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Angiotensin receptor blockade in juvenile male rat offspring: Implications for long-term cardio-renal health

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ABSTRACT

Inhibition of the renin-angiotensin system in early postnatal life is a potential therapeutic approach to prevent long-term cardiovascular and kidney diseases in individuals born small. We determined the long-term effects of juvenile losartan treatment on cardiovascular and kidney function in control male rat offspring and those exposed to uteroplacental insufficiency and born small. Bilateral uterine vessel ligation (Restricted) or sham (Control) surgery was performed in late gestation in Wistar Kyoto rats. At weaning, male offspring were randomly assigned to receive losartan in their drinking water or drinking water alone from 5 to 8 weeks of age, and followed to 26 weeks of age. Systolic blood pressure and kidney function were assessed throughout the study. Pressure myography was used to assess passive mechanical wall properties in mesenteric and femoral arteries from 26-week-old offspring. Losartan treatment for three weeks lowered systolic blood pressure in both Control and Restricted groups but this difference was not sustained after the cessation of treatment. Losartan, irrespective of birth weight, mildly increased renal tubulointerstitial fibrosis when assessed at 26 weeks of age. Mesenteric artery stiffness was increased by the early losartan treatment, and was associated with increased collagen and decreased elastin content. Losartan also exerted long-term increases in fat mass and decreases in skeletal muscle mass. In this study, untreated Restricted offspring did not develop hypertension, vascular dysfunction or kidney changes as anticipated. Regardless, we demonstrate that short-term losartan treatment in the juvenile period negatively affects postnatal growth, and kidney and vascular parameters in adulthood, irrespective of birth weight. The long-term effects of early-life losartan treatment warrant further consideration in settings where the potential benefits may outweigh the risks; *i.e.* when programmed adulthood diseases are apparent and in childhood cardiovascular and kidney diseases.

1. Introduction

Low birth weight is commonly used as a surrogate of intrauterine growth restriction (IUGR) and affects one in 10 births in the Western world [1,2]. IUGR is most commonly caused by uteroplacental insufficiency, whereby oxygen and nutrient delivery to the fetus is compromised [3]. An abundant literature has shown that low birth weight increases the risk of cardiovascular, kidney and metabolic diseases in later life [4,5]. Critical windows after birth are also independently susceptible to developmental programming [6,7]. In rodent studies, improved lactation in the immediate postnatal period [8–10] or lifestyle modifications such as a healthier diet [11] or exercise [12,13] in the juvenile period can ameliorate prenatally programmed changes to organ structure and function. Thus, interventions during postnatal or juvenile periods are feasible and potentially effective options to prevent programmed diseases.

The renin angiotensin system (RAS) plays a fundamental role in organ development, particularly of the kidneys and heart [14]. In adults, the RAS is critical for blood pressure control, and AT_1 receptor blockers (ARB) and angiotensin converting enzyme inhibitors (ACEi) are the most efficacious and widely used class of antihypertensive drugs. RAS blockade has also been demonstrated to attenuate age-

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related elevations in blood pressure and loss of kidney function [15,16]. For this reason, ARBs are being increasingly prescribed for adolescent hypertension and in children with albuminuria. In animal models of programmed cardiovascular and kidney disease [17], multiple studies have found that the RAS is inhibited during fetal life and upregulated in adulthood [17]. Therefore, studies have examined whether prenatal programming of hypertension could be prevented by postnatal pharmacological manipulation of the RAS. Treatment with the ACEi, enalapril, from 14 to 16 weeks of age in male IUGR rat offspring was reported to abolish hypertension within just one week [18]. In studies targeting the RAS in early postnatal life, long-lasting benefits have also been detected. In particular, RAS blockade with the ACEi captopril [12], ARB losartan [19] or aliskiren [16], between 2 and 4 weeks of age in rats, have all been reported to prevent hypertension in adulthood that was induced by various maternal diets.

Given that neonatal RAS blockade can impair nephrogenesis [14,20] and lead to irreversible tubular abnormalities [21,22], studies in rodents typically initiate treatment after two weeks postnatally, when the number of nephrons has been determined. However, functional and structural maturation of the kidney, particularly of the nephron tubules and collecting duct system, continues into puberty in humans [23] and beyond two months of age in rodents [24,25]. While seemingly beneficial for long-term blood pressure control, it is unknown whether short-term RAS blockade during early life, following cessation of nephrogenesis, can impact the kidneys and subsequently affect kidney function in adulthood. Indeed, these inhibitors of the RAS are known to target the kidneys in a manner that is independent of blood pressure lowering [26,27]. In this study, we investigated the effects of juvenile RAS blockade on long-term kidney function and pathology in rats. This was performed in both appropriately grown offspring and offspring born growth restricted (as a result of uteroplacental insufficiency) and thus at increased risk of adult-onset disease. Blood pressure, small artery passive mechanical wall properties, fat deposition and blood glucose levels were also measured. Growth restricted male offspring in this model have a permanent reduction in nephron [28] and cardiomyocyte [8] endowment, and mesenteric and femoral arterial dysfunction [9], which can [28], although not always [13], result in arterial hypertension. Kidney function remains unaffected at six months of age [28]. In the absence of major dysfunction in this current cohort of growth restricted rats, we hypothesized that treatment with the ARB, losartan, during a juvenile development stage would negatively impact cardiovascular and kidney function, irrespective of birth weight.

