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Losartan ameliorates renal injury, hypertension, and adipocytokine imbalance in 5/6 nephrectomized rats

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ABSTRACT

The mechanisms underlying insulin sensitivity and fat tissue distribution in chronic renal insufficiency remain unclear. Previous studies have shown the benefits of angiotensin II receptor blockers on moderately nourished to well-nourished patients with the metabolic syndrome. The current study explored the effect of losartan, the first selective angiotensin II receptor blocker, on insulin sensitivity and visceral fat tissue distribution in a 5/6 nephrectomized (N) rat model and investigated the expression of adipose tissue adipocytokines. Male Sprague-Dawley rats (200 g to 250 g) were subjected to 5/6 nephrectomy, and the adipocytes isolated from the visceral fat tissues were then studied. Results showed that desmin expression was significantly suppressed and systolic blood pressure was successfully normalized in the losartan-administered (NA) group. The weight of the visceral fat pad remarkably decreased in the N and NA groups (100 mg/500 ml drinking water) compared with the control group. The weight did not decrease further in the NA group compared with the N group. Insulin resistance was more remarkable in the N group compared with the control and NA groups. Moreover, the adipose tissue expression of adiponectin and leptin was downregulated whereas that of resistin was upregulated in the N group compared with the control group. However, the adiponectin, leptin, and resistin adipose tissue expression returned to their basal values in the NA group. These findings indicated that losartan administration ameliorated renal injury, systolic blood pressure, and adipocytokine imbalance of the adipose tissue in chronic renal insufficiency. Insulin sensitivity was not improved.

1. Introduction

Patients with chronic kidney disease present carbohydrate metabolism disorders and insulin resistance (Basturk and Unsal, 2012; Chen et al., 2003, 2004). Several studies have reported the association of metabolic syndrome and insulin resistance with a high risk for diabetes (Lorenzo et al., 2003) and kidney disease (Zhang et al., 2005). Diabetes and cardiovascular diseases are the main causes of end-stage renal disease, which is the dominant outcome in patients with type-2 diabetic nephropathy. End-stage renal disease is characterized by decreased kidney function and substantial proteinuria (Lewis et al., 2001; Packham et al., 2012; Shahinfar et al., 2002). Non-diabetic patients with end-stage renal disease also present mild fasting hyperglycemia and abnormal glucose tolerance, whereas others maintain normoglycemia but at the price of hyperinsulinemia (DeFronzo et al., 1981; Mak and DeFronzo, 1992; Mak et al., 1983). Malnutrition, inflammation, and atherosclerosis syndrome are commonly found in dialysis patients with diabetes (Abe et al., 2011; Tonbul et al., 2006), and these conditions provide insight into patients' susceptibility to cardiovascular disease (Kalaitzidis and Bakris, 2009). Impaired insulin sensitivity is frequently recognized in uremic patients. However, whether insulin resistance is a cause or a consequence of chronic kidney disease or end-stage renal disease remains unclear.

Hypertension is a key independent risk factor for kidney diseases and faster renal function loss. The pharmacological

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inhibitor of the renin–angiotensin–aldosterone system more effectively retards the progression of advanced proteinuric chronic kidney disease than other antihypertensive agents (Akdag et al., 2008). Previous studies have shown the impact of angiotensin II receptor blockers on insulin sensitivity in hypertensive patients (Moan et al., 1994; Ran et al., 2006). Angiotensin II receptor blockers reportedly act on fat distribution in patients with metabolic syndrome and hypertension (Chujo et al., 2007; Shimabukuro et al., 2007). Losartan, the first selective angiotensin II receptor blocker discovered, controls hypertension (Bakris et al., 2003; Parrinello et al., 2009), treats proteinuria (de Zeeuw et al., 2004), and alleviates hypercholesterolemia (Petnehazy et al., 2006) during the course of chronic kidney disease toward end-stage renal disease. Losartan also influences the proinflammatory mediator in atherogenesis (Han et al., 2007).

Abdominal obesity is an evident risk factor for cardiovascular complications and chronic inflammation in chronic kidney disease and dialysis patients (Behn and Ur, 2006; Ishimura et al., 2011). The waist circumference is associated with chronic kidney disease and mortality incidents (Elsayed et al., 2008; Kalaitzidis and Siamopoulos, 2011). Three major adipose tissue cytokines (leptin, adiponectin, and resistin) have been separately investigated in chronic kidney disease and dialysis patients (Baldasseroni et al., 2013; Zoccali and Mallamaci, 2011; Zoccali et al., 2011). Reduced kidney function manifests as low adiponectin, high leptin, and high resistin levels in the plasma (Baldasseroni et al., 2013; Zoccali and Mallamaci, 2011). Reduced kidney function is also positively correlated with the metabolic syndrome/inflammatory response in hemodialysis patients (Lee et al., 2011; Tsai et al., 2011). Meanwhile, leptin reverses metabolic alterations (Zoccali and Mallamaci, 2011), and resistin circulation levels are reportedly relation to the inflammatory biomarkers of chronic kidney disease (Axelsson et al., 2006). The role of angiotensin II type 1 (AT-1) receptor activation in vascular inflammation regulation is also associated with the upregulation of pro-inflammatory and pro-fibrotic pathways in obesity (Vaziri et al., 2005).

