European Journal of Pharmacology 622 (2009) 65–70

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Endocrine Pharmacology

Pancreatic tissue protective nature of D-Pinitol studied in streptozotocin-mediated oxidative stress in experimental diabetic rats

Selvaraj Sivakumar, Sorimuthu Pillai Subramanian *

Department of Biochemistry, University of Madras, Guindy campus, Chennai-600025, Tamilnadu, India

ARTICLE INFO

Article history:
Received 28 May 2009
Received in revised form 19 August 2009
Accepted 8 September 2009
Available online 16 September 2009

Keywords:
D-Pinitol
Diabetes mellitus
Streptozotocin
Lipid peroxidation
Oxidative stress

ABSTRACT

The present study was aimed to investigate the possible pancreatic tissue protective nature of D-Pinitol, a cyclitol present in soybean, against free radical-mediated oxidative stress in streptozotocin-induced diabetic rats by assaying the activity of pancreatic enzymatic antioxidants such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione-S-transferase (GST) and the levels of plasma non-enzymatic antioxidants such as vitamin E, vitamin C, ceruloplasmin and reduced glutathione (GSH). To assess the extent of oxidative stress, the levels of lipid peroxidation (LPO) and hydroperoxides in both plasma and pancreatic tissues were also measured. A significant increase in the levels of both lipid peroxides and hydroperoxides with a concomitant decrease in antioxidant status was observed in the diabetic rats when compared to control rats. Oral administration of D-Pinitol (50 mg/kg b.w./day for 30 days), a major cyclitol present in soybean, ameliorates the free radical-mediated alterations to near normalcy. The pancreatic tissue protective nature of D-Pinitol was further evidenced by histological observations. The results were statistically comparable with glyclazide, a standard hypoglycemic drug. Thus, the results of the present study suggest that D-Pinitol protects the pancreatic tissue from free radical-mediated oxidative stress in addition to its antidiabetic property.

1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting from insufficient secretion or action of endogenous insulin. The prevalence of diabetes is increasing throughout the world and is predicted to increase by two-fold from 150 million in the year 2000 to 300 million by the year 2030 (Wild et al., 2004). The larger share of this increase is mainly contributed by developing countries like India. Recently, India has been declared as the country with the largest number of diabetics in the world ahead of China and USA (Mohan et al., 2004). Moreover, it has been anticipated that approximately 57 million Indians will be diabetics by the year 2025. This obviously signifies that economic burden owing to diabetes and its complications in India will augment considerably in the near future (King et al., 1998).

Chronic hyperglycemia during diabetes leads to the accelerated generation of reactive oxygen species through oxidative phosphorylation during anaerobic glycolysis (Nishikawa et al., 2000), glucose autoxidation (Wolff and Dean, 1987; Hunt et al., 1988), glucosamine pathway (Kaneto et al., 2001) and decline in the antioxidant defense (Obrosova et al., 2002). The reactive oxygen species formed during hyperglycemia causes damage to the membrane lipids and proteins by lipid peroxidation (Sato et al., 1979; Baynes, 1991) and subsequent development of diabetic complications (Maritim et al., 2003; Obrosova et al., 2003). The increased production of reactive oxygen species may play a major role in the destruction of pancreas and the progression of β-cell dysfunction in diabetic condition. This is likely due to the susceptibility to oxidative stress as they possess low antioxidative capacity (Kajimoto and Kaneto, 2004). Reduction of hyperglycemia and improvement in the control of blood sugar reduce the oxidative stress and improve the metabolic function of β-cells (Ceriello, 2003).

The cellular antioxidant status determines the susceptibility to oxidative damage and is usually altered in response to oxidative stress. The antioxidants elicit its action on oxidative stress by a free radical scavenging mechanism. Recently there has been a considerable interest in finding natural antioxidants from plant materials to replace synthetic ones and the efforts to discover antioxidants as useful drug candidate to combat diabetic complications are going on relentlessly.

D-Pinitol (1D-3-*O*-methyl-chiro-inositol) is a major low molecular weight cyclitol present in developing soybean seed tissues, cotyledon and axis of the embryo (Phillips et al., 1982). Cyclitols are named so because of the cyclic structure in which all members of the ring are carbon atoms, in contrast to the cyclic structure of common sugars

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

0014-2999/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2009.09.021

that include oxygen in the ring. D-Pinitol has been suggested to possess various biological effects including anti-inflammatory (Singh et al., 2001), cardioprotective (Kim et al., 2005), feeding stimulant (Numata et al., 1979) and creatine retention promotion properties (Greenwood et al., 2001). Geethan and Prince (2008) have studied the antihyperlipidemic effect of D-Pinitol in streptozotocin-induced type II diabetic rats and have reported that D-Pinitol increases the utilization of glucose, thereby depressing the mobilization of fat. Bates et al. (2000) have studied the effect of oral administration of D-Pinitol at a concentration of 100 mg/kg b.w. to streptozotocin-induced diabetic mice and also the effect of D-Pinitol in insulin stimulated 2DG uptake by L6 cells and reported that oral administration maintained a reduction in plasma glucose concentration from about 14 to 10 mM and D-Pinitol did not increase insulin stimulated 2DG glucose uptake by L6 cells. They suggested that D-Pinitol may act via a post receptor pathway of insulin action. Recently, we have reported the antihyperglycemic nature of D-Pinitol by determining its modulatory role on the activities of hepatic key carbohydrate metabolizing enzymes in streptozotocin-induced experimental diabetes (Sivakumar and Subramanian, in press). Since, no systematic studies exist in the literature on the effect of D-Pinitol on oxidative stress in diabetes, the present study was aimed to vindicate the antioxidant potential of D-Pinitol on hyperglycemia-mediated lipid peroxidation and pancreatic β-cell protection in streptozotocin-induced experimental diabetic rats.

