Atherosclerosis 246 (2016) 98–105

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Vasoactive enzymes and blood flow responses to passive and active exercise in peripheral arterial disease

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ARTICLE INFO

Article history:
Received 8 October 2015
Received in revised form
2 December 2015
Accepted 21 December 2015
Available online 24 December 2015

Keywords:
Peripheral arterial disease
Leg blood flow
Vasoactive enzymes
NADPH oxidase

ABSTRACT

Background: Peripheral arterial disease (PAD) is characterised by impaired leg blood flow, which contributes to claudication and reduced exercise capacity. This study investigated to what extent vasoactive enzymes might contribute to altered blood flow in PAD (Fontaine stage II).
Methods: We compared femoral artery blood flow during reactive hyperaemia, leg-extension exercise and passive leg movement, and determined the level of vasoactive enzymes in skeletal muscle samples from the vastus lateralis in PAD (n = 10, 68.5 ± 6.5 years) and healthy controls (CON, n = 9, 62.1 ± 12.3 years). Leg blood flow was measured with Doppler ultrasound and muscle protein levels of phosphorylated endothelial nitric oxide synthase, NADPH oxidase, cyclooxygenase 1 and 2, thromboxane synthase, and prostacyclin synthase were determined.
Results: Leg blood flow during the initial 90 s of passive leg movement (242 ± 33 vs 441 ± 75 ml min^{-1}, P = 0.03) and during reactive hyperaemia (423 ± 100 vs 1255 ± 175 ml min^{-1}, P = 0.002) was lower in PAD than CON, whereas no significant difference was observed for leg blood flow during exercise (1490 ± 250 vs 1887 ± 349 ml min^{-1}, P = 0.37). PAD had higher NADPH oxidase than CON (1.04 ± 0.19 vs 0.50 ± 0.06 AU, P = 0.02), with no differences for other enzymes. Leg blood flow during exercise was correlated with prostacyclin synthase (P = 0.001).
Conclusion: Elevated NADPH oxidase indicates that oxidative stress may be a primary cause of low nitric oxide availability and impaired blood flow in PAD.

1. Introduction

Peripheral arterial disease (PAD) is characterised by reduced blood flow to the legs attributed to atherosclerotic lesions leading to stenosis and/or occlusion of the conduit arteries [1]. Typically, PAD patients who experience intermittent claudication have impaired muscle function and reduced exercise tolerance that limits daily physical activities [2,3]. While alterations in muscle morphology and metabolism are believed to contribute to these functional impairments, it is likely that the limb blood flow limitation is the primary cause of this impairment [4]. However, the extent to which limb blood flow is limited during leg exercise, and any contribution of endothelial dysfunction and the vasodilating systems, has not been established in PAD.

A recently developed test for the assessment of nitric oxide (NO) dependent vascular function is that of femoral blood flow response to passive movement of the lower leg [5]. Previous studies have shown that both aged individuals and individuals with PAD show a lower blood flow response to passive movement suggesting impaired NO function [5,6]. Similarly, reactive hyperaemia, which is also highly dependent on NO [7,8], is lower in the aged and in PAD compared to young healthy individuals [9]. This raises questions

Abbreviations: AUC, area under the curve; COX, cyclooxygenase; eNOS, endothelial nitric oxide synthase; GAPDH, glyceraldehyde 3-phosphase dehydrogenase; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; PAD, peripheral arterial disease; ROS, reactive oxygen species.

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http://dx.doi.org/10.1016/j.atherosclerosis.2015.12.029
0021-9150/

about the control of blood flow in PAD and presents the possibility that in addition to arterial stenosis, altered NO availability, and thereby limited vasodilation, might contribute to the impairment in leg blood flow of PAD patients during exercise.

Nitric oxide is a critical agent in the control of blood flow to skeletal muscle [7,10]. It is well established that NO availability declines with age [11,12] and can be further compromised by the presence of atherosclerotic disease, as observed in PAD [13]. However, the cause for low NO availability in PAD is unknown. Generally, NO availability is dependent on the amount of endothelial nitric oxide synthase (eNOS) protein, the state of activation of the enzyme, and the presence of reactive oxygen species (ROS) [11,14]. ROS reduce NO bioavailability by readily reacting with NO to form peroxynitrite, but ROS can also uncouple eNOS whereby the enzyme forms superoxide ions instead of NO [15]. One of the main contributors to ROS in the vasculature is NADPH oxidase and several inflammatory conditions and disease states have been associated with increased NADPH oxidase levels [16,17], but its expression in skeletal muscle of PAD patients is not known.

Although NO is known to be important for vascular function, other vasoactive systems, both vasodilating and constricting, are known to contribute to vascular conductance in skeletal muscle [10,18]. During exercise, blockade studies in healthy individuals demonstrate that NO and prostaglandins are interdependent vasodilating systems that can compensate for each other to maintain blood flow when one system is compromised [19,20]. This interaction may be acute, but there are also indications of more chronic redundancy between the systems; for example in diabetes and in hypertension, NO availability is low and vascular function is maintained by elevated levels of prostacyclin [21,22]. The impact of PAD on the prostaglandin system is unknown.

