Chemico-Biological Interactions 186 (2010) 200–210

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Resveratrol attenuates hyperglycemia-mediated oxidative stress, proinflammatory cytokines and protects hepatocytes ultrastructure in streptozotocin–nicotinamide-induced experimental diabetic rats

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ARTICLE INFO

Article history:
Received 17 February 2010
Received in revised form 9 March 2010
Accepted 12 March 2010
Available online 20 March 2010

Keywords:
Diabetic liver
Lipid peroxidation
Oxidative stress
Proinflammatory cytokines
Resveratrol

ABSTRACT

The present study was hypothesized to investigate the hepatoprotective nature of resveratrol in averting hyperglycemia-mediated oxidative stress by measuring extent of oxidant stress and levels of proinflammatory cytokines and antioxidant competence in the hepatic tissues of streptozotocin–nicotinamide-induced diabetic rats. After the experimental period of 30 days, the pathophysiological markers such as serum bilirubin and hepatic aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were studied in addition to hepatic TNF-α, IL-1β, IL-6, NF-κB p65 and nitric oxide (NO) levels in control and experimental groups of rats. The levels of vitamin C, vitamin E and reduced glutathione (GSH) and activities of superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione-S-transferase (GST) and glutathione reductase (GR) were determined in the liver tissues. Extent of oxidative stress was also assessed by hepatic lipid peroxides, hydroperoxides and protein carbonyls. A portion of liver was processed for histological and ultrastructural studies. Oral administration of resveratrol (5 mg/kg b.w.) to diabetic rats showed a significant decline in hepatic proinflammatory cytokines and notable attenuation in hepatic lipid peroxides, hydroperoxides and protein carbonyls. The diminished activities of hepatic enzymic antioxidants as well as the decreased levels of hepatic non-enzymic antioxidants of diabetic rats were reverted to near normalcy by resveratrol administration. Moreover, the histological and ultrastructural observations evidenced that resveratrol effectively rescues the hepatocytes from hyperglycemia-mediated oxidative damage without affecting its cellular function and structural integrity. The findings of the present investigation demonstrated the hepatocyte protective nature of resveratrol by attenuating markers of hyperglycemia-mediated oxidative stress and antioxidant competence in hepatic tissues of diabetic rats.

1. Introduction

Diabetes mellitus, a pervasive and multifactorial metabolic syndrome, is characterized by imperfection in insulin secretion and insulin receptor or postreceptor events with derangement in carbohydrate, protein and lipid metabolism and results in chronic hyperglycemia, a clinical hallmark of diabetes [1]. Chronic hyperglycemia, in contrary to normoglycemia, has negative impacts on various organs and tissues, such as pancreas, liver, kidneys, muscles, adipose tissues, etc. thereby fostering progression of early diabetic complications by distressing secretory competence of pancreatic β-cells, which consequently results in glucotoxicity. Glucotoxicity eventually leads to progressive β-cell dysfunction, impaired insulin gene transcription and permanent β-cell loss due to apoptosis, ensuing in a vicious cycle with exasperation of the hyperglycemia [2].

Indeed, hyperglycemia is contemplated to generate reactive oxygen species through diverse pathways, such as mutilation of the redox equilibrium, augmentation of advanced glycation products, activation of protein kinase C or overproduction of mitochondrial superoxides that eventually leading to oxidative stress in a variety of tissues [3]. The vulnerability of each tissue to oxidative stress can vary depending upon their expressed antioxidant enzymes. In addition to the pancreatic β-cells, supraphysiological glucose is notorious to provoke oxidative stress in hepatocytes which can cause hepatic tissue damages [4].

Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; GPx, glutathione peroxidase; GR, glutathione reductase; GST, glutathione-S-transferase; GSH, reduced glutathione; HbA1c, glycosylated hemoglobin; LSD, least significant difference; NO, nitric oxide; SOD, superoxide dismutase.

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doi:10.1016/j.cbi.2010.03.028

Liver is one of the chief storage organs for glucose reserve in the body and plays a crucial role in maintenance of blood glucose homeostasis, because it consents to amass the superfluous blood glucose and to demobilize it in hypoglycemic states. Chronic hyperglycemia impedes normal inhibition of hepatic glucose production persuaded by an acute escalation of blood glucose level and enhances phosphoenolpyruvate carboxykinase gene expression, which is an imperative enzyme for the regulation of gluconeogenesis [5]. Further, liver is the focal organ of oxidative and detoxifying processes as well as free radical reactions and the biomarkers of oxidative stress are elevated in the liver at an early stage in many diseases, including diabetes mellitus [6]. In experimental diabetes, streptozotocin exerts its toxic effects on liver and other organs in addition to pancreatic β-cells. The insulin insufficiency and hyperglycemia that result from β-cell necrosis further augment liver damage through reactive free radicals mediated lipid peroxidation of hepatocellular membrane [7].