2. Materials and methods

2.1. Chemicals

D-Pinitol and streptozotocin were purchased from Sigma Chemicals Co., St. Louis, MO, USA. All other chemicals used in this study were of analytical grade available commercially.

2.2. Animals and diet

The experiments were conducted in accordance with the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines (IAEC approval No. 01/017/08). Male Wistar rats weighing 160–180 g, procured from Tamilnadu Veterinary and Animal Sciences University, Chennai, India were used in this study. The rats were maintained under standard laboratory conditions at 25 ± 2 °C, relative humidity 50 ± 15% and normal photoperiod (12 h light/dark cycle). Throughout the experimental period, the rats were fed with balanced commercial pellet diet (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. Diet and water were provided *ad libitum*.

2.3. Experimental induction of diabetes

The overnight fasted rats were induced by a single intraperitoneal injection of streptozotocin (50 mg/kg b.w.) dissolved in freshly prepared 0.1 M cold citrate buffer, pH 4.5 (Rakieten et al., 1963). Since, streptozotocin is capable of inducing fatal hypoglycemia as a result of massive pancreatic insulin release, the streptozotocin-treated rats were provided with 10% glucose solution after 6 h for the next 24 h to prevent hypoglycemia. Neither death nor any other adverse effect was observed. After a week in time for the development and aggravation of diabetes, rats with moderate diabetes (i.e. blood glucose concentration, > 14 mM) that exhibited glycosuria and hyperglycemia were selected for the experiment.

2.4. Experimental design

The rats were divided into four groups, each group comprises of six rats, as detailed below:

Group 1	Control rats
Group 2	Streptozotocin-induced diabetic rats
Group 3	Diabetic rats treated with D-Pinitol (50 mg/kg b.w./rat/day) orally for 30 days once a day
Group 4	Diabetic rats treated with glyclazide (5 mg/kg b.w./rat/day) orally for 30 days once a day

During the experimental period, respiratory changes, distress, abnormal locomotion, catalepsy and blood glucose levels of all the rats were determined at regular intervals. At the end of the experimental period, the rats were fasted overnight, anaesthetized and sacrificed by cervical decapitation. The blood was collected with or without ethylenediamine-tetra-acetic acid (EDTA) for plasma or serum separation, respectively.

2.5. Biochemical estimations

Fasting blood glucose level was estimated by the method of Sasaki et al. (1972) using *O*-toluidine reagent. Plasma insulin was determined using ELISA kit (for rats) supplied by Lincoplex Ltd., USA. Hemoglobin and glycosylated hemoglobin levels were estimated by the method of Drabkin and Austin (1932) and Nayak and Pattabiraman (1981), respectively. The activities of serum aspartate transaminase (AST), serum alanine transaminase (ALT) and serum alkaline phosphatase (ALP) were assayed by the method of King (1965a,b). The levels of plasma non-enzymatic antioxidants such as vitamin C (Omaye et al., 1979), vitamin E (Desai, 1984), reduced glutathione (Sedlak and Lindsay, 1968) and ceruloplasmin (Ravin, 1961) were estimated. Further, the levels of LPO and hydroperoxides and the activities of enzymatic antioxidants were determined in the pancreatic tissue homogenate. Tissue homogenate was prepared by excising the pancreatic tissues followed by rinsing with ice-cold saline and homogenizing in Tris–HCl buffer, pH 7.4 with a Teflon homogenizer at 4 °C. The homogenate was used for the determination of LPO (Ohkawa et al., 1979) and hydroperoxides (Jiang et al., 1992). The activities of SOD and catalase were determined by the method of Misra and Fridovich (1972) and Takahara et al. (1960), respectively. GPx was assayed by the method of Rotruck et al. (1973), GST was assayed by the method of Habig et al. (1974) and the tissue protein was estimated by the method of Lowry et al. (1951).

2.6. Histological study

A portion of pancreas was fixed in 10% formalin for a week at room temperature. Then the specimens were dehydrated in a graded series of ethanol, cleared in xylene and embedded in paraffin wax. The blocks were then sectioned into 5 μm thick using a rotary microtome. Sections were stained by modified aldehyde fuchsin and photomicrographs were obtained under light microscope.

2.7. Statistical analysis

The results were expressed as mean ± S.E.M. of six rats per group and the statistical significance was evaluated by one-way analysis of variance (ANOVA) using the SPSS/15.0 software followed by LSD. Values were considered statistically significant when $P < 0.05$.

3. Results

Table 1 depicts the levels of fasting blood glucose, plasma insulin, hemoglobin and glycosylated hemoglobin in control and experimental