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Original Article

Resveratrol protects diabetic kidney by attenuating hyperglycemia-mediated oxidative stress and renal inflammatory cytokines via Nrf2–Keap1 signaling

P. Palsamy, S. Subramanian *

Department of Biochemistry, University of Madras, Guindy Campus, Chennai, 600 025 Tamilnadu, India

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ABSTRACT

Hyperglycemia-mediated oxidative stress plays a crucial role in the progression of diabetic nephropathy. Hence, the present study was hypothesized to explore the renoprotective nature of resveratrol by assessing markers of oxidative stress, proinflammatory cytokines and antioxidant competence in streptozotocin–nicotinamide-induced diabetic rats. Oral administration of resveratrol to diabetic rats showed a significant normalization on the levels of creatinine clearance, plasma adiponectin, C-peptide and renal superoxide anion, hydroxyl radical, nitric oxide, TNF-α, IL-1β, IL-6 and NF-κB p65 subunit and activities of renal aspartate transaminase, alanine transaminase and alkaline phosphatase in comparison with diabetic rats. The altered activities of renal aldose reductase, sorbitol dehydrogenase and glyoxalase-I and elevated level of serum advanced glycation end products in diabetic rats were also reverted back to near normalcy. Further, resveratrol treatment revealed a significant improvement in superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and glutathione reductase activities and vitamins C and E, and reduced glutathione levels, with a significant decline in lipid peroxides, hydroperoxides and protein carbonyls levels in diabetic kidneys. Similarly, mRNA and protein analyses substantiated that resveratrol treatment notably normalizes the renal expression of Nrf2/Keap1and its downstream regulatory proteins in the diabetic group of rats. Histological and ultrastructural observations also evidenced that resveratrol effectively protects the kidneys from hyperglycemia-mediated oxidative damage. These findings demonstrated the renoprotective nature of resveratrol by attenuating markers of oxidative stress in renal tissues of diabetic rats.

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1. Introduction

Diabetes mellitus is a heterogeneous metabolic disorder essentially characterized by insufficiency of insulin secretion and insulin receptor or postreceptor events with derangement in carbohydrate, protein and lipid metabolism resulting in chronic hyperglycemia. Hyperglycemia is a well distinguished pathogenetic factor of long-term complications in diabetes mellitus not only engenders excessive free radicals but also attenuates antioxidative machineries through glycation of the antioxidant enzymes. Hence, oxidative stress has been considered to be a general pathogenic factor of diabetic complications including nephropathy [1]. Diabetic nephropathy, an imperative complication of diabetes mellitus, is characterized by the protuberance of the glomerular mesangium due to amassing of extracellular matrix proteins synthesized by the mesangial cells with basement membrane thickening, glomerular and tubular hypertrophy, glomerulosclerosis and tubulointerstitial fibrosis [2]. As hyperglycemia-mediated oxidative stress plays a central role in the development and progression of diabetic nephropathy, glycemic control remains the focal target in the treatment of diabetic nephropathy.

Agents with significant antioxidant potentials have been reported to elicit protection on diabetic kidney damage. The antioxidants such as taurine, melatonin, vitamin C and vitamin E are reported to reduce the renal complications like glomerular hypertrophy, albuminuria, glomerular collagen and renal protein kinase C activity in experimental diabetes [3,4]. Glyclazide, the second generation sulfonylurea, has also been shown to possess a notable antioxidant potential due to its azabicyclo-octyl ring independent of glycemic control by scavenging free radicals, reducing the expression of NAD(P)H oxidase, enhancing renal expression of Mn-superoxide dismutase and endothelial nitric oxide synthase, suppressing glomerular macrophage migration, thereby ameliorating glomerular matrix expansion and albuminuria [5]. Hence, it is recommended that therapy with antioxidants may signify a useful pharmacologic overture to the management of diabetes. Therefore, the present study was aimed to assess the antioxidant defensive as well as renal tissue protective

Abbreviations: AGEs, advanced glycation end products; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; Ccr, creatinine clearance; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; γ-GCS, γ-glutamylcysteine synthetase; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GST, glutathione-S-transferase; HO-1, heme oxygenase-1; Keap1, Kelch-like ECH-associated protein 1; LSD, least significant difference; NO, nitric oxide; Nrf2, nuclear factor (erythroid-derived 2)-like 2; Pcr, plasma creatinine; PMSF, phenylmethylsulfonyl-fluoride; SOD, superoxide dismutase; Ucr, urinary creatinine

* Corresponding author. Fax: +91 44 22300488.
E-mail address: subbus2020@yahoo.co.in (S. Subramanian).

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nature of resveratrol (3,5,4′-trihydroxystilbene) in streptozotocin–nicotinamide-induced diabetic rats.

