Chemico-Biological Interactions 240 (2015) 292–303

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Gallic acid ameliorates renal functions by inhibiting the activation of p38 MAPK in experimentally induced type 2 diabetic rats and cultured rat proximal tubular epithelial cells

Amjid Ahad [a], Haseeb Ahsan [b], Mohd. Mujeeb [c], Waseem Ahmad Siddiqui [a, *]

[a] Lipid Metabolism Laboratory, Department of Biochemistry, Faculty of Science, Jamia Hamdard, Hamdard Nagar, New Delhi 110062, India
[b] Department of Biochemistry, Faculty of Dentistry, Jamia Millia Islamia, New Delhi 110025, India
[c] Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi 110062, India

ARTICLE INFO

Article history:
Received 16 April 2015
Received in revised form
14 July 2015
Accepted 31 August 2015
Available online 1 September 2015

Keywords:
Diabetic nephropathy
Hyperglycemia
Oxidative stress
Inflammation
Gallic acid
p38 MAPK

ABSTRACT

Diabetic nephropathy (DN) is one of the leading causes of morbidity and mortality in diabetic patients that accounts for about 40% of deaths in type 2 diabetes. p38 mitogen activated protein kinase (p38 MAPK), a serine–threonine kinase, plays an important role in tissue inflammation and is known to be activated under conditions of oxidative stress and hyperglycemia. The role of p38 MAPK has been demonstrated in DN, and its inhibition has been suggested as an alternative approach in the treatment of DN. In the present study, we investigated the nephroprotective effects of an anti-inflammatory phenolic compound, gallic acid (GA, 3,4,5-trihydroxybenzoic acid), in high fat diet/streptozotocin (HFD/STZ) induce type 2 diabetic wistar albino rats. GA (25 mg/kgbw and 50 mg/kgbw, p.o.) treatment for 16 weeks post induction of diabetes led to a significant reduction in the levels of blood glucose, HbA1c, serum creatinine, blood urea nitrogen and proteinuria as well as a significant reduction in the levels of creatinine clearance. GA significantly inhibited the renal p38 MAPK and nuclear factor kappa B (N-κB) activation as well as significantly reduced the levels of renal transforming growth factor beta (TGF-β) and fibronectin. Treatment with GA resulted in a significant reduction in the serum levels of proinflammatory cytokines viz. interleukin 1 beta (IL-1β), IL-6 and tumor necrosis factor alpha (TNF-α). Moreover, GA significantly lowered renal pathology and attenuated renal oxidative stress. In cultured rat NRK 52E proximal tubular epithelial cells, GA treatment inhibited high glucose induced activation of p38 MAPK and NF-κB as well as suppressed proinflammatory cytokine synthesis. The results of the present study provide *in vivo* and *in vitro* evidences that the p38 MAPK pathway plays an important role in the pathogenesis of DN, and GA attenuates the p38 MAPK-mediated renal dysfunction in HFD/STZ induced type 2 diabetic rats.

1. Introduction

Long-term hyperglycemia causes metabolic, hemodynamic and physiological abnormalities in the nephrons that can lead gradually to insufficiency and dysfunction of renal cells. Diabetes-induced nephropathy is the leading cause of end-stage renal disease (ESRD) across the world, and the major reason for morbidity and mortality in diabetic patients [1]. Diabetic nephropathy (DN) is characterized typically by molecular and physiological changes coherent with inflammation including upregulation of nuclear factor-kappaB (NF-κB), inducible nitric oxide synthase (iNOS), transforming growth factor-beta (TGF-β) and pro-inflammatory cytokines; infiltration and accumulation of immune cells,

Abbreviations: DN, diabetic nephropathy; HFD/STZ, high fat diet/streptozotocin; GA, gallic acid; p38 MAPK, p38 mitogen activated protein kinase; NF-κB, nuclear factor kappa B; TGF-β, transforming growth factor beta; iNOS, inducible nitric oxide synthase; T2DM, type 2 diabetes mellitus; GBM, glomerular basement membrane; TNF-α, tumor necrosis factor-alpha; AST, aspartate transaminase; ALT, alanine transaminase; SOD, superoxide dismutase; CAT, catalase; HOMA-IR, homeostasis model assessment-insulin resistance; HbA1c, glycated haemoglobin; BUN, blood urea nitrogen; bw, body weight.

