%)	IR group	17.3 ± 3.5	11.9 ± 2.5*
	IR+ISHI group	18.5 ± 4.0	14.7 ± 2.5*,#
PI	IR group	8.1 ± 2.1	5.3 ± 1.5*
	IR+ISHI group	8.9 ± 1.8	6.8 ± 1.6*,#

Note: T0: prior to anesthesia; T1: 60 min after reperfusion; FMD: flow-mediated dilation; PI: perfusion index. *P < 0.05 vs. baselines, #P < 0.05 vs. IR group.

Table 3. Serum levels of oxidative stress indicators in two groups (mean ± SD, n=30)

	Group	T0	T1
MDA (nmol/mL)	IR group	2.3 ± 0.9	5.2 ± 1.2*
	IR+ISHI group	2.5 ± 0.8	3.7 ± 1.5*,#
SOD (U/L)	IR group	220 ± 79	155 ± 58*
	IR+ISHI group	214 ± 85	189 ± 68*,#

Note: T0: prior to anesthesia; T1: 60 min after reperfusion; MDA: malondialdehyde; SOD: superoxide dismutase. *P < 0.05 vs. baselines, #P < 0.05 vs. IR group.

Table 4. Serum levels of vWF, NO and ET-1 in two groups (mean ± SD, n=30)

	Group	T0	T1
vWF (%)	IR group	92 ± 28	162 ± 41*
	IR+ISHI group	96 ± 24	126 ± 35*,#
NO (mmol/L)	IR group	49 ± 10	30 ± 13*
	IR+ISHI group	47 ± 11	38 ± 12*,#
ET (ng/L)	IR group	9.2 ± 2.6	16.2 ± 4.1*
	IR+ISHI group	9.9 ± 3.0	13.1 ± 3.9*,#

Note: T0: prior to anesthesia; T1: 60 min after reperfusion; vWF: von Willebrand factor; NO: nitric oxide; ET-1: endothelin-1. *P < 0.05 vs. baselines, #P < 0.05 vs. IR group.

found in gender, age, body mass index, tourniquet duration, and volume of fluid (P > 0.05, **Table 1**).

Measurements of FMD and PI

Compared with baselines, FMD and PI values in both IR and IR+ISHI group were significantly decreased at 60 min after reperfusion (P < 0.05) (Supplementary Table 1). However, FMD and PI values were increased at 60 min after reperfusion in IR+ISHI group as compared to IR group (P < 0.05, **Table 2**).

Indicators of oxidative stress

Table 3 showed increased serum MDA level and decreased SOD activity after reperfusion in both two groups (P < 0.05). Serum MDA level was lower, whereas, SOD activity was higher in IR+ISHI group than in IR group (P < 0.05).

Levels of serum vWF, NO and ET-1

As shown in **Table 4**, serum levels of vWF and ET-1 were remarkably increased and NO level was decreased at 60 min after reperfusion in both two groups (P < 0.05). Compared to IR group, however, the levels of vWF and ET-1 were decreased, whereas NO level was increased in IR+ISHI group (P < 0.05).

Systemic biomarkers of skeletal muscle injury

Serum levels of CK, CK-MB and LDH at 60 min after reperfusion were significantly higher than baselines in both IR and IR+ISHI group. Pretreatment with ISHI attenuated the increase of these systemic biomarkers (P < 0.05, **Table 5**).

Discussion

The main findings of this study were that 1) Tourniquet-induced IR led to endothelial dysfunction and skeletal muscle injury. 2) We demonstrated the beneficial effect of ISHI pretreatment in inhibiting oxidative stress, ameliorating endothelial dysfunction and attenuating skeletal muscle injury in lower extremity undergoing IR injury.

Studies have well demonstrated that reperfusion causes notable changes in microvascular structures in the skeletal muscle, with vascular endothelial dysfunction being one of the dominating characteristics of IR injury [7]. Reperfusion sets off the xanthine-xanthine oxidase reaction and phospholipase activation, leading to reactive oxygen species (ROS) production, which will cause endothelium injury and subse-

Table 5. Serum levels of muscle injury biomarkers in two groups (mean ± SD, n=30)

	Group	T0	T1
CK (U/L)	IR group	112 ± 29	749 ± 136*
	IR+ISHI group	125 ± 30	540 ± 122*,#
CK-MB (U/L)	IR group	12 ± 3	38 ± 10*
	IR+ISHI group	14 ± 3	28 ± 9*,#
LDH (U/L)	IR group	56 ± 15	122 ± 32*
	IR+ISHI group	54 ± 13	101 ± 28*,#

