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Changes in biomechanical properties of the coronary artery wall contribute to maintained contractile responses to endothelin-1 in atherosclerosis

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ABSTRACT

Aims: Our aim was to determine whether alterations in biomechanical properties of human diseased compared to normal coronary artery contribute to changes in artery responsiveness to endothelin-1 in atherosclerosis.
Main methods: Concentration–response curves were constructed to endothelin-1 in normal and diseased coronary artery. The passive mechanical properties of arteries were determined using tensile ring tests from which finite element models of passive mechanical properties of both groups were created. Finite element modelling of artery endothelin-1 responses was then performed.
Key findings: Maximum responses to endothelin-1 were significantly attenuated in diseased (27 ± 3 mN, $n = 55$) compared to normal (38 ± 2 mN, $n = 68$) artery, although this remained over 70% of control. There was no difference in potency (pD_2 control $= 8.03 \pm 0.06$; pD_2 diseased $= 7.98 \pm 0.06$). Finite element modelling of tensile ring tests resulted in hyperelastic shear modulus $\mu = 2004 \pm 410$ Pa and hardening exponent $\alpha = 22.8 \pm 2.2$ for normal wall and $\mu = 2464 \pm 1075$ Pa and $\alpha = 38.3 \pm 6.7$ for plaque tissue and distensibility of diseased vessels was decreased. Finite element modelling of active properties of both groups resulted in higher muscle contractile strain (represented by thermal reactivity) of the atherosclerotic artery model than the normal artery model. The models suggest that a change in muscle response to endothelin-1 occurs in atherosclerotic artery to increase its distensibility towards that seen in normal artery.
Significance: Our data suggest that an adaptation occurs in medial smooth muscle of atherosclerotic coronary artery to maintain distensibility of the vessel wall in the presence of endothelin-1. This may contribute to the vasospastic effect of locally increased endothelin-1 production that is reported in this condition.

Introduction

The endothelin (ET) system is up-regulated in human coronary atherosclerosis with elevated levels of ET-1 detected in plasma (Lerman et al. 1991), correlating to the extent of disease (Salomone et al., 1996) and with increased ET-1 expression within the atherosclerotic plaque (Zeiher et al., 1995; Bacon et al., 1996). Interestingly, in response to this increased synthesis of ET peptide in human atherosclerosis detected *in vitro* (Maguire and Davenport., 1998a; Ihling et al., 2001) and *in vivo* (Böhm et al., 2002) we find no corresponding increase in density of ET receptors in the medial smooth muscle layer of coronary artery and indeed there is a profound down-regulation of ET receptor expression in the non-contractile intimal smooth muscle layer (Bacon et al., 1996; Maguire and Davenport, 2000; Katugampola et al., 2002). Histological analysis of diseased coronary artery has demonstrated marked atrophy of the contractile medial smooth muscle in the remodelled arterial wall that is predicted to contribute to plaque instability (Burke et al., 2002).

The mechanical properties of atherosclerotic artery have been studied both *in vitro* (Beattie et al., 1998; Baldewsing et al., 2008) and *in vivo* (van Popele et al., 2001; Baldewsing et al., 2008; Wykretowicz et al., 2009), where the diseased artery has been shown to be stiffer than healthy artery in clinical studies. Holzapfel and Ogden (2010) have provided a review of the studies on the theoretical modelling of arterial smooth muscle function. These include the study of Yang et al. (2003) on a model integrating the electro- and mechano-chemical functions of smooth muscle cell, as well as that of Zulliger et al. (2004) on a pseudo-strain energy function describing the arterial mechanical properties with the coupling of collagen, elastin, and smooth muscle active properties. Despite the well-modelled smooth muscle functions

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in these studies, the changes of arterial active properties with atherosclerotic disease remain unclear.

Our hypothesis was that the contractile responses to ET-1 in isolated rings of human atherosclerotic coronary artery should be markedly attenuated compared to histologically normal vessels. This should be a consequence of the relative reduction in the amount of contractile smooth muscle present, compounded by a decrease in arterial distensibility resulting from gross changes in the vessel structure, particularly intimal thickening, increased fibrosis and the presence of lipid laden and calcified plaque (Stary et al., 1994, 1995). Our aim was to compare contractile responses to ET-1 in human normal and atherosclerotic coronary artery *in vitro* and to develop finite element (FE) models to compare the passive and active mechanical properties of these vessels in health and disease. We first performed mechanical tests and FE modelling to obtain the passive mechanical properties (stretch and distensibility in response to mechanical loading in the absence of chemically mediated smooth muscle cell activation) of both the normal and diseased arteries. We then used these determined passive properties in further FE modelling of the arterial active response to ET-1 to understand how atherosclerosis-induced changes in arterial structure and mechanical properties may affect this active response to ET-1.

Unexpectedly we find that compared to histologically normal coronary artery, *in vitro* contractile responses to ET-1 are well maintained in atherosclerotic vessels with FE modelling demonstrating the capacity of medial smooth muscle cells in diseased arteries to develop elevated contractile strains in response to ET-1 compared to normal arteries.

