Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Novel method to assess arterial insufficiency in rodent hind limb

Matthew A. Ziegler, MD,[a] *Matthew R. DiStasi, PhD,*[b] *Steven J. Miller, PhD,*[a,c] *Michael C. Dalsing, MD,*[a] *and Joseph L. Unthank, PhD*[a,c,*]

[a] *Department of Surgery, Indiana University School of Medicine, Indianapolis, Indiana*
[b] *Departments of Pediatrics, and Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, Indiana*
[c] *Department of Cellular and Integrative Physiology, Indiana University School of Medicine, Indianapolis, Indiana*

ARTICLE INFO

Article history:
Received 21 May 2015
Received in revised form
25 September 2015
Accepted 23 October 2015
Available online 31 October 2015

Keywords:
Arterial insufficiency
Arterial reserve capacity
Arterial occlusion
Preclinical models
Venous flow

ABSTRACT

Background: Lack of techniques to assess maximal blood flow capacity thwarts the use of rodent models of arterial insufficiency to evaluate therapies for intermittent claudication. We evaluated femoral vein outflow (VO) in combination with stimulated muscle contraction as a potential method to assess functional hind limb arterial reserve and therapeutic efficacy in a rodent model of subcritical limb ischemia.

Materials and methods: VO was measured with perivascular flow probes at rest and during stimulated calf muscle contraction in young, healthy rats (Wistar Kyoto, WKY; lean Zucker rats, LZR) and rats with cardiovascular risk factors (spontaneously hypertensive [SHR]; obese Zucker rats [OZR]) with acute and/or chronic femoral arterial occlusion. Therapeutic efficacy was assessed by administration of Ramipril or Losartan to SHR after femoral artery excision.

Results: VO measurement in WKY demonstrated the utility of this method to assess hind limb perfusion at rest and during calf muscle contraction. Although application to diseased models (OZR and SHR) demonstrated normal resting perfusion compared with contralateral limbs, a significant reduction in reserve capacity was uncovered with muscle stimulation. Administration of Ramipril and Losartan demonstrated significant improvement in functional arterial reserve.

Conclusions: The results demonstrate that this novel method to assess distal limb perfusion in small rodents with subcritical limb ischemia is sufficient to unmask perfusion deficits not apparent at rest, detect impaired compensation in diseased animal models with risk factors, and assess therapeutic efficacy. The approach provides a significant advance in methods to investigate potential mechanisms and novel therapies for subcritical limb ischemia in preclinical rodent models.

* *Corresponding author*. Department of Surgery, Indiana University School of Medicine, 975 West Walnut, IB341, Indianapolis, IN 46202 5251. Tel.: +1 317 274 7339; fax: +1 317 962 0289.
E-mail address: junthank@iupui.edu (J.L. Unthank).
0022-4804/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2015.10.033

1. Introduction

Peripheral arterial disease (PAD) of the lower extremity is a significant and serious health problem associated with increased morbidity, mortality, and decreased quality of life [1,2]. It afflicts ~8 million Americans [3], including ~20% of the population aged >55 y [4] and ~60% of those ≥85 y [5,6]. Its prevalence is increasing significantly [7,8] as the population lives longer with chronic disease and risk factors such as diabetes. Approximately 10%–20% of the population with PAD eventually experience pain during walking (intermittent claudication, IC) because of arterial insufficiency or inadequate perfusion during increased metabolic demand [5,9]. Within 10 y of diagnosis with IC, 20%–30% progress to critical limb ischemia characterized by rest pain or tissue loss (ulceration or gangrene) generally in the foot. This number increases to 45%–60% if the patients are diabetic [10].

