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Noradrenaline has opposing effects on the hydraulic conductance of arterial intima and media

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ABSTRACT

The uptake of circulating macromolecules by the arterial intima is thought to be a key step in atherogenesis. Such transport is dominantly advective, so elucidating the mechanisms of water transport is important. The relation between vasoactive agents and water transport in the arterial wall is incompletely understood. Here we applied our recently-developed combination of computational and experimental methods to investigate the effects of noradrenaline (NA) on hydraulic conductance of the wall (L_p), medial extracellular matrix volume fraction (ϕ^{ECM}) and medial permeability (K_1^1) in the rat abdominal aorta. Experimentally, we found that physiological NA concentrations were sufficient to induce SMC contraction and produced significant decreases in L_p and increases in ϕ^{ECM}. Simulation results based on 3D confocal images of the extracellular volume showed a corresponding increase in K_1^1, attributed to the opening of the ECM. Conversion of permeabilities to layer-specific resistances revealed that although the total wall resistance increased, medial resistance decreased, suggesting an increase in intimal resistance upon application of NA.

1. Introduction

The uptake of lipid-carrying plasma macromolecules by the arterial wall is thought to be a critical factor in the development of atherosclerosis (Weinberg, 2004; Tarbell, 2003, 2010). The characteristically patchy accumulation of such macromolecules is the result of complex transport mechanisms into and within the arterial wall which are only partially understood. Given that such macromolecular transport is dominantly advective (Tedgui and Lever, 1985), elucidating the mechanisms of water transport is a key step towards understanding macromolecule accumulation. Our previous studies have demonstrated that medial hydraulic resistance accounts for most of the total wall hydraulic resistance within the physiological pressure range, even in the relatively thin-walled rat aortic bifurcation (Chooi et al., 2016). The medial permeability to water in atheroprone arteries is therefore of interest.

The medial layer of the arterial wall consists of vascular smooth muscle cells (SMCs) surrounded by a complex network of elastin, collagen, proteoglycans and glycosaminoglycans. Changes to the structure of this layer are likely to have an impact on the transport of water and solutes across the wall. Our previous study (Chooi et al., 2016) investigated influences of changes in structure resulting from alteration of transmural pressure. It was found that the structural rearrangement of the solid components of the media gives rise to a nonlinear relation between permeability and wall strain. However, there is also an active mechanical mechanism – SMC contraction – that could alter medial (and hence wall) permeability through effects on structure.

When stimulated, SMCs alter their tone or actually contract (i.e. shorten along their long axis) depending on the transmural pressure gradient and hence stretch of the wall (Zulliger et al., 2002); under isobaric conditions, the luminal diameter decreases and wall thickness increases (Rachev and Hayashi, 1999). SMC contraction can be induced by the nervous system, by chemical signals transported in the blood and by locally-released paracrine mediators (Ludmer et al., 1986). Hypertension and obesity are examples of systemic conditions associated with increased SMC tone (Fridez et al., 2001; Meyer et al., 2013); both are important risk factors for atherosclerosis, and may act at least in part by influencing medial transport properties. Examples of paracrine mediators are the endothelium-derived constrictor endothelin (ET-1) and dilator nitric oxide (NO) (Bourque et al., 2011). More recently, a role of perivascular adipose tissue in SMC tone control has been reported; it acts via another set of vasoactive molecules yet to be identified

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(Meyer et al., 2013). An important implication of these sources of paracrine signalling is the potential existence of heterogeneous distributions of vasodilators and vasoconstrictors within the tunica media, leading to spatially varying medial permeability. This could account in part for the patchy distribution of macromolecular accumulation and atherosclerosis.

Here, we have applied our combined numerical/experimental method (Comerford et al., 2015) to investigate the effects of noradrenaline (NA) on water transport properties of the whole arterial wall and its component layers. Effects on transmural water flux were obtained by direct measurement, effects on medial permeability were obtained by numerical methods using experimentally-derived boundary conditions, and intimal hydraulic resistance was obtained by subtraction.

