Nutrition, Metabolism & Cardiovascular Diseases (2014) **24**, 623–631

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

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

Feeding of banana flower and pseudostem to diabetic rats results in modulation of renal GLUTs, TGFβ, PKC and extracellular matrix components

J.B. Jamuna, C.D. Nandini*

Department of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore 570 020, Karnataka, India

Received 2 July 2013; received in revised form 17 October 2013; accepted 4 December 2013
Available online 25 December 2013

KEYWORDS
Glucose transporters;
Diabetes;
Banana flower;
Banana pseudostem;
Kidney;
Extracellular matrix components

Abstract *Background and aims:* Sustained hyperglycemia as a result of diabetes mellitus results in over-expression of glucose transporters (GLUTs/SGLTs), protein kinase C-α (PKC-α) and transforming growth factor-β (TGF-β) in kidney which increases synthesis and accumulation of extracellular matrix (ECM) components leading to diabetic nephropathy. Previous results from our laboratory showed that banana flower (BF) and pseudostem (BS) ameliorated diabetic complications and reduced formation of advanced glycation end-products (AGEs). In this study, attempts were made to delineate the changes observed in GLUTs and ECM components in kidney by feeding BF and BS at the molecular level.

Methods and results: Diabetes was induced in male Wistar rats by injecting streptozotocin. Rats were fed with standard AIN-76 diet or diet supplemented with 5% BF or BS. Rats fed with diet supplemented with aminoguanidine (0.05%) were used as a positive control. Effect of BF and BS on expression of GLUTs/SGLTs, PKC and TGF β in kidney was evaluated by RT-PCR and accumulation of ECM components in kidney was quantitated by ELISA and immunohistochemistry. BF and BS modulated the over-expression of GLUT 1, 2, 5, SGLT 1, 2 and factors such as PKC-α and TGF-β to various extents. This impinged on the synthesis of ECM components like laminin, fibronectin and type-IV collagen.

Conclusion: The results suggest that BF and BS reduce the diabetic nephropathy complications which are accompanied by changes at the molecular level.

© 2013 Elsevier B.V. All rights reserved.

Introduction

Diabetic nephropathy (DN) is manifested clinically as a result of secondary complications of both Type 1 and Type 2 diabetes mellitus [1]. In hyperglycemic conditions, glucose transport in diabetic kidney is upregulated. The resultant enhancement in glucose metabolic flux leads to activation of a number of metabolic pathways that result in increased advanced glycation end products (AGEs) and oxidative stress generation [2]. This inturn activates a number of signaling pathways that lead to enhanced extracellular matrix (ECM) production directly via protein kinase-C (PKC) and transforming growth factor-β (TGF-β) synthesis [3]. TGF-β stimulates its signaling pathways to enhance synthesis of ECM proteins like fibronectin, laminin, thrombospondin and various forms of collagens, primarily type-IV and type-I [4]. The accumulation of ECM proteins in the mesangial areas and the associated encroachment on neighboring capillaries, with loss of filtration surface area, leads to decline in glomerular function, mesangial expansion and glomerulosclerosis [5].

Kidney has various glucose transporters. High extracellular glucose induces an increase in glucose uptake via

* Corresponding author. Tel.: +91 (0)821 2514876; fax: +91 821 2517233.
E-mail address: cdnandini@yahoo.com (C.D. Nandini).

0939-4753/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.numecd.2013.12.003

increased expression of glucose transporters (GLUTs/SGLTs) [6]. In diabetic hyperglycemic rats, modulation of GLUT 1, 2 and 5, has been reported in response to the intracellular concentration of glucose [7]. Streptozotocin (STZ)-induced diabetes has been reported to stimulate the insertion of GLUT2 into the basement membrane of Bowman's capsule of the proximal tubule and to increase facilitative glucose uptake [8].

One of the strategies employed in preventing diabetic complications is through dietary management. Diet rich in dietary fiber, antioxidants, nutraceuticals, etc. are highly beneficial for diabetics. Banana plant is known for its medicinal properties since ages and furthermore, it is reported to contain various biologically active compounds, such as dopamine, nor-adrenaline, serotonin and anti-hyperglycemic factors [9].

