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Glibenclamide improves kidney and heart structure and function in the adenine-diet model of chronic kidney disease

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ABSTRACT

The development of chronic kidney disease (CKD) and associated cardiovascular disease involves free radical damage and inflammation. Addition of adenine to the diet induces inflammation followed by CKD and cardiovascular disease. NOD-like receptor protein-3 (NLRP-3) is pro-inflammatory in the kidney; glibenclamide inhibits production of NLRP-3. Male Wistar rats were fed either control rat food or adenine (0.25%) in this food for 16 weeks. Glibenclamide (10 mg/kg/day) was administered to two groups with and without adenine for the final 8 weeks. Kidney function (blood urea nitrogen/BUN, plasma creatinine/PCr, plasma uric acid, proteinuria), kidney structure (fibrosis, inflammation), cardiovascular parameters (blood pressure, left ventricular stiffness, vascular responses and echocardiography) and protein expression of markers for oxidative stress (HO-1), and inflammation (TNF-α, NLRP-3) were assessed. In adenine-fed rats, glibenclamide decreased BUN (controls: 6 ± 0.6; adenine: 56.6 ± 5.4; adenine + glibenclamide: 19.4 ± 2.7 mmol/L), PCr (controls: 42 ± 2.8; adenine: 268 ± 23; adenine + glibenclamide: 81 ± 10 μmol/L), proteinuria (controls: 150 ± 7.4; adenine: 303 ± 19; adenine + glibenclamide: 220 ± 13 μmol/L) (all $p < 0.05$). Glibenclamide decreased infiltration of chronic inflammatory cells, fibrosis, tubular damage and expression of HO-1, TNF-α and NLRP-3 in the kidney. Glibenclamide did not alter plasma uric acid concentrations (controls: 38 ± 1; adenine: 63 ± 4; adenine + glibenclamide: 69 ± 14 μmol/L). Cardiovascular changes included decreased systolic blood pressure and improved vascular responses although cardiac fibrosis, left ventricular stiffness and hypertrophy were not reduced. Glibenclamide improved kidney structure and function in CKD and decreased some cardiovascular parameters. Inflammatory markers and cell populations were attenuated by glibenclamide in kidneys.

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1. Introduction

Inflammation plays a key role in the progression of chronic kidney disease (CKD) both in experimental animals [1] and in humans [2]. Inflammation in the kidney may be caused by glomerular and tubular injury that, in turn, initiates production of pro-inflammatory cytokines and oxidative stress, and immune cell infiltration [3]. The inflammatory responses involve distinct families of soluble and cellular receptors that activate pro-inflammatory signalling pathways, including the NOD-like receptors [4]. NOD-like receptor protein-3 (NLRP-3) is an intracellular protein involved in cell proliferation and regeneration, differentiation and apoptosis by activating mediators such as caspase-1 [4,5]. It is also pro-inflammatory via its stimulation of release of interleukins [5]. In non-diabetic CKD, increased NLRP-3 expression was associated with progression of the disease [5]. The pathogenesis of CKD and other inflammatory diseases is known to result from increased uric acid [6] and this may occur via induction of NLRP-3 [7,8].

Glibenclamide is a K_{ATP} channel blocker with known anti-inflammatory properties [9,10], reducing the infiltration of inflammatory cells during ischaemia/reperfusion injury in the kidney [10]. In the brain, reduced production of TNF-α by glibenclamide correlated with anti-oxidant and anti-inflammatory responses against ischaemia/reperfusion injury [9]. Glibenclamide inhibited the production of NLRP-3 [11] which is involved in inflammatory processes in CKD [4]. The adenine-induced model of kidney disease in rats was developed by Yokozawa and coworkers [12]. These rats fed 0.75% adenine developed marked structural changes in the kidney with a severe decline in function within 4 weeks, demonstrating acute rather than chronic kidney disease. Our recent studies with a range of adenine doses show that addition of 0.25% adenine to the rat diet produces changes in kidney structure and

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function that mimic human CKD together with the appropriate cardiovascular symptoms [13]. In the present investigation, we hypothesised that the adenine-induced increase in uric acid would stimulate NLRP-3 expression and inflammation, and that glibenclamide would improve kidney and heart structure and function by normalising kidney expression of NLRP-3.

2. Materials and methods

2.1. Animal studies

All experimental protocols were approved by the Animal Experimentation Ethics Committee of the University of Southern Queensland, under the guidelines of the National Health and Medical Research Council of Australia. Male Wistar rats aged 9–10 weeks ($n = 48$; body weight 335 ± 3 g) were divided into 4 experimental groups, each $n = 12$, treated for 16 weeks with powdered rat food alone as control or with glibenclamide 10 mg/kg/day for the last 8 weeks, or 0.25% adenine (Carbosynth Limited, Compton, Berkshire, UK) (approximately 150 mg/kg/day) in powdered food [13], with or without glibenclamide (Sigma–Aldrich) 10 mg/kg/day for the last 8 weeks. The dose of glibenclamide was based on previous literature reports [14–16].