2. Methods

2.1. Overview

The effect of NA-induced vasoconstriction on arterial wall hydraulic conductance, L_p, was investigated using an *ex vivo* preparation of the rat aortic bifurcation (Fig. 1(a) and (b)) described previously (Chooi et al., 2016). The aortic bifurcation is a common site for atherosclerosis (Mitchell and Schwartz, 1965); stenosis at this location is a major cause of peripheral arterial disease. To distinguish between SMCs and extracellular matrix (ECM) of the wall, and hence to provide the microstructure for the numerical simulations of medial transport, bovine serum albumin (BSA) labelled with the fluorescent dye Lissamine™ rhodamine (Rh-BSA), was added to the luminal fluid and its transport was allowed to reach a steady state across the arterial wall. Following completion of the L_p measurements, the Rh-BSA was chemically fixed by perfusion at pressure and its distribution was imaged by confocal microscopy (Fig. 1(c)). Image volumes were transformed onto a structured computational grid and SMCs and other areas inaccessible to the albumin tracer were removed from the domain using a penalty parameter,[1] effectively treating the SMCs and fibres with pores sufficiently small to exclude albumin as impermeable objects. This gave realistic geometries for flow simulations.

Flow was simulated in medial tissue blocks driven by pressure gradients imposed in each of the three orthogonal axes and the intrinsic permeability was calculated (Fig. 1(e) and (f)). The permeability of the ECM was assumed to remain unchanged under the influence of NA; the implications of this assumption are discussed below. The ECM volume fraction was also quantified in each medial block (Fig. 1(d)). Medial thickness was measured from confocal images that were rotated and aligned with the radial direction. Finally, the total wall hydraulic resistance was decomposed into medial and intimal components by subtracting the computationally-obtained medial resistance from the experimentally-measured whole wall resistance, thus elucidating the effects of NA on medial and intimal hydraulic resistance (Fig. 1(g)).

2.2. Animals

All animal procedures were approved by the Local Ethical Review Panel of Imperial College London and complied with the Animals (Scientific Procedures) Act 1986. Eight male Sprague Dawley rats (271.5 ± 6.5 g; mean ± SEM; Charles River, UK) were fed a normal laboratory diet (LBS Biotechnology Ltd, UK) *ad libitum* and housed under a 12 h light cycle at 20–25 °C.

2.3. Vessel isolation

The *ex vivo* methods used in this study were based on previous work, described in Chooi et al. (2016). Briefly, animals were anaesthetised with isoflurane and the distal abdominal aorta and proximal iliac arteries were cannulated and removed. A system of reservoirs provided a constant hydrostatic pressure (Tedgui and Lever, 1984; Forster and Weinberg, 1997) and prevented collapse or overpressurisation of the arteries during the isolation. The cannulae were tied to a stereotactic tripod before removal of the vessels from the body to maintain arterial segment lengths and the bifurcation angle at their *in vivo* values. The entire preparation was placed into a temperature-controlled bath of Tyrode's Salt Solution (TSS; composition in g/l was 8 NaCl, 0.2 KCl, 0.2 $CaCl_2$, 0.1 $MgCl_2$, 0.05 NaH_2PO_4, 1 $NaHCO_3$, 1 glucose; pH 6.5) at 37 °C that had been pre-equilibrated with 95% air and 5% CO_2.

Fig. 2 shows the system used to perfuse the vessel at pressure *ex vivo*. TSS supplemented with 1% Rh-BSA and 3% unlabelled BSA was introduced into the lumen and the abluminal TSS was replaced with TSS containing 4% unlabelled BSA.

[1] This parameter drives the permeability towards zero.

2.4. Hydraulic conductance experiments

Steady state L_p was measured in arteries exposed to an increasing concentration of NA using methods described previously (Chooi et al., 2016). Baseline L_p in the absence of NA was measured in each specimen. NA concentration in the abluminal bath was then increased stepwise (1 nM, 100 nM, 10 μM), allowing water transport to reach steady state after each increase in concentration before re-assessing L_p.

