THE EFFECTS OF LEPTIN ADMINISTRATION ON RENAL FUNCTION IN SPONTANEOUSLY HYPERTENSIVE RATS

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Received: 18 February 2016, Revised and Accepted: 29 September 2016

INTRODUCTION

Leptin is an adipokine with pleiotropic effects acting through leptin receptors in several body tissues. Apart from its well-established role in appetite and energy homeostasis, leptin is being increasingly implicated in several pathophysiological processes although much remains to be confirmed. Leptin levels are usually elevated in obese individuals, and massively obese individuals are also known to have glomerulopathy including changes such as glomerulosclerosis, glomerulomegaly, and also marked by proteinuria [1–4]. The role of leptin in this, however, is unknown. Raised blood pressure is often also seen in obese individuals, which has been attributed to increased sympathetic nervous system activity, stimulated by elevated leptin [5]. Leptin administration to normotensive rats has been shown to increase blood pressure [6]. Raised leptin level in obese hypertensive individuals could, therefore, worsen the complication of hypertension and might even be responsible for chronic kidney disease (CKD) and its progression to end-stage renal disease and renal failure [7,8].

Cytokines of the transforming growth factor-β (TGF-β) family, particularly those regulating proliferation and growth of tissues, have been implicated in tissue fibrosis [9]. TGF-β1, a member of this family of proteins, stimulates excessive production of extracellular matrix material via activation of Smad2 [7,10]. Structural and functional damage to tissues result in progression of the disease. Smad7, which is activated by Smad3, is inhibitory in nature [11]. TGF-β1 might also be activated by Smad-independent pathways involving bone morphogenic protein 7 (BMP7) and offers the prospect of new therapeutic targets for treatment in CKD [9]. The involvement of leptin in obesity-related glomerulopathy (ORG) is unknown, and a link with leptin might describe a pathway that facilitates tissue damage and eventually CKD in conditions characterized by hyperleptinemia.

In addition to leptin, adipose tissue also releases numerous other adipokines, and it might not be possible to investigate the role of leptin in renal disease in an obese model. To study the role of leptin and raised blood pressure in glomerulosclerosis requires the use of a nonobese hypertensive animal model. This study, therefore, investigated the effects of exogenous leptin administration on glomerular function in spontaneously hypertensive rats (SHRs), which would be a novel pathway in the study of obesity/leptin-related glomerular disease pathogenesis. The central hypothesis of this study is that leptin has a direct role in the pathogenesis of kidney disease in hypertension.

METHODS

Male SHRs, aged 12–14 weeks, were given daily subcutaneous injections of either leptin (BioVision Milpitas, CA, USA) (60 µg/kg/day; n=8) or saline (vehicle; n=8) for 42 days. All rats were housed singly in ventilated cages and maintained on a 12:12 hrs day-night cycle at the Laboratory Animal Care Unit, Universiti Teknologi MARA (UiTM), Sungai Buloh Campus. The study design was approved by the Animal Users and Care Committee of the Faculty of Medicine, (UiTM), Malaysia. They were fed standard rat chow and had free access to tap water. Blood pressure was measured once a week using a tail-cuff plethysmography (Kent Scientific Corporation, Torrington, CT, USA) [12]. Urine was collected over 24 hrs once a week in a metabolic cage and analyzed for urine electrolytes, urea, creatinine, and protein excretion. Blood was...
collected on the last day for the measurement of serum electrolytes, urea, creatinine, and albumin. On day 43, animals were euthanized, and kidneys were harvested and stored at −80°C until analysis for determination TGF-β1/Smad pathway expression.

Quantitative real-time polymerase chain reaction (RT-PCR) was used to determine messenger ribonucleic acid (mRNA) expression of TGF-β, Smad2, 3, 7, and BMP7 using the CYBR-green technique in a single-step reaction. RNA was extracted from the right kidney tissue using trizol reagent (Invitrogen, Life Technologies) and then subjected to the RT-PCR assay. The ratio (i.e. fold-change) of gene expression levels in the leptin treatment groups to control rats was calculated relative to the housekeeping control genes - β-actin and glyceraldehyde 3-phosphate dehydrogenase.

The other (left) kidneys were stored in neutral buffered 10% formalin until they were processed and stained with hematoxylin and eosin [13]. Prepared slides were analyzed by light microscopy. Digital images were captured using an imaging system (NIS-Element BR 3.1 image analysis system, Nikon, Tokyo, Japan). The tissue area was divided into three parts (upper, middle, and lower zone). 30 glomeruli in each part were measured consecutively for a count of total nuclei as well as total area (µm²). Total number of nuclei was counted manually by counting hematoxylin stained areas inside the glomerulus. On the other hand, the total area was measured by tracing the outer limit of bowman’s space using the image analysis software.

All data are expressed as mean±standard error of mean and comparisons between the means were made using the independent t-test, and a p<0.05 was accepted as statistically significant.

RESULTS

All animals had a steady increase in body weight throughout the study period, and neither of the groups differed in body weight both at the start and at the end of the study period (Table 1).

