European Journal of Pharmacology 777 (2016) 96–103

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Endocrine pharmacology

Mast cell stabilizers obviate high fat diet-induced renal dysfunction in rats

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ARTICLE INFO

Article history:
Received 26 August 2015
Received in revised form
26 February 2016
Accepted 29 February 2016
Available online 2 March 2016

Keywords:
Mast cells
Kidney
High fat diet
Obesity
cromoglycate
ketotifen

ABSTRACT

The present study investigated the infiltration of mast cells into the kidney tissue and the preventive role of mast cell stabilizers against high fat diet (HFD)-induced renal injury in rats. The animals were fed on HFD (30% fat) for 12 consecutive weeks to induce renal injury. The HFD-induced obesity was assessed by calculating obesity index, adiposity index, and estimation of total cholesterol, triglycerides, and high density lipoproteins in plasma. The renal dysfunction was evaluated by measuring creatinine clearance, blood urea nitrogen, uric acid, electrolytes and microproteinuria. The oxidative stress in renal tissues was determined by myeloperoxidase activity, thiobarbituric acid reactive substances, superoxide anion generation and reduced glutathione level. The systolic blood pressure (SBP) was monitored using non-invasive blood pressure measuring apparatus. Histamine and hydroxyproline contents were quantified in renal tissues. Gross histopathological changes, mast cell density and collagen deposition in the renal tissue was determined by means of histopathology. The mast cell stabilizers, sodium cromoglycate and ketotifen were administered daily for 12 weeks. The HFD fed rats demonstrated significant increase in lipid profile, kidney injury with marked increase in renal oxidative stress, SBP, mast cell density, histamine content and hydroxyproline content that was attenuated by sodium cromoglycate and ketotifen treatment. Hence, the novel findings of this investigation suggest that HFD induced mast cells infiltration into kidney tissue seems to play an important role in renal pathology, and treatment with mast cell stabilizers serves as potential therapy in management of HFD induced renal dysfunction in rats.

1. Introduction

Obesity is a fast growing public health problem which affects almost every tenth person globally (WHO, 2012). It is characterized by excessive accumulation of fat in different regions of the body due to an imbalance between food intake and energy expenditure, subsequently affecting health of an individual (Clement and Ferre, 2003) Initially, obesity was considered to be the problem of rich and middle aged people, however, its prevalence is escalating among children and adults in both developed and developing countries (Imes and Burke, 2014). Over weight and obesity is considered to be one of the major risk factors for cardiovascular and metabolic disorders as well as some cancers (Meyer et al., 2011). Recently, obesity-related renal abnormalities have also caught the attention of researchers. Obesity has been reported to aggravate IgA-induced glomerulonephritis and diabetes-induced nephropathy in humans (Praga et al., 2001; Gonzalez et al., 2005). Studies in experimental animals have shown marked renal damage with morphological and functional alterations due to obesity (Altunkaynak et al., 2008).

Obesity is recognized as an inflammatory disorder with significant increase in inflammatory cytokines, including interleukin (IL-6), tumour necrosis factor-α (TNF-α) and interferon-γ (Rocha and Folco, 2011). The insulin resistance observed in obesity has been correlated with increased levels of inflammatory cytokines (McArdle et al., 2013). Resistin is the inflammatory marker produced by adipose tissues and macrophages that up-regulates IL-6 and TNF-α (Reilly et al., 2005). In patients with chronic kidney disease, the increased levels of resistin are associated with increase in inflammation and decline in kidney function (Marouga et al., 2013).

Mast cells play a major role in inflammation and allergic reactions. These cells originate from bone marrow precursor cells and are recruited in peripheral tissues where they find their

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http://dx.doi.org/10.1016/j.ejphar.2016.02.066
0014-2999/

differentiation and maturity. The mast cells are sensitized by IgE or complement system that leads to the release of potent bioactive substances from their cytoplasmic granules, resulting in anaphylactic shock. The biologically active substances present in mast cells are histamine, serotonin, TNF-α, leukotrienes and prostaglandins. The role of mast cells in non allergic disorders including cardiovascular and renal disorders is well recognized. The activation of mast cell proteases such as tryptase and chymase are responsible for renal fibrosis (Li et al., 2010). Many studies have shown correlation between mast cells density and disease severity in renal pathology such as IgA nephropathy, glomerulonephritis, diabetic nephropathy, protein overload induced nephropathy and acute as well as chronic rejections (Ehara and Shigematsu, 2003; Li et al., 2010). Moreover, the mast cell stabilizers are documented to provide protection against unilateral obstruction-induced nephropathy by preventing the degranulation of renal mast cells (Summers et al., 2012). The role of mast cells in obesity-induced renal damage has never been studied before. The present study was designed to explore the role of mast cells in the pathogenesis of HFD-induced renal injury in rats and to investigate role of mast cell stabilizers as possible therapy against obesity-induced renal injury.

