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Cardiovascular Pathology 19 (2010) e91 – e98

CARDIOVASCULAR PATHOLOGY

Original Article

Reduced cyclic stretch, endothelial dysfunction, and oxidative stress: an ex vivo model

Tyler Thacher[a,*], Veronica Gambillara[a], Rafaela F. da Silva[a,b], Paolo Silacci[a], Nikos Stergiopulos[a]

[a]LHTC, Institute of Bioengineering, Swiss Federal Institute of Technology Lausanne (EPFL), Lausanne, Switzerland
[b]Neurovascular Lab, Department of Neurosurgery, Geneva University Medical Center and Faculty of Medicine, University of Geneva, Geneva, Switzerland

Received 9 January 2009; received in revised form 23 June 2009; accepted 30 June 2009

Abstract

Background: The objective of this study was to investigate whether reduction of cyclic circumferential stretch will impair endothelial function and elevate basal levels of oxidative stress, both known risk factors linked to cardiovascular disease. **Methods:** Ex vivo and in vitro models were used to perfuse porcine carotid arteries and porcine endothelial cells, respectively, for 24 h. In both cases, one group was allowed to stretch naturally when exposed to a pulse shear stress (6±3 dynes/cm^2) combined with a pulse pressure of 80±10 mmHg, yielding a physiological cyclic stretch of 4–5%. This group was compared to a reduced stretch group, achieved by wrapping the arterial segment with a silicon band or by seeding the endothelial cells inside less compliant tubes, decreasing cyclic stretch to 1%. **Results:** The experimentally reduced compliance caused a significant decrease in bradykinin-dependent vascular relaxation. Reduced compliance significantly decreased the phosphorylation of serine 1177 (Ser1177) on eNOS, suggesting the activity of eNOS was decreased. Overall production of reactive oxygen species was increased by reducing compliance, as visualized with DHE. Finally, p22-phox and p47-phox, key players in the superoxide-generating NAD(P)H oxidase, were also up-regulated by reduced compliance. **Conclusions:** These findings point out how reduced arterial compliance increases the risk of arterial disease by creating a less functional endothelium, interrupting the eNOS activation pathway, and increasing the vascular levels of oxidative stress.

Keywords: Arterial compliance; Endothelial dysfunction; Oxidative stress; eNOS

1. Introduction

Reduction of arterial compliance typically correlates well with aging and is known to contribute to cardiovascular events such as coronary artery disease and stroke [1,2]. Reduction of systemic arterial compliance augments vascular impedance, wave reflection, and leads to an increase in systolic and pulse pressure. Augmented pulse pressure is easily measured and is currently recognized as a strong predictor of coronary heart disease [3]. At a more local level, reduced arterial compliance manifests itself by reducing arterial pulsation in the radial direction over the course of the heart cycle, commonly referred to as reduced cyclic stretch.

We suspect that reduction of cyclic circumferential stretch could increase the risk of cardiovascular disease by impairing endothelial functionality and elevating basal levels of oxidative stress, marked by the production of reactive oxygen species (ROS). Elevated levels of ROS are known to damage cardiovascular tissue and react with nitric oxide (NO), therefore lowering the bioavailability of NO [4] and increasing the risk of cardiovascular disease [5]. A useful marker of oxidative stress is dihydroethidium (DHE), which reacts with all ROS, providing a measure of total oxidative stress. The predominant system producing ROS in vascular

This work was supported by the Swiss National Science Foundation: grant 310000118274.

* Corresponding author. Laboratiore d'Hémodynamique et de Technologie Cardiovasculaire, École Polytechnique Fédéral de Lausanne, Bâtiment AI 1241, Station 15, 1015 Lausanne, CH, Switzerland. Tel.: +41 21 693 9654; fax: +41 21 693 9635.

E-mail address: tyler.thacher@epfl.ch (T. Thacher).

1054-8807/09/$ – see front matter
doi:10.1016/j.carpath.2009.06.007

smooth muscle and endothelial cells is the membrane-bound NAD(P)H oxidase and is regulated by the expression of p22-phox [6] and p47-phox [7].

We also suspect that reduction of cyclic stretch could affect the production of NO, an essential regulator of vascular reactivity and tone, produced by the endothelium and regulated by the expression as well as the activation of eNOS. Phosphorylation of eNOS serine 1177 (Ser1177) is a hallmark of eNOS activation [8,9]. By interrupting the phosphorylation of eNOS on Ser1177 one diminishes the amount of active vascular NO, increasing the risk of cardiovascular disease.