In our previous studies, we have shown that banana flower (BF) and pseudostem (BS), when fed at 5% level individually to diabetic rats, significantly reduced parameters associated with diabetes and AGEs formation [10]. Recently, we have reported that BF and BS are rich sources of dietary fibers and antioxidants [11]. Different extracts of BF and BS were able to promote glucose uptake in Ehrlich Ascites tumor (EAT) cells indicating that its consumption will be beneficial in diabetic condition [12]. The objective of the present study was to determine the effects of BF and BS feeding on expression of GLUTs/SGLTs, PKC-α and TGF-β in kidney of streptozotocin-induced diabetic rats and their effect on synthesis of ECM constituents namely, laminin, type-IV collagen and fibronectin.

Methods

Chemicals

Albumin Blue 580 dye, primers for β-actin, GLUT 1, 2 and 5, SGLT 1 and 2, TGF-β, PKC-α, type IV collagen and laminin from Engelbreth–Holm–Swarm murine sarcoma basement membrane, fibronectin from bovine plasma, were procured from Sigma–Aldrich Pvt. Ltd., St. Louis, USA. RT-PCR kits, bovine serum albumin (BSA) anti-fibronectin antibody, ALP- and FITC-conjugated secondary antibodies were procured from Genei Pvt. Ltd., India. Glucose oxidase/peroxidase (GOD/POD) and creatinine estimation kits were purchased from Span Diagnostics Pvt. Ltd., Surat, India. Anti-type-IV collagen and anti-laminin antibodies were procured from Abcam, USA. Vitamins and minerals used in animal experiments were from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Casein was from Nimesh Corporation, Mumbai, India. All other chemicals and reagents used were of analytical grade.

Plant material

Fresh banana flowers and pseudostems of *Musa* sp. var. elakki bale were obtained from the Mysore local market and identified by the Department of Horticulture, Government of Karnataka, Mysore, India. Banana flowers were separated from inflorescence by discarding the spathe and tender pseudostems were separated from thick outer leaf sheaths. Plant material was cleaned and cut into pieces, dried in an oven at 40 °C and powdered. The powdered plant materials were kept in air tight containers and stored in refrigerator at 4 °C until use.

Animals and experimental design

The study had approval of Institutional Animal Ethical Committee (IAEC), CFTRI, Mysore, India. Male Wistar rats (OUTB-Wistar, IND cftri) weighing 120–140 g were selected for the study and divided into eight groups based on fasting blood glucose levels. The groups were tentatively named as starch fed control and diabetic (SFC/SFD), banana flower fed control and diabetic (FFC/FFD), banana pseudostem fed control and diabetic (TFC/TFD) and aminoguanidine fed control and diabetic (AFC/AFD) groups. In the beginning of the experiment, control groups had 6 rats and diabetic groups had 14 rats each. Rats were housed in individual cages and had free access to diet and water. Rats were rendered diabetic by a single i.p. injection of streptozotocin (48 mg/kg body weight) in freshly prepared citrate buffer (pH 4.5, 0.1 M) [10]. BF and BS at 5% and aminoguanidine at 0.05% levels were incorporated, at the expense of corn starch in AIN-76 basal diet [13], containing 65.3% corn starch, 20% protein, 10% fat, 3.5% AIN-76 mineral mix, 1% AIN-76 vitamin mix, 0.2% choline chloride and was stored at 4 °C. Diet and water was given *ad libitum*. Experiment was carried out for a period of sixteen weeks after the induction of diabetes.

Analytical methods

Fasting blood sugar (FBS) was measured in retro-orbital blood after overnight fasting by glucose oxidase/peroxidase method (GOD/POD) using commercially available kit from Span diagnostics, India [10]. Urine sugar was estimated by dinitrosalicylic acid method [14]. Creatinine levels in urine and serum were estimated by Bower's method using commercially available kit from Span diagnostics, India [15]. Glomerular filtration rate (GFR) was calculated using the formula as detailed earlier [16]. Albumin in the urine was estimated to determine microalbuminuria using Albumin Blue 580 dye [17]. Protein was estimated in kidney homogenates by Lowry's method [18]. Kidney index (KI) was calculated using the formula:

$$\mathrm{KI} = \frac{\text{kidney weight(g)}}{\text{body weight(g)}} \times 1000$$

Isolation of total RNA

Total RNA was isolated from 100 mg of freshly harvested rat kidneys by Trizol method. The concentration of RNA was determined by the absorption ratio at 260 and 280 nm, and quality was checked by using formaldehyde gel electrophoresis for 28S and 18S RNA. The isolated RNA

Download English Version:

https://daneshyari.com/en/article/3002046

Download Persian Version:

https://daneshyari.com/article/3002046

Daneshyari.com