Systolic blood pressure was not significantly different between the two groups after 42 days of treatment (Table 1). This might be possible that leptin contributes to the pathogenesis of these disorders. SHR was selected as the experimental model as it is an accepted animal model of essential hypertension [14]. This model was especially suitable to the proposed experimental design since these animals develop hypertension by genetic mechanisms [15] and do not express the obese phenotype.

Table 1: Body weight (g) of control and leptin-treated rats for 42 days treatment

| Group | Body weight g Day 0 | Body weight in g Day 42 |
|-------|---------------------|-------------------------|
| Control | 25±8.09 | 291±8.87 |
| Leptin  | 25±3.09 | 277±6.46 |

Table 2: Mean systolic blood pressure of control and leptin-treated rats for 42 days treatment

| Group (mmHg) | Blood pressure Day 0 | Blood pressure Day 42 |
|--------------|----------------------|-----------------------|
| Control      | 180±4.07             | 184±5.38              |
| Leptin       | 175±3.73             | 189±4.56              |

Table 3: Renal parameters of control and leptin-treated rats on day 42 of treatment

| Groups         | Creatinine clearance (µl/min) | Urine output (ml/day) | Urine Albin (g/L) | Total protein (g/L) | Urinary Sodium excretion (µmol/min) | Potassium excretion (µmol/min) |
|----------------|------------------------------|-----------------------|------------------|---------------------|-------------------------------------|---------------------------------|
| Control        | 799±64.3                    | 9±1.20                | 0.98±0.18        | 3.40±0.45           | 0.73±0.13                           | 1.04±0.17                      |
| Leptin         | 716.8±51.8                  | 10±1.29               | 0.70±0.25        | 2.24±0.35           | 0.56±0.10                           | 0.65±0.10                      |
Table 4: Total number of nuclei inside a glomerulus and area of glomeruli in control and leptin-treated rat kidneys after 42 days of treatment

| Group     | Mean number of nuclei per glomerulus | Area of glomeruli (μm²) |
|-----------|-------------------------------------|------------------------|
| Control   | 4297±169                            | 6537.2±5.1±2442.0      |
| Leptin    | 4012±186                            | 6132.12±2111.4         |

obesity might be involved. In addition, it also suggests that leptin might not be directly involved in the hypertrophy of the glomeruli or obesity-related glomerulopathy.

While little difference was evident in renal function and histology between control and leptin-treated groups, TGF-β1, Smad2, and BMP7 mRNA expressions were higher, whereas Smad3 and Smad7 levels were lower in leptin-treated rats. TGF-β1 is believed to be the link between leptin and the development of glomerulosclerosis [18]. Transgenic mice with higher levels of TGF-β1 in the plasma had progressed glomerulosclerosis [19]. TGF-β1 expression was also higher, along with changes in mesangial and glomerular basement membrane, in db/db mice similar to changes seen in nephropathy of Type 2 diabetes mellitus [20]. Similar evidence has also been reported from human studies where increased TGF-β1 is associated with a higher risk for liver cirrhosis [21,22]. TGF-β1 effects are mediated through TGF-β1 Type II receptors and the Smad pathway. Activation leads to the formation of Smad2/3 complex, which translocates to the nucleus and triggers target gene transcription. Smad2 expression was also significantly higher in leptin-treated rats. The exact mechanism responsible for leptin-stimulated increase in TGF-β1 expression is unclear, but evidence from studies on nonalcoholic steatohepatitis suggests that leptin-mediated oxidative stress might be responsible for the elevated TGF-β1 levels [23]. Decreased Smad7 might be associated with increased renal fibrosis as Smad7 knockout mice were found to show a more severe progression of renal dysfunction and renal failure than wild-type control animals, suggesting a protective role for Smad7 in renal fibrosis [21]. BMP7 activation occurs by pathways independent of TGF-β1, and it is possible that the administration simultaneously triggered BMP7 expression. BMP7 is a major inhibitor of fibrosis [24]. It might explain why there is a lack of worsening of renal dysfunction and histological evidence of renal dysfunction in the leptin-treated animals.

CONCLUSION

Administration of leptin at a dose of 60 μg/day for 42 days did not affect kidney function and histopathology in SHR. However, it seems to have increased the expression of TGF-β1, Smad2, and BMP7. Whether the elevated BMP7 expression may be responsible for lack of effect of leptin on renal function is a question that remains to be answered. Slight higher doses of leptin and perhaps a longer duration of administration could help further clarify the role of leptin in glomerular sclerosis.

ACKNOWLEDGMENT

The authors thank all the technical staff at the Institute of Medical and Molecular Biology, Laboratory Animal Care Unit, Faculty of Medicine, UTM and Research Laboratory, UPNM, for their assistance. This research was funded by the Ministry of Higher Education, Malaysia, under the RAG Scheme [600-BMI/RAGS 5/3 (B7/2013), RAGS/2012/ UPMN/SK01/1], and [MSH] (100-RMI/PLR 16/2/71[2010]).

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