2. Materials and methods

2.1. Animal handling

All experiments were approved by The University of Melbourne Animal Ethics Committee and were conducted in accordance with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes*. Female Wistar Kyoto rats were maintained under standard conditions with food and water provided *ad libitum*. Rats were mated and uteroplacental insufficiency (offspring termed Restricted) or sham (offspring termed Control) surgery was performed at embryonic day (E) 18 as previously described [29]. Uteroplacental insufficiency was induced under anesthesia by bilateral uterine vessel (artery and vein) ligation, which restricts the transport of oxygen and nutrients to the fetuses. Rats delivered at term (E22) and birth weights and litter size were recorded. Litter size was not standardized (total pups per litter: Control 10 ± 1; Restricted 6 ± 1). Offspring were separated from their mother at postnatal day 35 (P35) as per routine practice with this model due to compromised lactogenesis in Restricted dams [30]. Food and water were provided *ad libitum* to all dams and offspring.

2.2. Losartan treatment

From 5 to 8 weeks of age, Control and Restricted male offspring were housed individually and randomly assigned to receive losartan in their drinking water (30 mg/kg body weight per day) or drinking water alone (resulting in four groups; n = 7–11 offspring per group with no more than one offspring per litter per group). The losartan dose in the drinking water was adjusted every three to four days according to body weight and the observed drinking pattern. Water consumption over the treatment period did not differ between Control and Restricted animals (ml: Control-Untreated: 500 ± 17, Restricted-Untreated: 444 ± 30, Control-Losartan: 505 ± 41, Restricted-Losartan: 502 ± 47, n = 6–11/group) and thus all rats randomized to losartan received the same drug dose relative to body weight. Restricted rats were ~15% lighter than Controls throughout the treatment period and thus the absolute drug dose was 15% less in Restricted *vs* Control rats. After the treatment period, all rats were provided with drinking water alone, until post-mortem at 26 weeks of age.

2.3. Blood pressure

Blood pressure was measured using non-invasive tail-cuff plethysmography (ADInstruments Pty. Ltd., Castle Hill, NSW, Australia) at 8, 12, 16 and 26 weeks of age in rats acclimatized to the restraint procedure, as previously described [31,32]. The last five of ten acquired traces were recorded and averaged to determine systolic blood pressure.

2.4. Food and water intake, and renal function

Animals were weighed and placed in individual metabolic cages for 24 h measurements of food and water intake, and urine production at 8 (conclusion of losartan treatment) and 26 weeks of age. Rats were acclimatized to the metabolic cages twice prior to the 24 h urine collection to minimize stress. Measurements of urinary albumin, total protein, sodium, potassium and creatinine were performed using a COBAS Integra 400 (Roche Diagnostics, West Sussex, UK). Creatinine clearance ($ml.min^{-1}$) was calculated as follows: (urinary creatinine [$\mu mol.l^{-1}$] × 24 h urine production [ml])/(plasma creatinine [$\mu mol.l^{-1}$] × 1440 [min]).

2.5. Tissue and blood collection

Blood glucose (random non-fasted; tail vein sample) was measured using a glucometer. All rats were euthanized with an intraperitoneal injection of illium Xylazil-20 (30 mg/kg) and ketamine (225 mg/kg). A subset of rats was euthanized at 5 weeks of age and small mesenteric and femoral arteries were isolated to obtain baseline values of passive mechanical wall properties (prior to losartan treatment). Remaining rats were euthanized at 26 weeks of age, and a blood sample was collected *via* cardiac puncture. Small mesenteric and femoral arteries were isolated from these adult rats, along with kidneys, heart, liver, adrenal glands, and dorsal fat which were weighed. Kidneys and mesenteric artery segments were immersion fixed in 4% neutral buffered formalin for histological analyses.

2.6. Passive mechanical wall properties at 5 and 26 weeks of age

Leak-free segments of arteries from rats at P35 and 26 weeks of age were mounted on a pressure myograph (Living Systems Instrumentation), as described previously [33,34], and superfused with Ca^{2+}-free, EGTA-containing physiological salt solution (PSS, mM): 120 NaCl, 5 KCl, 25 $NaHCO_3$, 1 KH_2PO_4, 1.2 $MgSO_4$, 11 glucose, and 2 EGTA, bubbled with carbogen (95% O_2, 5% CO_2) at ~35 °C. Intraluminal pressure was increased stepwise from 5 to 110 mmHg. Artery outside diameter (OD) and wall thickness (WT) were measured at