Thus, the aims of this study were: 1) to establish femoral arterial blood flow responses in PAD patients compared to healthy controls during reactive hyperaemia, passive leg movement, and active knee extensor exercise; 2) to compare the amounts of vasoactive enzymes in the vastus lateralis muscle in PAD patients and healthy controls, and 3) to explore the relationships between levels of vasoactive enzymes and the blood flow responses.

2. Methods

2.1. Participants

Ten patients with a confirmed diagnosis of PAD (Fontaine stage II) and nine healthy control participants of similar age and weight were recruited to participate in the study (see Table 1 for participant characteristics). All participants were screened for inclusion. PAD patients were included if they had: 1) diagnosis of PAD with a medical record of stenosis and occlusion locations, 2) clinically stable (>6 months) intermittent claudication [23], and 3) an ankle-to-brachial systolic blood pressure index (ABI) <0.90 in the study leg [24]. Patients were excluded if they had: 1) ABI > 0.9, 2) unstable or poorly controlled conditions, such as unstable angina or poorly-managed diabetes, and 3) communicable diseases. Four patients had previously (>12 months) undergone revascularisation procedures for PAD, but stenosis had reoccurred and inclusion criteria were clinically confirmed with haemodynamic measures and vascular imaging. Medication use was not an exclusion criteria in this study. Participants in the healthy group were included if they had: 1) an ABI >1.0 in both legs, 2) no history of PAD, 3) no unstable or poorly managed conditions, and 4) no communicable diseases. All participants provided a full medical history including smoking and medication use (Table 1), and completed a 7-day physical activity recall and the walking impairment questionnaire [25]. All participants provided written, informed consent prior to the study, and all procedures were approved by the Royal Brisbane and Women's Hospital and University of the Sunshine Coast human research ethics committees.

Table 1
Participant characteristics. Values are mean ± SD or percent distribution. PAD = peripheral arterial disease, ABI = ankle brachial index, Peak power (W) was determined during an incremental single leg kick test, ACE = angiotensin converting enzyme, ARB = angiotensin receptor blocker. *denotes significant difference between the PAD group and the healthy control group, $P < 0.05$.

	Healthy	PAD
N	9	10
Age (years)	62 ± 12	69 ± 7
Gender (M/F)	7/2	8/2
Height (cm)	173.9 ± 8.8	176.9 ± 4.7
Weight (kg)	83.8 ± 13.9	80.0 ± 16.0
Body mass index (kg m^{-2})	25.4 ± 3.9	27.4 ± 2.7
Current smokers (%)	0	20
Past smokers (%)	89	100
Study leg ABI	1.22 ± 0.15	0.79 ± 0.15*
Contralateral leg ABI	1.16 ± 0.11	0.83 ± 0.18*
Peak power (W)	38 ± 14	23 ± 7*
Previous lower limb revascularisation surgery (%)	0	40
Previous myocardial infarction (%)	11	30
Obese (%)	0	10
Diabetic (%)	11	10
Hypertensive (%)	33	70
Hyperlipidemia (%)	44	40
Diuretic use (%)	0	30
β-blocker use (%)	0	50
Ca^{2+} channel blocker use (%)	22	10
ACE/ARB use (%)	22	60
Statin use (%)	30	80
Antiplatelet use (%)	33	80
Aspirin use (%)	33	60

Healthy controls reported no limitation in their walking ability, while the PAD group reported walking impairment (49 ± 9 out of 100, where 0 = unable and 100 = fully able). There were no significant differences in reported habitual activity level between groups (PAD: 6.1 ± 2.1; Healthy: 4.4 ± 2.2 h $week^{-1}$, $P = 0.58$). All PAD participants were past smokers and two were current smokers at the time the experiment was conducted. Eight of the nine controls had also smoked previously. PAD patients tended to report a larger pack-year smoking history (PAD: 42 ± 12; Healthy: 14 ± 5 pack-years, $P = 0.057$).

One individual was excluded from the study due to a history of hepatitis C. Four PAD patients were excluded from the reactive hyperaemia test due to the presence of stent grafts in the common or superficial femoral arteries.

3. Study overview

Following screening and test familiarisation, participants visited the laboratory on two occasions separated by at least 7 days (PAD: 25 ± 7; Healthy: 28 ± 12 days). All participants were instructed to avoid exercise, caffeine, and alcohol for 24 h before their appointments. During the first visit, each participant underwent a resting biopsy of the vastus lateralis muscle of the study leg for the determination of vasoactive mediators. The second visit involved measurement of resting leg blood flow and reactive hyperaemia at the femoral artery. This was followed by the assessment of femoral artery blood flow during 5-min bouts of passive and active isolated single-leg extension exercise.

4. Experimental procedures

4.1. Ankle-brachial index (ABI)

Ankle and brachial systolic blood pressures were measured in