Materials

Human tissue

Anonymised human coronary artery samples were used in this study with local ethical approval (REC 05/Q0104/142). Samples were obtained from Papworth Hospital Research Tissue Bank (Cambridgeshire 1 Research Ethics Committee reference 08/H0304/56) and were collected with written informed patient consent. Histologically normal arteries (no visible evidence of atherosclerosis) were from 68 patients transplanted for cardiomyopathies (Dec and Fuster, 1994) and atherosclerotic artery samples with evidence of coronary artery disease (CAD, visible plaque present) were from 55 patients transplanted for ischaemic heart disease. *n*-Values refer to the number of patients from whom tissue was obtained. Artery samples for the contractile functional experiments were transferred to the laboratory in Krebs solution and used within 12 h of retrieval. ET-1 was purchased from the Peptide Institute (Osaka, Japan). Stock solutions (10^{-4} M) were made up in 0.1% acetic acid and stored at −70 °C. Krebs solution was of the following composition: (mM) NaCl 90, KCl 5, $MgSO_4.7H_2O$ 0.5, Na_2HPO_4 1, $NaHCO_3$ 45, $CaCl_2$ 2.25, glucose 10, Na pyruvate 5, fumaric acid 5, and L-glutamic acid 5.

Methods

Human coronary artery functional assay

Experiments were carried out as previously described (Maguire, 2002). Briefly, 4 mm rings of histologically normal ($n = 68$) or atherosclerotic ($n = 55$) endothelium-denuded human coronary artery were set up for isometric force recordings in 5 ml organ baths containing oxygenated Krebs solution at 37 °C. For diseased arteries all 4 mm segments contained visible atherosclerotic plaque, although plaque size was variable between samples. Following an active force normalisation procedure to determine optimum resting force, successful removal of the endothelium was confirmed by lack of reversal of the contractile response to 10 μM phenylephrine by 1 μM acetylcholine. After 30 minute equilibration, cumulative concentration–response curves (CRC) were then constructed to ET-1 (10^{-10}–10^{-6} M) and experiments were completed by addition of 100 mM KCl to determine maximum possible contractile response for each tissue. ET-1 responses were expressed as force developed (mN) or % terminal KCl response. Data were analysed using the non-linear iterative curve-fitting programme GraphPad Prism (GraphPad Software Inc., La Jolla, USA) to determine values of pD_2 ($-\log_{10}$ of the EC_{50} (the concentration of ET-1 that produces 50% of the maximum ET-1 response)) and maximum response (E_{MAX}).

Passive tensile ring tests

The passive mechanical properties of normal and atherosclerotic human coronary arteries were determined using tensile ring tests. The arterial samples were snap frozen to maintain structural integrity, stored at -70 °C and defrosted before use. The defrosted samples were expected to have negligible smooth muscle cell activation induced by stretching. The artery rings were set up as above, uniaxial loadings were applied to the rings and the reaction forces and arterial deformations in response to mechanical stretch were recorded in both normal ($n = 5$) and atherosclerotic ($n = 6$) arterial rings. The mechanical stretch λ of the ring was calculated as:

$$\lambda = \frac{2L}{C} \tag{1}$$

where L is the distance between the two wires that were used to stretch the arterial ring, and C is the inner circumference of the ring without loading, which was measured from the histology image of the artery.

Finite element models of the passive mechanical properties of normal and atherosclerotic coronary artery

For each normal artery sample the FE model was created using Abaqus (Dassault Systèmes, Vélizy-Villacoublay, France), comprising a single circular ring with diameter and wall thickness estimated from the vessel histology (Fig. 1A). The models were assigned with first order Ogden hyperelastic properties with shear modulus μ and hardening exponent α (Abaqus Theory Manual), assuming incompressible material. The model reaction forces were matched with those of the corresponding tensile ring tests using an error minimisation approach with MATLAB (MathWorks, Natick, USA) to determine the optimum μ and α that provide the best matching. Mean values of μ and α of the normal artery models were then calculated and expressed as mean ± sem.

A similar approach was applied to the atherosclerotic artery models, with an additional inner vessel wall layer included to represent plaque tissue (Fig. 1B). For atherosclerotic artery the wall outer layer was assigned with the same mean values of μ and α determined for normal artery and the additional inner plaque layer was assigned with different optimum values of μ and α determined by matching the modelled force–stretch results with those of the passive tensile ring tests for the diseased artery samples.

In order to compare the overall stiffness of the normal and atherosclerotic arteries, each of the FE models was loaded with internal physiological pressures of 80 mm Hg and 120 mm Hg. The distensibility D of each arterial sample was determined from the geometry changes of the model using Eq. (2):

$$D = \frac{A_{120} - A_{80}}{A_{80}(P_{120} - P_{80})} \tag{2}$$

where A_{80}, A_{120}, P_{80}, and P_{120} are the lumen area A and pressure P at 80 mm Hg and 120 mm Hg, respectively. The stiffness of the arteries was examined by this normalised D (i.e. change of lumen area normalised by the initial area) rather than the non-normalised D to prevent the

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