Vascular Pharmacology 81 (2016) 42–52

Contents lists available at ScienceDirect

Vascular Pharmacology

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

Progressive vascular remodelling, endothelial dysfunction and stiffness in mesenteric resistance arteries in a rodent model of chronic kidney disease

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ARTICLE INFO

Article history:
Received 25 September 2015
Received in revised form 2 December 2015
Accepted 31 December 2015
Available online 5 January 2016

Keywords:
Mesenteric resistance artery
Hypertension
Polycystic kidney disease
Endothelium
Vascular remodelling

ABSTRACT

Chronic kidney disease (CKD) and hypertension are co-morbid conditions both associated with altered resistance artery structure, biomechanics and function. We examined these characteristics in mesenteric artery together with renal function and systolic blood pressure (SBP) changes in the Lewis polycystic kidney (LPK) rat model of CKD. Animals were studied at early (6-weeks), intermediate (12-weeks), and late (18-weeks) time-points ($n = 21$), relative to age-matched Lewis controls ($n = 29$). At 12 and 18-weeks, LPK arteries exhibited eutrophic and hypertrophic inward remodelling characterised by thickened medial smooth muscle, decreased lumen diameter, and unchanged or increased media cross-sectional area, respectively. At these later time points, endothelium-dependent vasorelaxation was also compromised, associated with impaired endothelium-dependent hyperpolarisation and reduced nitric oxide synthase activity. Stiffness, elastic-modulus/stress slopes and collagen/elastin ratios were increased in 6 and 18-week-old-LPK, in contrast to greater arterial compliance at 12 weeks. Multiple linear regression analysis highlighted SBP as the main predictor of wall–lumen ratio ($r = 0.536$, $P < 0.001$ $n = 46$ pairs). Concentration–response curves revealed increased sensitivity to phenylephrine but not potassium chloride in 18-week-LPK. Our results indicate that impairment in LPK resistance vasculature is evident at 6 weeks, and worsens with hypertension and progression of renal disease.

1. Introduction

Impaired function of the resistance vasculature is associated with hypertension in chronic kidney disease (CKD) [1,2] and as a group, CKD patients have an increased risk of mortality due to cardiovascular disease [3]. Resistance arteries provide more than 80% of the resistance to blood flow in the body and, as a consequence, modified structural properties play a significant role in persistent increases in total peripheral resistance [4]. The structural, biomechanical and functional attributes of resistance vessels are hypothesised as being one of the first sites of target organ damage due to increased intraluminal pressures from hypertension [5,6].

In hypertension, resistance arteries typically undergo inward vascular remodelling, characterised by increased medial wall thickness and decreased lumen diameter that may be “eutrophic” or “hypertrophic”, corresponding to unchanged or increased media cross sectional area (MCSA), respectively [7]. Eutrophic remodelling is typically seen in essential hypertension [2] and uraemic conditions [8], and typically occurs in the absence of stiffening [6]. In contrast, hypertrophic remodelling has been reported in renovascular hypertension [9]. Vascular remodelling may initially be an adaptive compensatory mechanism to buffer pressure changes in the arteries; however, it eventually becomes maladaptive and compromises organ function, contributing to the cardiovascular complications of hypertension [2,10].

Structural alterations result in biomechanical property changes, which in turn worsen the degree of structural alterations, including a loss of distensibility, increase in wall stiffness, and modifications in the vessel composition (collagen–elastin ratios), consequently impairing maximal passive dilatation ability and leading to further structural remodelling [11,12]. Arterial stiffness is increasingly being recognised as an important prognostic index and potential therapeutic target in patients with hypertension [13].

Abbreviations: EC_{50}, 50% effective concentration; ACh, acetylcholine hydrochloride; CKD, chronic kidney disease; EDH, endothelium-dependent hyperpolarisation; Indo, indomethacin; LPK, Lewis polycystic kidney; MSB, Martius/Scarlet Blue; R_{max}, maximum response; MCSA, media cross sectional area; L-NAME, N^{Ω}-nitro-L-arginine methyl ester hydrochloride; NO, nitric oxide; PE, phenylephrine hydrochloride; KCl, potassium chloride; SNP, sodium nitroprusside; SBP, systolic blood pressure; VK, Von Kossa.

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http://dx.doi.org/10.1016/j.vph.2015.12.004
1537-1891/

Resistance artery vascular remodelling may also exacerbate hypertension through its effects on vascular function, such as magnifying normal vasoactive inputs (vascular amplifier effect) [14] and vasorelaxation dysfunction, which is a well-established phenomenon in hypertension, being described in the subcutaneous resistance arteries of patients with end-stage renal disease [15]. Vasorelaxation in resistance arteries involves three components: nitric oxide (NO), prostanoids, and endothelium-dependent hyperpolarisation (EDH) [15], with the contribution of each dependent upon the vascular bed under study, and the varying degrees of relative impairment depending on the disease state [16–18].

We have previously established in the Lewis polycystic kidney (LPK) rodent model of CKD that animals develop large vessel stiffness, calcification, and vascular remodelling by 12 weeks of age [19,20]. The LPK model is a result of an autosomal recessive mutation in NimA (Never In Mitosis Gene A)-Related Kinase 8 (*Nek8*) [21], which in humans is responsible for nephronophthisis (NPHP) 9 [22,23]. Within the nephrocystin family of proteins, multiple NPHP gene mutations have been identified and are the leading genetic cause of end-stage renal disease in children and young adults [23]. In the juvenile cystic kidney (jck) mouse [24] and LPK rat [25], the *Nek8* mutation leads to a phenotypic presentation of cystic kidney disease resembling human autosomal recessive polycystic kidney disease. The LPK animals present with established hypertension by 6 weeks of age, renal dysfunction by 12 weeks of age, and progression to marked renal disease by 18–24 weeks of age [25]. We recently demonstrated that in addition to sympathetic overactivity, a vascular amplifier effect was likely to contribute to the increased blood pressure observed in the LPK [26], consistent with the prediction that resistance artery vascular remodelling is occurring in this model. Therefore, we hypothesised that significant structural, biomechanical and functional changes would be evident in the mesenteric artery as a representative resistance vessel in the LPK rat, and further, show progressive change in parallel with the decline in renal function. Thus, the present study investigated temporal changes of resistance artery structural, functional and biomechanical properties in association with blood pressure and renal function in a model of CKD.