* Corresponding author. Department of Biochemistry, Lipid Metabolism Laboratory, Jamia Hamdard (Hamdard University), New Delhi 110062, India.
E-mail addresses: wasiddiqui01@gmail.com, was.sid@gmail.com (W.A. Siddiqui).

http://dx.doi.org/10.1016/j.cbi.2015.08.026
0009-2797/

glomerular basement membrane (GBM) thickening, mesangial expansion and gross anatomical and structural alterations in renal cells. Inhibition of this inflammatory process at any of its multiple steps could lead to amelioration of DN, suggesting the role of inflammation in the development of DN [2].

p38 mitogen activated protein kinase (p38 MAPK), a member of ser/thr family of MAP kinases, is known to play a regulatory role in inflammation in various diseases. The p38 MAPK pathway is involved in the activation and induction of NF-κB, tumor necrosis factor-alpha (TNF-α), interleukins (IL-1, IL-6), chemokines, TGF-β and iNOS [3–6]. Suppression of p38 MAPK activity by various agents has been shown to be beneficial in the prevention of various diseases including lung inflammation, rheumatoid arthritis, Alzheimer's disease and inflammatory bowel disease [7–13]. Furthermore, recent experimental evidences have suggested the role of p38 MAPK in the development of DN, and its inhibition has been proposed to be an effective therapeutic strategy for the treatment of DN [14–16].

Plants and their products have been shown to be more dependable sources for the development of new drugs against various diseases including diabetes mellitus (DM). Gallic acid (GA, 3,4,5-trihydroxybenzoic acid), a bioactive compound present in numerous plants and fruits, has been reported to possess antioxidant, anti-allergic, anti-cancer, anti-inflammatory and antidiabetic activities [17–25]. Moreover, GA has been reported to inhibit NF-κB activation in various experimental models [26,27].

No major research work has been carried out till date regarding the preventive effects of GA on DN. Therefore, the present study was designed to investigate the efficacy of GA against renal dysfunction in HFD/STZ-induced type 2 diabetic rat model, and to evaluate the pathogenic role of p38 MAPK signalling in the development of DN.

2. Materials and methods

2.1. Chemicals and reagents

Gallic acid, Dulbecco's modified Eagle's medium (DMEM), foetal bovine serum (FBS), antibiotics, isobutyl methylxanthine (IBMX), and dexamethasone (DEX) were purchased from Sigma Chemicals Pvt. Ltd. (New Delhi, India). NRK 52E proximal tubular epithelial cells were obtained from National Centre for Cell Sciences, Pune. Streptozotocin (STZ), Coomassie blue G250, reduced glutathione (GSH), oxidized glutathione (GSSG) and pyrogallol were purchased from Sigma–Aldrich Chemicals Pvt. Ltd. (New Delhi, India). Phosphoric acid (H_3PO_4), napthylethylenediamine dihydrochloride (NEDD), thiobarbituric acid (TBA) were purchased from Sisco Research Laboratories Ltd. (Mumbai, India). Bovine serum albumin (BSA), hydrogen peroxide, dimethyl sulphoxide (DMSO), ethylenediamine-tetra-acetic acid, tris-buffer, thiobarbituric acid (TBA) and tricarboxylic acid (TCA) were purchased from E Merck Ltd. (Mumbai, India). Other chemicals used in the study were of analytical grade of highest purity, and procured from standard commercial sources.