2. Materials and methods

The experiments were performed in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments (CPCSEA), Ministry of Environment and Forest, Government of India. Adult male Wistar rats of 14-16 week age (225-275 g body weight) were employed in the present study. They were maintained on group specific diet and water ad libitum. Normal diet contained fat (5.0%), carbohydrates (85%) and proteins as macronutrients (10%), whereas the high fat diet (HFD) consisted of fat (30%), carbohydrates (60%) and proteins as macronutrients (10%).

2.1. Experimental protocol

Four groups of randomly divided rats were employed in this study, each containing 6 rats. Sodium cromoglycate and ketotifen were suspended in 0.5% carboxy methyl cellulose (CMC). Group 1 (Control): Normal diet (rat chow) was given to the animals for 12 weeks. Group 2 (HFD, 30% fat): HFD was given to the animals for 12 weeks. Group 3 (HFD+Sodium cromoglycate): Sodium cromoglycate (24 mg/kg/day, intraperitoneal, i.p.) was administered to rats fed on HFD once daily for 12 weeks. Group 4 (HFD+Ketotifen): Ketotifen (1 mg/kg/day, i.p.) was administered to rats fed on HFD once daily for 12 weeks.

2.2. Drugs and chemicals

Sodium cromoglycate, ketotifen and histamine were procured from Sigma Aldrich, India. All other reagents used in the study were of analytical grade.

2.3. Induction of renal injury

The renal injury was induced by feeding HFD to rats for 12 weeks. At the end of 12 weeks, the rats were placed in metabolic cage for collection of urine. The rats were anaesthetized with ketamine (50 mg/kg, i.p.). Blood samples were collected using retro-orbital puncture and animals were killed by cervical dislocation. The perirenal fat, retroperitoneal fat, and epididymal fat were collected and weighed. Plasma isolated from whole blood was used for the estimation of total cholesterol, triglycerides, high density lipoprotein (HDL), and glucose concentration. Creatinine, blood urea nitrogen (BUN), uric acid, sodium and potassium levels were quantified to assess renal function. The creatinine, sodium, and protein content were estimated in urine. The kidneys were removed, weighed and washed with 1.17% potassium chloride (KCl) solution. Small portions isolated from the kidneys were used for estimation of superoxide anion generation (SAG), histamine and hydroxyproline content, while the remaining kidney tissue was minced and homogenized (10% w/v) in 1.17% KCl solution using teflon homogenizer. The contents were centrifuged at $800 \times g$ for 20 min. The pellet obtained was used for estimation of myeloperoxidase (MPO) activity, whereas, clear supernatant was used to estimate lipid peroxides and reduced glutathione (GSH) levels. A small portion of kidneys was preserved in 10% neutral buffered formalin for histological examination.

2.4. Morphological assessment of obesity

The average feed intake of rats in various groups was noted at different intervals through out the study. The nasoanal length of each animal was measured to determine the obesity index. Obesity index was calculated as cube root of body weight (g)/nasoanal length (mm) $\times 10^4$. The adiposity index was calculated as per formulae: [Adiposity index=(perirenal fat+retroperitoneal fat+epididymal fat/body weight) $\times$ 100]. The percentage change in body weight and kidney to body weight ratio was also calculated.

2.5. Estimation of plasma lipid level

The total cholesterol, triglyceride level, HDL as well as glucose levels were determined in plasma samples by using commercially available kits by Medsource Ozone Biomedicals Ltd. and Transasia Bio-medicals Ltd., India. The results were expressed as milligram per decilitre (mg/dl).

2.6. Estimation of renal parameters

The estimation of creatinine in plasma and urine samples was done by using kit by Angstrom Biotech Pvt. Ltd., India. The creatinine clearance (CrCl) was calculated as: CrCl=urine creatinine $\times$ urine volume/plasma creatinine $\times 24 \times 60 \times$ animal weight. The results were expressed as millilitre per min per kilogram of body weight. The BUN and uric levels were estimated in plasma by using kit by Angstrom Biotech Pvt. Ltd., India. The results were expressed as milligram per decilitre of plasma. The level of potassium in plasma and of sodium in plasma as well as in urine was estimated using kit by Crest Biosystems, India. The potassium level was expressed as millimoles per litre of plasma. The fractional excretion of sodium (FeNa) was calculated by using formula [FeNa=100 $\times$ (urine sodium $\times$ plasma creatinine/plasma sodium $\times$ urine creatinine)]. The results were expressed as percentage changes. The microproteins were estimated in urine samples using kit by Crest Biosystems, India and expressed as milligram per day.

2.7. Estimation of renal oxidative stress parameters

The MPO activity was measured as described earlier (Pundir et al., 2013; Brar et al., 2014). The MPO activity was expressed as unit per gram of kidney tissue weight. The quantitative measurement of TBARS, an index of lipid peroxidation in kidney was performed according to established method (Pundir et al., 2013; Brar et al., 2014). The total protein content was determined in the tissue homogenate using Lowry's method. The results were expressed as nanomoles per milligram of protein. The SAG in renal tissue was