Based on these findings, abdominal obesity and hypertension are considered to be inflammatory responses to the development of chronic kidney disease, and AT-1 reportedly regulates the inflammatory process. However, previous studies have been performed on moderately nourished to well-nourished subjects. Accordingly, the present study investigated the effects of angiotensin II receptor blocker on insulin sensitivity, kidney pathology, metabolic factors, and adipocytokines in a 5/6 nephrectomized (N) rat model, which is a model of impaired renal function that mimics chronic kidney disease in a poorly nourished environment (Shobeiri et al., 2010). The anti-inflammatory effects of losartan on obesity, especially in the adipose tissue expression of adipocytokines, were also explored.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats weighing 200 g to 250 g (6 weeks old) were purchased from a local breeder. The rats were housed four to a cage at 20 °C to 22 °C in a light-controlled room on an alternating 12 h light/dark cycle. The rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) after one week of acclimatization, and ventral laparotomy was performed under aseptic conditions. The 5/6 nephrectomy was performed through a surgical resection of the upper and lower thirds of the left kidney followed by right nephrectomy one week later. The 5/6-nephrectomized rat model is the common model of impaired renal function to mimic advanced chronic kidney disease and a poorly nourished environment (Shobeiri et al., 2010). Control rats were subjected to a mock operation. All procedures were carried out in accordance with the guidelines of the Taiwan Government Guide for the Care and Use of Laboratory Animals. The study protocol was approved by the animal welfare committees. The rats were bred for one month and grouped into three: 5/6 nephrectomized rats (N group; $n=8$), 5/6 nephrectomized rats administered with losartan (NA group; $n=8$), and mock control rats (control group; $n=8$). Losartan was administered to the rats by dissolving it in their drinking water (100 mg/500 ml) as described by Yang et al. (2002). Blood pressure was measured by the non-invasive tail-cuff method. Renal function was determined by measuring the plasma creatinine and blood urea nitrogen levels. Insulin sensitivity was measured by the oral glucose tolerance test (OGTT). Whole body fat distribution was evaluated by computed tomography (CT) scanning. Renal impairment was evaluated by histological examination. Isolated adipocytes were used to evaluate the mean and distribution of fat cell size. Adipocytokine expression in adipocytes (adiponectin, leptin, and resistin) was measured by reverse transcription (RT)–polymerase chain reaction (PCR).

2.2. Blood pressure measurement

Blood pressure was measured using a programmable sphygmomanometer (BP-98A; Softron, Japan) by the tail-cuff method. The small animal study unit of the system has a rat-holder base with a built-in warming element to raise the ambient temperature to 37 °C and maintain adequate circulation in the tail for indirect blood pressure measurements. The animal was positioned in Lucite housing with its tail firmly held outside. The occluding metal tubular cuff (9 mm to 12 mm internal diameter) and the pneumatic pulse sensor-transducer were then placed on the tail and connected to the sphygmomanometer. The occluding cuff pressure was controlled at a pre-adjusted inflation–deflation rate until the first pulse was recorded. All measurements were carried out in a quiet room starting at 10:00 AM because the normal blood pressure shows an intrinsic diurnal variation and may be disturbed by environmental conditions. The order of testing for the different groups was varied on subsequent testing days. An experienced technician took three to five measurements per rat from 20 min to 30 min.

2.3. Plasma measurement

Blood sampling was carried out in a quiet room starting at 10:00 AM. The order of testing for the different groups was varied on subsequent testing days. Blood samples for the glucose and insulin measurements (approximately 500 μl) were collected by tail bleeding into a 1.5 ml heparin-coated polyethylene microfuge tube on ice. Trunk blood was collected from each rat after decapitation. Plasma was separated by centrifugation and stored at –20 °C until analysis. Plasma glucose was measured on a glucose analyzer (Model 23A; Yellow Springs Instrument Company, Yellow Springs, OH, USA). Plasma insulin, triglyceride, nonesterified fatty acids, creatinine, and urea were measured with commercial kits.

2.4. Measurement of visceral fat tissues

A CT scan was performed on the rats in three different regions at an ultra-high resolution setting. Three slices were examined at the level of the sacroiliac joint, the upper part of the iliac crest, and 1.5 cm above the second slice. A density range between –150 and –40 Hounsfield units was used to define the fat area, and a range between –40 and 250 Hounsfield units was used to define the muscle area (water density = 0). All CT scans were performed with the anesthetized rats in a prone position. The total adipose and