2.2. Animals and sub-acute toxicity assessment study

Male albino rats (Wistar strain) of male sex weighing 130–180 g (8–10 weeks) were used for the present study. Animals were housed in the Animal house of Jamia Hamdard, New Delhi and were kept in a controlled environment under standard conditions of temperature and humidity with an alternating 12hr light and dark cycle. The animals had free access to food (Amrut Laboratory Rat Feed, Navamaharashtra, Pune, India) and water *ad libitum*. The animals were maintained in accordance with guidelines prescribed by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the study was approved by the animal ethics committee of Jamia Hamdard, New Delhi, India (Project No. 721).

Sub-acute toxicity assessment study of test drugs was determined in albino Wistar rats following the protocol described by organization for economic co-operation and development guideline number 407 for the conduct of repeated dose 28 day oral toxicity study in rodents [28]. The test drug, GA, was dissolved in 0.1% DMSO (vehicle) before its administration into the rats. The animals were divided into six groups with six animals in each group. Rats in group I served as normal control (vehicle control) while those in group II, III, IV, V and VI were fed orally with test drug at, 25, 50, 75, 100 and 150 mg/kgbw, respectively, for 28 days. After the experimental period, animals in different groups were sacrificed by cervical dislocation under local ether anaesthesia. Blood was collected in two different tubes from retro-orbital plexus. One tube had anticoagulant (for plasma separation) and the other tube without anticoagulant (to separate serum for various biochemical estimations). The liver was dissected out, washed in ice-cold saline, blotted dry, and weighed. A 10% w/v homogenate was prepared in phosphate buffer, pH 7.4 and used for the biochemical analyses. Hemoglobin content, red blood cell (RBC) and white blood cell (WBC) counts were measured from freely flowing blood [29]. Serum and liver protein was determined by the Bradford method [30] using Bovine Serum Albumin (BSA) as standard. Serum urea content was estimated by a commercial assay kit (Span diagnostics Pvt Ltd, Surat, India). Hepatospecific markers, viz. activities of Aspartate transaminase (AST) and Alanine transaminase (ALT) were assayed by the commercial assay kits (Span diagnostics Pvt Ltd, Surat, India). Lipid peroxidation in liver was estimated by measuring MDA by the standard method [31]. Enzymatic antioxidant, Superoxide dismutase (SOD) activity was determined by the standard method [32]. Another antioxidant, Catalase (CAT) activity was assayed by the standard method [33].

2.3. Experimental design

The rats were divided into five groups comprising of six animals in each group as follows: Group I (NC): Normal control rats, received 0.1% DMSO (vehicle) per os (p.o.) once a day along with standard pellet diet throughout the experiment. Group II (DC): Diabetic control rats, received HFD initially for 5 weeks followed by a single i.p. injection of STZ (35 mg/kg bw, in citrate buffer; pH 4.5). The animals were kept on normal pellet diet for the rest of experimental period. Group III (GA-N): Normal rats, received gallic acid (50 mg/kg bw/day dissolved in 0.1% DMSO, p.o.) for 16 weeks post induction of diabetes. Group IV (GA-25): Diabetic rats, received gallic acid (25 mg/kg bw/day dissolved in 0.1% DMSO, p.o.) for 16 weeks post induction of diabetes. Group V (GA-50): Diabetic rats, received gallic acid (50 mg/kg bw/day dissolved in 0.1% DMSO, p.o.) for 16 weeks post induction of diabetes. Gallic acid was dissolved in 0.1% DMSO and given orally once a day with the help of an intragastric tube until the end of the study (16 weeks post-induction of diabetes) to groups III, IV and V animals. GA doses (25 mg/kgbw and 50 mg/kgbw) were selected on the basis of preliminary sub-acute toxicity assessment study.

2.4. Induction of type 2 diabetes mellitus (T2DM)

The male Wistar rats were selected and weighed then marked for individual identification. Prior to dietary manipulation, all rats were fed standard pellet rodent diet and water ad libitum and maintained on a 12-h light/dark (06:00/18:00) cycle. After acclimatization, the rats (160–200 g) were fed high fat diet, HFD (60.3%