2. Material and methods

2.1. Animals

Mixed sex LPK at 6, 12 and 18 weeks ($n = 7$ for each age group), and age-matched control strain Lewis rats (6 weeks $n = 8$; 12 weeks $n = 14$; 18 weeks $n = 7$) were obtained from the Animal Resource Centre in Western Australia, Australia. A total of 43 rats were used for this study. Animals were housed in the animal house facility of Macquarie University under a 12-h light/dark cycle at 20.5 °C and 57% humidity, and offered rat chow and water ad libitum. All experimental protocols and procedures were approved by the Animal Ethics Committee of Macquarie University and adhered to the National Health and Medical Research Council of Australia's Australian code for the care and use of animals for scientific purposes (8th Edition 2013).

2.2. Tail cuff plethysmography and urine collection

Systolic blood pressure (SBP) was measured using tail-cuff plethysmography (ADInstruments, Sydney, NSW, Australia) as described previously [25]. At least 72 h prior to euthanasia, urine samples were collected from animals over a period of 24 h while held individually in metabolic cages, during which animals were offered chow and water ad libitum, and their water consumption was measured.

2.3. Tissue harvesting and biochemical analyses

Animals were deeply anaesthetised with 5% isoflurane (VCA I.S.O., Sydney, NSW, Australia) in 100% O_2 and decapitated. Trunk blood samples were collected in pre-cooled EDTA containing tubes (BD Microtainer®, Becton, Dickinson and Company, Macquarie Park, NSW, Australia) and centrifuged (3000 RPM for 5 min at 4 °C). Plasma and urine were analysed for biochemical parameters using an IDEXX VetLab analyser (IDEXX Laboratories Pty Ltd., Rydalmere, NSW, Australia). The mesenteric vasculature, kidneys and heart were removed. The kidneys, heart, and left ventricle were weighed and respective indices (HI and KI; %) were calculated as heart or kidney weight (g) / body weight (BW) (g) × 100.

2.4. Mesenteric artery isolation

Immediately following dissection, the mesenteric vasculature was placed in ice-cold Krebs physiological solution (in mM: NaCl 118.2, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11.7, $NaHCO_3$ 25, and EDTA, 0.026), continuously bubbled with carbogen (95% O_2; 5% CO_2; BOC Ltd., North Ryde, NSW, Australia) to achieve a pH value of 7.4–7.45 [12].

After cleaning of adherent connective tissue under a dissecting microscope, a second order mesenteric artery segment (200–400 μm external diameter; ~4 mm in length) was mounted in a pressure myograph (Living Systems Instrumentation, Burlington, VT, USA) in 5 mL of carbogen-bubbled ice-cold Kreb's solution. Artery ends were cannulated with glass micropipettes (1 × 0.25 mm glass capillaries, A-M Systems, Inc., Sequim, WA, USA), and secured with 10–0 sutures (Ethilon® Nylon Suture LLC., CA, USA). The remaining mesenteric vasculature was placed in 4% formalin for 24 h, followed by 70% ethanol until further processing for histology.

2.5. Pressure myography

Average intraluminal pressure in the artery was increased to 120 mm Hg, and the vessel length stretched until there was no lateral bowing of the vessel [27]. Pressure was then decreased to 60 mm Hg and the vessel left to equilibrate for 60 min, with Kreb's buffer superfused at a flow rate of 3 mL/min. During this period, the vessel warmed to 37 °C and constantly bubbled in carbogen via a miniature gas dispersion tube (Living Systems Instrumentation). Lumen and wall thickness dimensions were measured at a constant intraluminal pressure of 60 mm Hg using the video dimension analyser within the pressure myography system for all experimental conditions. Following equilibration, vessel integrity was determined, with vessels considered viable if the α_1-adrenergic receptor agonist phenylephrine (PE; 10^{-6} M) elicited >50% constriction relative to resting lumen diameter [28,29]. Vascular functional and structural investigations were then performed sequentially. All data were recorded using Spike 2 v7.07a software (Cambridge Electronic Design, Cambridge, UK).

2.5.1. Vascular function investigations

Cumulative concentration–response curves to (i) the α_1-adrenergic receptor agonist PE and (ii) the direct muscle depolariser potassium chloride (KCl) were obtained in order to study vasoconstrictor mechanisms.

After preconstriction with PE, cumulative concentration–response curves to (i) the endothelium-dependent vasodilator acetylcholine (ACh) and (ii) -independent vasodilator sodium nitroprusside (SNP) were obtained in order to study endothelial integrity.

After preconstriction to PE, cumulative concentration–response curves to ACh were obtained after arteries had been incubated in (i) either N-$^{\Omega}$nitro-L-arginine methyl ester (L-NAME) alone or (ii) indomethacin (Indo) and L-NAME to further delineate vasorelaxation mechanisms.

The 50% effective concentration (EC_{50}) and maximum response (R_{max}) were calculated from the concentration–response curves. The differences between R_{max} responses to ACh and that in the presence of L-NAME alone were considered the NO-dependent [30] component of the ACh-induced response; the ACh–R_{max} remaining in the presence