Based on these statistics, there is widespread agreement that PAD is a malady in need of new medical treatments. Indeed, the development of therapies to improve function and quality of life in all stages of PAD has been identified as a great and unmet medical need [11]. Although multiple clinical trials with critical limb ischemia patients have been conducted in recent years, few clinical studies have focused on the much more prevalent condition of IC. Moreover, there is a significant lack of medications effective in relieving symptoms and improving function in claudicants [12]. Recent clinical reports using Ramipril and other angiotensin converting enzyme inhibitors [13,14] have demonstrated the potential of pharmacologic therapy to significantly improve muscle function and perfusion in claudicants. Although the results are impressive and promising, significant questions have been raised regarding mechanisms and drug specificity [12]. Consequently, additional studies are warranted. Furthermore, appropriate preclinical studies could provide important direction and insight regarding mechanisms and novel treatments for claudicants. However, the evaluation of potential therapeutics to improve subcritical limb ischemia requires assessment of maximal blood flow capacity or flow reserve and the lack of such techniques in small animal models is a major limitation for their use in preclinical studies [15–17].

The purpose of this study was to evaluate femoral vein outflow (VO) in combination with stimulated muscle contraction as a potential method to assess functional hind limb arterial reserve and therapeutic efficacy in a small rodent model of subcritical limb ischemia. Distal femoral VO was measured with ultrasound transit time perivascular flow probes at rest and during electrically stimulated skeletal muscle contraction to assess functional reserve capacity. We first established the potential utility of this technique in young, healthy rats. Then, rats with vascular risk factors and known impairments to vascular compensation were used to assess the ability of this technique to detect perfusion deficits that were not apparent at rest. Finally, the ability of this method to assess potential therapeutic efficacy was tested in rats with risk factors that also received pharmacologic therapy.

2. Methods

2.1. Animals

These animal studies were approved by the Indiana University School of Medicine Institutional Animal Care and Use Committee. The animal care was in compliance with the Guide for the Care and Use of Laboratory Animals. Only male animals were used to minimize variability in these experiments to assess the feasibility and utility of the VO technique.

The first series of experiments were performed in young Wistar Kyoto rats (WKY, 8–10 wk old) obtained from Harlan Laboratories, Inc (Indianapolis, IN) to assess the feasibility of measuring VO, in the right hind limb 2 wk after femoral artery ligation (ligated) and in the left limb before (nonoccluded) and after acute clamping (acute) of the distal femoral artery (Fig. 1), both at rest and during electrically induced calf muscle contraction.

After establishing feasibility, the next experiments were performed to evaluate the utility of this technique to assess differences between the diseased models of obese Zucker rats (OZR; Charles River Laboratories, Wilmington, MA) and retired breeder spontaneously hypertensive rats (SHR; Harlan Laboratories Inc) compared with lean, normotensive control rats (lean Zucker rats [LZR]). The OZR and SHR were selected because they are the most common rat strains used in studies of hind limb ischemia [18–31] and have disorders typically associated with vascular disease in humans. We used femoral artery excision in these animals as most previous studies in these strains have used this method to induce arterial insufficiency. In these animals, VO was measured in both left and right hind limbs at rest and during electrically induced calf muscle contraction, 2 wk after right femoral artery excision (excised). In the left limb, the measurements were made before (nonoccluded) and after acute (acute) clamping of the distal femoral artery (Fig. 1).

The final set of rat experiments was conducted to evaluate the ability of the VO technique to assess the efficacy of potential therapies. SHR were treated with renin–angiotensin system suppressing agents because this class of drug has been shown to provide remarkable benefit to human claudicants with vascular risk factors including hypertension [13] and has demonstrated similar effects in prior rodent studies [28,32]. Treatment was initiated 2 wk after femoral artery excision, and VO was measured in the right hind limb of SHR 2-wk later (4 wk after excision).

Finally, acute studies were performed in mice to evaluate the potential application of VO studies during skeletal muscle stimulation in this species. Adult C57BL/6 mice aged ≥12 wk were obtained from an established colony.

2.2. Model creation of arterial insufficiency

2.2.1. Femoral artery ligation in rats

In the initial studies to develop the protocol for the VO method and test the feasibility of unmasking a perfusion deficit with electrically stimulated skeletal muscle contraction, a single ligation of the femoral artery was performed in WKY rats. After induction of anesthesia with isoflurane, a transverse