This pathophysiological sequence sets the scene for considering antioxidant therapy as an adjunct in the management of diabetes. Several studies have recently dealt with either maintenance of antioxidant defense of diabetic liver or reduction of peroxidative stress induced hepatic damage in experimental models [8–10]. 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 hepatocyte protective nature of resveratrol (3,5,4′-trihydroxystilbene) in streptozotocin–nicotinamide-induced diabetic rats.

Resveratrol is a naturally occurring polyphenol found in grapes, berries and peanuts that reveals numerous pleiotropic pharmacological actions including antiplatelet, anticancer and anti-inflammatory properties [11]. It is reported to avert the progression of a wide array of pathologies such as cardiovascular diseases, ischemic injuries, liver toxicity and neurodegenerative processes [12]. It is believed to be the active ingredient in red wines that might provide answer to the "French paradox", i.e., the apparent compatibility of a high-fat diet with a low incidence of coronary atherosclerosis [13]. Moreover, the cardioprotective efficacy of resveratrol is partly because of its antioxidant, antiapoptotic and antiarrhythmic effects [14,15]. Interestingly, resveratrol acquires significant antioxidant properties like scavenging intracellular free radicals in an assortment of cell types [16]. It acts as an estrogen receptor agonist and triggers a significant decline in estrogen level both in male and female rats due to a negative feedback effect [17].

Recently, studies have shown that resveratrol offers protection against the metabolic changes associated with hypercaloric diets in obese Zucker rats with induced insulin resistance, hyperglycemia and dyslipidemia [18]. Similarly, resveratrol is reported to act as an insulin-secretagogue in different β-cell insulinoma lines which might contribute to its glucose lowering effect [19]. Conversely, a few literature data indicate that resveratrol shows reversible inhibitory effects on insulin secretion from freshly isolated rat pancreatic islets [20,21]. These effects are, however, not fully elucidated and some results are contradictory.

Recently, Su et al. have demonstrated the antihyperglycemic effect of resveratrol in experimental diabetic rats [22]. More recently, we have reported the antihyperglycemic properties of resveratrol and its modulatory effects on the activities of key carbohydrate metabolizing enzymes in the liver and kidney tissues of streptozotocin–nicotinamide-induced diabetic rats [23,24]. However, no study has investigated the hepatocytes ultrastructure protective nature of resveratrol in streptozotocin–nicotinamide-induced diabetic rats. Hence, the present study is aimed to investigate ameliorative potential of resveratrol on proinflammatory cytokines, hyperglycemia-mediated oxidative stress and hepatocyte dysfunction in streptozotocin–nicotinamide-induced diabetic rats and the efficacy of resveratrol was compared with glyclazide, a standard oral antihyperglycemic drug.

2. Materials and methods

2.1. Chemicals

Resveratrol, streptozotocin, nicotinamide, epinephrine, thiobarbituric acid, GSH, vitamin E and sodium azide were procured from Sigma Chemicals Co., St. Louis, MO, USA, 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

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 standard rat chow (Hindustan Lever Ltd., Bangalore, India) and water. The animals were acclimatized to the laboratory for 2 weeks prior to the inception of experiments. Animal experimentations were premeditated and executed in compliance 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).

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 cold citrate buffer (100 mM, pH 4.5) [25], 15 min after the intraperitoneal administration of nicotinamide (110 mg/kg body weight) [26]. 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 [27]. 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 six rats as detailed follows. Group 1 served as control rats; Group 2 served as control rats administered with resveratrol (5 mg/kg body weight/day) in aqueous suspension orally for 30 days; Group 3 served as streptozotocin–nicotinamide-induced diabetic rats; Group 4 served as diabetic rats administered with resveratrol (5 mg/kg body weight/day) in aqueous suspension orally for 30 days, and Group 5 served as diabetic rats administered with glyclazide, a well known oral antihyperglycemic drug, (5 mg/kg body weight/day) in aqueous suspension orally for 30 days (to compare the efficacy of resveratrol).

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 b.w. 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