2.5. Microscopy and image processing

2.5.1. Fixation of arteries at pressure and embedding

Steady state tracer distributions were obtained after completion of L_p measurements. Vessels were placed into a fresh abluminal saline bath containing 100 nM NA until steady state transmural flux of tracer was reached. Fixation and dehydration followed immediately as described by Chooi et al. (2016). The deformation induced by the 100 mmHg transmural pressure and the original vessel lengths and angles were maintained by performing the fixation without removing the vessel from the stereotactic and perfusion apparatus. The use of formal sublimate (6% $HgCl_2$ in 15% formaldehyde) prevented elastic recoil of the vessel when it was released from the apparatus; our previous study (Chooi et al., 2016) showed that preserved length was ~100% of the original vessel length with this fixative but not with formaldehyde on its own.

2.5.2. Confocal microscopy

The lateral walls were imaged in 3D at a position 2 mm proximal to the apex of the bifurcation. (For full details, see Comerford et al. (2015).) Briefly, embedded arteries were cut in the frontal plane so that the cut face showed a longitudinal section. The cut face was imaged using an inverted laser scanning confocal microscope (Leica, TCS SP5) with the *z*-axis of the *z*-stack aligned perpendicularly to the cut face. Rhodamine fluorescence was excited at 575 nm; emission was imaged at 585–595 nm.

2.5.3. Image processing

Five cuboidal blocks were extracted from images of three pieces of tissue from the baseline group. A further four blocks were extracted from images of three pieces of tissue fixed at 100 nM NA. An example and coordinate orientation of a block is shown in Fig. 3. A correction for intensity attenuation with depth was performed using Fiji (Schindelin et al., 2012) as described previously (Comerford et al., 2015) and three image volume rotations were applied to align the imaging axes to the cylindrical coordinates of the aorta. Medial thickness was measured after image rotations were applied.

2.6. Effective permeability

To determine the effective permeability of a porous medium, the flow field must be determined. Flow around solid objects embedded in a porous matrix is described by Brinkman's equation (see Wang and Tarbell (1995), Huang and Tarbell (1997), and Comerford et al. (2015)). In the arterial media the solid objects are the SMCs and impervious fibrous proteins, and the surrounding medium is the porous ECM. The chosen isotropic value for ECM permeability, $k_{ECM} = 1.32 \times 10^{-18}$ m^2, was taken from the mean of published values (Wang and Tarbell, 1995; Huang and Tarbell, 1997; Dabagh et al., 2009). Although these published values were measured in rabbit tissue, ECM structure and behaviour are similar between vertebrate species (Wagenseil and Mecham, 2009).

We recently outlined an efficient approach to determine the effective permeability of the arterial media using Brinkman's equation (Comerford et al., 2015) and implemented it in the spectral/hp element framework Nektar++ (Cantwell et al., 2015). Briefly, we first determine the flow around SMCs in a representative region of the realistic microstructure obtained from 3D confocal imaging data (Fig. 3, the green tissue represents the ECM and the blue regions the SMCs and impervious fibrous proteins). The method treats the impermeable objects by applying a penalty parameter that ensures flow travels around rather than through them. The flow field is determined in each of the main coordinate directions of each block taken from the arterial wall (coordinates shown in Fig. 3) subject to a pressure drop in that direction. From these simulations we can determine mean volumetric velocity ($\langle \mathbf{u} \rangle$) and pressure gradients ($\langle \nabla p \rangle$) using Darcy's law:

$$\langle \mathbf{u} \rangle = \frac{\mathbf{k}}{\nu} \langle \nabla p \rangle, \tag{1}$$

where ν is the kinematic viscosity and $\mathbf{k}$ is the permeability tensor:

$$\mathbf{k} = \begin{bmatrix} k_{rr} & k_{rz} & k_{r\theta} \\ k_{rz} & k_{zz} & k_{z\theta} \\ k_{r\theta} & k_{z\theta} & k_{\theta\theta} \end{bmatrix} \tag{2}$$