Note: T0: prior to anesthesia; T1: 60 min after reperfusion; CK: creative kinase; CK-MB: creative kinase-MB; LDH: lactate dehydrogenase. *P < 0.05 vs. baselines, #P < 0.05 vs. IR group.

quently impair endothelium-dependent vasodilatation [8]. ROS also induces the expression of adhesion molecules that facilitates endothelium-neutrophil interaction leading to further neutrophils infiltration and "no-reflow phenomenon" after reperfusion [8, 9]. Numerous studies have shown that endothelial function after IR is improved by agents that reduce oxygen radical generation or activity [9, 10].

Endothelial dysfunction could be evidenced by changes in circulatory endothelial cells-derived molecules. High levels of circulatory vWF, which promotes platelet adhesion to the endothelium of oxidative stressed arterial vessels, are observed in patients undergoing reperfusion after thrombolytic therapy or coronary arterial expansion during percutaneous transluminal coronary angioplasty, and the elevated vWF level is related to free radicals production [11, 12]. In addition to its vasoconstriction properties, ET-1 may also acts as an inflammatory mediator by activating neutrophils, stimulating the release of cytokines and inducing adhesion molecule expression [13, 14], thus, ET-1 may play an important role in the endothelial dysfunction, and ET-1 levels are persistently raised and may even be augmented during reperfusion [15, 16]. Endothelium-derived NO plays a critical role in regulating endothelial function due to its vasodilating effect, inhibition of platelet aggregation, oxygen radical scavenging and reduction of leukocyte-endothelial cell adhesion [17]. The reperfusion of the muscle resulted in a progressive reduction in NO production, which could be explained by the decrease in endothelial and neural nitric oxide synthase activities [18].

Endothelial function can also be quantified by FMD, using ultrasound methods, which represents the endothelium-dependent vasorelaxation of the artery in response to increased blood flow. It has been proved that FMD impairment is associated with the oxidative stress and oxygen radicals' production, whereas, FMD improvement correlates to increased total antioxidant status [19]. Based on analysis of the pulse oximetry signal, PI can also be employed as an indicator of peripheral microvascular perfusion. Our results showed that reperfusion to the ischemic extremity resulted in high levels of vWF and ET-1, significant decreases in NO level, and impaired FMD and PI measurements. Furthermore, the activities of the enzymes CK, CK-MB and LDH, sensitive and reliable biomarkers for muscle damage, were increased. These results, taken together, suggested that IR leads to endothelial dysfunction and further muscle damage.

ISHI is an injectable formulation containing raw extract of Ixeris sonchifolia Hance and has been traditionally used to treat coronary heart disease, angina and cerebral infarction. Flavonoids in Ixeris sonchifolia Hance, in particular luteolin, might be responsible for the protective effects of this herb. It has been demonstrated that flavonoids including luteolin would inhibit contraction of isolated thoracic aorta due to entry of extracellular Ca^{2+} in a concentration-dependent manner [5, 20]. In addition to their relaxant property, flavonoids are also believed to be antioxidants or free radical scavengers in the biological systems. Flavonoids could also inhibit various enzymes responsible for radicals production in a concentration-dependent manner, and scavenge superoxide radicals and peroxyl radicals [21, 22]. It is well demonstrated that endothelium-derived NO would rapidly react with oxygen radicals and its relaxation property would be remarkably impaired [23]. Owing to its potent antioxidant activity, luteolin might prevent NO from interacting with superoxide radical, thereby enhancing NO bioavailability [24]. Flavonoids can also scavenge peroxynitrite which damages endothelium and impairs and showing endothelium-dependent relaxation, leading ultimately to improved endothelial function [25]. Thus, by scavenging such reactive species, flavonoids may help prevent endothelial dysfunction during reperfusion. As demonstrated in our study, ISHI pretreatment significantly decreased the levels of MDA, vWF

and ET-1, increased NO level and SOD activity, improved FMD and PI measurements, and attenuated skeletal muscle injury, which was evidenced by decreased levels of CK, CK-MB and LDH. Furthermore, no obvious side effects of ISHI were noticed in our study, indicating that ISHI could be considered as a safe therapy in treating with clinical IR injury.

In conclusion, through inhibition of oxidative stress, pretreatment with ISHI could alleviate endothelial dysfunction and attenuate skeletal muscle injury in patients undergoing tourniquet-induced IR.

Disclosure of conflict of interest

None.

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