2.2. Systolic blood pressure measurement

Systolic blood pressure was measured in rats under light sedation with an i.p. injection of Zoletil (tiletamine 15 mg/kg, zolazepam 15 mg/kg). Measurements were taken using an MLT1010 Piezo-Electric Pulse Transducer (AD Instruments, Sydney, Australia) with an inflatable tail-cuff connected to a MLT844 Physiological Pressure Transducer (AD Instruments) and Power Lab data acquisition unit (AD Instruments) [17].

2.3. Echocardiography

Echocardiography was performed by trained cardiac sonographers at the small animal theatre of The Prince Charles Hospital, Brisbane. Rats were anaesthetised via i.p. injection with Zoletil (tiletamine 15 mg/kg, zolazepam 15 mg/kg) mixed with Ilium Xylazil (xylazine 10 mg/kg). Echocardiographic images of rats were obtained using the Hewlett Packard Sonos 5500 (12 MHz frequency foetal transducer) at an image depth of 3 cm using two focal zones. Measurements of left ventricular posterior wall thickness and internal diameter were made using two-dimensional M-mode taken at mid-papillary level [18].

2.4. Collection of urine and plasma

Rats were kept in metabolic cages for 24 h to collect urine. The urine was used for the estimation of protein by the Bradford method. Before the terminal experiments, rats were anaesthetised and blood was collected and centrifuged to collect plasma which was stored at −20 °C until further analysis [17]. Blood urea nitrogen (BUN), and plasma concentrations of creatinine (PCr), uric acid, phosphate, triglycerides, total cholesterol and non-esterified fatty acids (NEFA) were determined by The University of Queensland Veterinary Pathology Services, using an Olympus AU400 autoanalyser [17].

2.5. Isolated heart preparation

After taking blood samples as above, the heart was excised and placed in cooled (0 °C) crystalloid perfusate (modified Krebs–Henseleit solution). A cannula was then placed in the aorta immediately above the coronary ostia of the aortic stump. The heart was perfused and isovolumetric ventricular function was measured by inserting a latex balloon catheter into the left ventricle connected to a Capto SP844 MLT844 physiological pressure transducer and Chart software on a Maclab system (AD Instruments, Australia) and PowerLab data acquisition unit (AD Instruments) as previously described [17]. At the end of this experiment, the atria and right ventricle were dissected away leaving the left ventricle and septum, which was blotted dry, then weighed. Myocardial diastolic stiffness was calculated as the diastolic stiffness constant (κ) [17].

2.6. Organ bath studies for vascular response

Thoracic aortic rings (4 mm in length) were placed in an organ bath chamber with a resting tension of 10 mN. Cumulative concentration–response (contraction) curves were measured for noradrenaline; concentration–response (relaxation) curves were measured for acetylcholine and sodium nitroprusside in the presence of a submaximal (70%) contraction to noradrenaline [17].

2.7. Histology

Immediately after removal, slices of kidney and heart (left ventricle) were fixed in 10% buffered formalin for 3 days with a change of formalin solution every day. The samples were then dehydrated in ethanols and embedded in paraffin wax. Thin sections (4 μm) of the kidney and left ventricle were cut and stained with haematoxylin and eosin (H&E) or Masson's trichrome stain for kidney and picrosirius red for heart as previously described [17]. The Aperio digital pathology system for imaging was used for H&E and Masson's trichrome and image morphometry was analysed using ImagePro Plus image analysis software.

2.8. Western blot analysis

The following antibodies were used as primary antibodies: monoclonal mouse anti-heme-oxygenase-1 (HO-1) (AB13248, Abcam; 1:1000); polyclonal goat anti-tumour necrosis factor (TNF)-α (N-19, Santa Cruz Biotechnology; 1:1000); polyclonal rabbit NLRP-3/Cryopyrin(H-66) (SC 66846, Santa Cruz Biotechnology; 1:500). A portion of the cortex and medulla from each kidney and a portion of left ventricle from each heart was homogenised in ice-cold radio-immunoprecipitation (RIPA) lysis buffer containing protease inhibitors (50 mmol/L Tris–HCl [pH 7.5], 150 mmol/L NaCl, 5 mmol/L EDTA, 1 μg/mL leupeptin, 1 mmol/L phenylmethylsulfonyl fluoride and aprotinin lμg/mL) as described previously [19]. Equal loading of protein was determined by Western immunoblot using a rabbit GAPDH antibody as a protein loading control (rabbit polyclonal, Sigma–Aldrich Pty Ltd., Sydney, Australia, 1:5000). Band intensities were quantified by using an image analyser and Scion software (Scion Corp., Frederick, MD, version release alpha 4.0.3.2).

2.9. Statistical analysis

The data were expressed as mean ± standard error of the mean (SEM) and were analysed using one way analysis of variance (ANOVA) followed by Tukey's test as post hoc analysis. $p < 0.05$ was considered significant. Statistical analyses were performed using Graph Pad Prism version 5.00 for Windows.

3. Results

3.1. Kidney structure and function

Dietary supplementation with 0.25% adenine for 16 weeks induced structural changes in the kidney shown as infiltration of

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