Studying the regulation of NO and oxidative stress in relation to altered arterial wall mechanics may provide insight into how reduced stretch affects endothelial functionality and its contribution to cardiovascular disease.

2. Methods

2.1. Arterial groups

Left internal carotid arteries of 6-month-old pigs weighing 120–150 kg were obtained from the local slaughterhouse (Bell SA, Cheseaux-sur-Lausanne, Switzerland) shortly after sacrifice. Adventitial tissue was removed and a 3.5-cm segment, 1 cm distal to the bifurcation, was excised. The arterial segments were then mounted onto the ex vivo arterial support system (EVASS, see description below). The segments were stretched longitudinally to 1.3 times the unstretched and unpressurized length. To simulate decreased compliance, a silicon cuff (Statice Sante, Besançon, France) of 6.0 or 8.0 mm (depending on the outer diameter) and of 0.2±0.05 mm thickness was placed around the arterial segment (Fig. 1). The reduction in circumferential cyclic stretch obtained with the cuff was roughly 80%, when compared to the uncuffed arterial segment.

2.2. Ex vivo arterial perfusion system

The ex vivo arterial perfusion system used in this study enables the perfusion of isolated arterial segments under precise control of perfusion pressure and flow. Details on EVASS have been given previously [10–12]. The arterial segments were perfused for 24 h with M199-EBS (Amersham) containing 5% fetal calf serum, 10 mmol/l HEPES (Sigma), 20 μg/ml Gentemicin (Gibco), 100 U/ml penicillin-streptomycin solution (Sigma), and 0.75 μg/ml Amphoterin B (Gibco). Eight percent medical-grade dextran (Sigma) was added to increase the viscosity of the medium to that of blood (~0.04 N s/m^2). The medium was constantly infused with 5% CO_2 and 95% air. Perfusion flow was adapted to create a pulsatile unidirectional shear stress with a mean value of 6 dyne/cm^2, amplitude of 3 dyne/cm^2, and frequency of 1 s. Perfusion pressure was set to 80 mmHg with a pulse pressure amplitude of ±10 mmHg. Resulting strains were 4–5% for the uncuffed segment, which is in the physiological range of pulsatile stretch for the porcine carotid, and less than 1% for the cuffed segment, simulating a less compliant arterial segment.

Initially, we feared that by introducing a cuff we would locally affect the hydrostatic pressure felt by the vascular

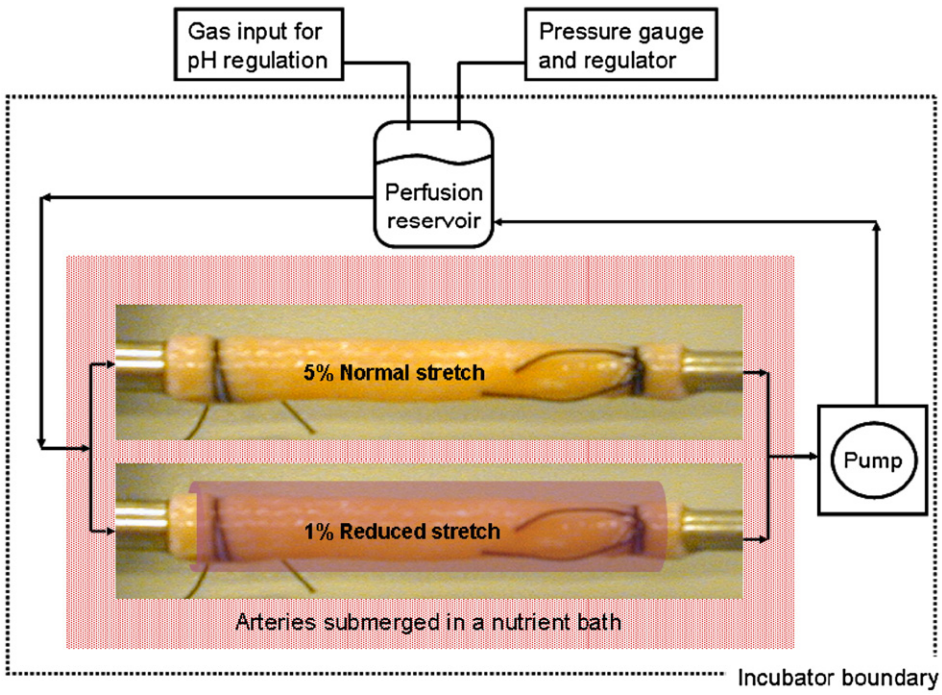

Fig. 1. Illustration depicting perfusion circuit and showing normal and reduced stretch arterial segments.