Resveratrol is a polyphenolic phytoalexin that occurs naturally in many plant species, including grapevines and berries, and exhibits surfeit of pharmacologic health benefits including antioxidant [6], antimutagenic [7], anti-inflammatory [8], estrogenic [9], antiplatelet [10], anticancer [11] and cardioprotective properties [12]. There has been copious epidemiologic and clinical evidence confirming that resveratrol may act as an antioxidant through enhancing hydrogen peroxide tolerance and inhibiting cyclooxygenase-2 activity. Recently, resveratrol has been reported to possess antihyperglycemic effect in experimental diabetes [13], which is mediated by modulating the activities of key carbohydrate metabolizing enzymes in the hepatic and renal tissues of experimental diabetic rats [14,15]. More recently, we have reported the pancreatic β-cell [16] as well as hepatocyte protective nature of resveratrol from oxidative damage in streptozotocin–nicotinamide-induced diabetic rats [17]. Moreover, very limited studies have demonstrated the effect of resveratrol on diabetic kidney by assessing the changes in phosphorylation of histone H3, levels of the oxidative markers such as malondialdehyde and glutathione and antioxidant enzymes such as superoxide dismutase and catalase in the renal tissues of diabetic rats [18,19] and none of them have explored the protective nature of resveratrol on renal tissue ultrastructure during hyperglycemia- as well as proinflammatory cytokine-mediated oxidative damage in streptozotocin–nicotinamide-induced diabetic rats. Hence, the present study was aimed to investigate the ameliorative potential of resveratrol on proinflammatory cytokines, hyperglycemia-mediated oxidative stress and renal dysfunction in streptozotocin–nicotinamide-induced diabetic rats and the efficacy of resveratrol was compared with glyclazide, an oral antihyperglycemic drug with antioxidant potential.

2. Materials and methods

2.1. Chemicals

Resveratrol, streptozotocin, nicotinamide, epinephrine, thiobarbituric acid, reduced glutathione (GSH), vitamin E and sodium azide were procured from Sigma Chemicals Co. (St. Louis, MO), stored at 2–4 °C and protected from sunlight. All other chemicals were of analytical grade and were obtained from standard commercial suppliers.

2.2. Experimental animals

Animal experiments were premeditated and executed in accordance with the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines (Approval No.01/036/07). Six-week-old, male, Wistar rats weighing 160–180 g, procured from Tamilnadu Veterinary and Animal Sciences University, Chennai, India, were housed in clean, sterile, polypropylene cages under standard vivarium conditions (12 h light/dark cycle) with ad libitum access to water and standard rat chow (Hindustan Lever Ltd., Bangalore, India) with a composition of 5% fat, 21% protein, 55% nitrogen-free extract and 4% fiber (w/w) with adequate mineral and vitamin levels for the animals. The animals were acclimatized to the laboratory conditions for 2 weeks prior to the inception of experiments.

2.3. Induction of experimental diabetes

Experimental diabetes was induced in 12 h fasted rats by single i.p. injection of streptozotocin (50 mg/kg body weight) dissolved in 100 mM cold citrate buffer, pH 4.5 [20], 15 min after the intraperitoneal administration of nicotinamide (110 mg/kg body weight) [21]. Since streptozotocin is capable of inducing fatal hypoglycemia as a result of massive pancreatic insulin release, the rats were supplied with 10% glucose solution after 6 h of streptozotocin administration for the next 24 h to prevent hypoglycemia [22]. After a week in time for the development and aggravation of diabetes, rats with moderate diabetes (i.e. blood glucose concentration ≥ 14 mM) were selected for the experiment.

2.4. Experimental design

The experimental animals were divided into five groups, each group comprising of a minimum of six rats detailed as follows. Group 1 served as control rats; group 2 served as control rats daily administered with resveratrol (5 mg/kg body weight) in aqueous suspension orally for 30 days; group 3 served as streptozotocin–nicotinamide-induced diabetic rats; group 4 served as diabetic rats daily administered with resveratrol (5 mg/kg body weight) in aqueous suspension orally for 30 days; and group 5 served as diabetic rats daily administered with glyclazide (5 mg/kg body weight) in aqueous suspension orally for 30 days.

During the experimental period, body weight, blood glucose, food and water consumption and physical examinations were determined at regular intervals. The dosage was adjusted every week according to any change in body weight to maintain similar dose per kg body weight of rat over the entire period of study for each group. At the end of the treatment period, the rats were fasted overnight, anaesthetized with ketamine (80 mg/kg body weight; i.p.) and killed by cervical decapitation. The blood was collected with or without anticoagulant for plasma or serum separation, respectively. Fasting blood glucose, HbA1c and plasma insulin levels were determined to confirm the antihyperglycemic property of resveratrol [14,15].

2.5. Preparation of kidney tissue homogenate

Kidney tissues from control and experimental groups of rats were excised, rinsed with ice-cold saline and homogenized in 100 mM Tris–HCl buffer (pH 7.4) using Teflon homogenizer and centrifuged at 12,000*g* for 30 min at 4 °C. The supernatant was pooled and used for the estimations. The protein content in the tissue homogenate was also estimated [23].

2.6. Determination of intraperitoneal insulin tolerance

At the end of the experimental period, fasting blood samples were withdrawn from the control and experimental groups of rats. Four more blood samples were collected at 30, 60, 90 and 120 min intervals after the intraperitoneal administration of a bolus of insulin (2 units/kg body weight). All the blood samples were collected with ethylenediaminetetraacetic acid (EDTA) for the determination of glucose by using glucose oxidase peroxidase diagnostic enzyme kit (Span Diagnostic Chemicals, Surat, India).

2.7. Assessment of renal dysfunction

The levels of plasma and urinary creatinine (Pcr and Ucr) were determined by alkaline picrate method using creatinine assay kit (Span Diagnostics Ltd., Surat, India). Urine samples (24 h) were collected using metabolic cages. Creatinine clearance (Ccr) was computed using the following formula: Ccr (μl/min) = (Ucr/Pcr) × urine volume (μl/min). The activities of pathophysiological enzymes such as aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were also assayed in the kidney tissue homogenate of control and experimental groups of rats [24,25].

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