Nutrition 32 (2016) 898–903

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

Retinoids have therapeutic action in type 2 diabetes

Dilnasheen Meerza Ph.D. [a], Sarah Iqbal M.Sc. [a], Sufian Zaheer M.D. [b], Imrana Naseem Ph.D. [a,*]

[a] Department of Biochemistry, Aligarh Muslim University, Aligarh, India
[b] Department of Pathology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, India

ARTICLE INFO

Article history:
Received 12 August 2015
Accepted 2 February 2016

Keywords:
Type 2 diabetes
Vitamin A
Hyperglycemia
Oxidative stress
DNA damage

ABSTRACT

Objective: Uncontrolled diabetes is associated with a compromised antioxidant state of the body. Consequentially, the reactive oxygen species generated lead to oxidative insult and associated complications. Based on this paradigm, exogenous antioxidant supplementation is thought to exert a therapeutic role in type 2 diabetes (T2-D) biology.
Methods: In the present study, the effect of vitamin A supplementation was assessed on disease progression in T2-D BALB/c mice. Animals were divided into three groups. With the exception of control, the mice in remaining groups were induced with experimental T2-D. After a 15-day treatment protocol, the mice were sacrificed and various parameters were analyzed.
Results: The treated group evidenced a considerable improvement in total antioxidant potential and glycemic control. A therapeutic effect on beta cell degeneration as compared to the diabetic group was also found.
Conclusions: The study illustrates the antihyperglycemic and antioxidant potential of vitamin A in vivo, which has potential to serve as a dietary intervention in T2-D.
© 2016 Elsevier Inc. All rights reserved.

Introduction

Vitamin A comprises a group of fat-soluble organic compounds, involved in a multitude of biological functions [1]. Among the vitamin A metabolites, retinol, retinaldehyde, and retinoic acid (RA) exert a pivotal biological activity and are commonly named as retinoids. These molecules are widely known to regulate ocular activity [1,2]. Recently, they have been discovered to play pleiotropic roles in cell signaling, endocrine development, and even pancreas function [3], thus serving as a potential and attractive treatment for type 2 diabetes (T2-D). Chertow showed the role of vitamin A in pancreatic regeneration in 1978 but its use in diabetes therapy remains largely unexplored [3–5]. The growing incidence of T2-D has renewed interest in retinoid biology.

T2-D is characterized by peripheral insulin resistance, dysfunctional regulation of hepatic glucose production, and impaired beta cell activity that contributes to hyperglycemia [6, 7]. Persistent hyperglycemia augments the production of reactive oxygen species [8,9]. The present therapeutic approaches have little or even no effect in ameliorating the oxidative status of the cell. Conventional medical intervention involves control of hyperglycemia, but this strategy becomes ineffective over years [10]. An insulin treatment regime, by itself, has been unable to inhibit protein glycation, which further overwhelms the redox system [11]. Research has led to the discovery of a relationship between increased oxidative stress and decreased insulin sensitivity, elucidating the putative role of antioxidant therapy in disease control [12,13].

Vitamin A boasts the highest antioxidant potential among all vitamins [14]. Literature suggests its involvement in radical trapping at lower oxygen partial pressures, yet it remains considerably unexplored, compared with other antioxidant vitamins such as C and E, in disease management [14,15]. Various researchers have investigated the role of retinoids in islet health, repair, and replication [16]. Retinoids, particularly retinoic acid (RA), have displayed promising results in vitro, and are currently under investigation for remedial manipulation [16,17]. Alarmingly, vitamin A deficiency is widespread among malnourished diabetics and in lower economic strata of the U.S. population and developing Asian nations, where T2-D is on the rise [1,18]. Thus, this study aims to investigate the role of exogenous vitamin A supplementation on T2-D pathophysiology.

Dilnasheen Meerza and Sarah Iqbal contributed equally to this report.
* Corresponding author. Tel.: 91-9719069125; fax: 0571-2706002.
E-mail address: Imrananaseem2009@gmail.com (I. Naseem).

http://dx.doi.org/10.1016/j.nut.2016.02.003
0899-9007/© 2016 Elsevier Inc. All rights reserved.

Material and methods

The present study was conducted on female albino mice housed in the animal center at the Department of Biochemistry, Aligarh Muslim University. All procedures performed in studies involving animals were in accordance with the ethical standards of the Institutional Animal Ethical Committee, Department of Biochemistry, Aligarh Muslim University. All applicable national and institutional guidelines for the care and use of animals were followed.The animals were divided into three groups of eight each and kept under a 12-h light-dark cycle in ventilated plastic cages at 25°C (±2°C). The first group was control (C) and given no treatment. The animals in remaining groups were administered alloxan at a dose of 150 mg/kg body weight and their fasting blood glucose (FBG) was monitored until they became diabetic. One set of diabetic animals was then maintained on saline treatment; this group is referred to as D. The animals in the remaining group (D + A) were subjected to intraperitoneal vitamin A administration (retinyl palmitate at 86 μg/kg body weight) for a regular period of 15 days. All mice were given free access to food and water ad libitum.

Sample collection

After overnight fasting, mice were sacrificed by cervical dislocation at the end of the treatment period. Blood and tissue samples were collected for analysis.

Biochemical analysis

FBG, hexokinase, fructose 1,6-bisphosphatase (FBPase), and glucose transporter 4 levels were analyzed in treated animals to study glucose homeostasis. For antioxidant status, the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR) were assayed. The levels of lipid peroxidation product- malondialdehyde (MDA)- were also determined, and the associated DNA and tissue damages were assessed by comet assay and histopathology, respectively.

FBG estimation

Glucose levels were estimated in blood samples using the glucose oxidase-peroxidase method of Trinder [19] and the Cogent Autospan Glucose kit (Span Diagnostics Ltd., Surat, India). Glucose concentration in the samples was determined using a standard curve.

Hexokinase activity

The hexokinase activity was measured by the method of Crane and Sols [20]. A 1 mL reaction mixture containing Tris HCl (50 μM), $MgCl_2$ (10 μM), adenosine triphosphate (5 μM), and glucose (2 μM) was added to 1 mg to 1.5 mg of sample protein. The reaction was stopped after 1 h by adding 0.5 mL each of 10% barium hydroxide and zinc sulphate solutions. After centrifugation at 2000*g* for 10 min, glucose was estimated in the supernatant (free from phosphorylated derivatives) by the method of Nelson [21].

FBPase activity

The liver FBPase activity was measured by the method of Freedland and Harper [22]. The reaction mixture containing Tris HCl (50 μM), $MgCl_2$ (10 μM), cysteine HCl (12 μM), and fructose 1,6-diphosphate (10 μM) in 1.5 mL was added to the sample containing 0.6 mg to 0.8 mg protein. The reaction was stopped by adding 1 mL of 10% tricarboxylic acid after 60 min. Samples were centrifuged at 2000*g* for 10 min followed by estimation of phosphate released in the supernatant by the method of Tausky and Shorr [23].

SOD activity

SOD activity was assayed according to the method of Marklund and Marklund [24]. One enzyme unit is defined as the amount of enzyme required to cause 50% inhibition to auto-oxidation of pyrogallol per 3 mL of assay mixture.

CAT activity

CAT activity (Enzyme Commission number 1.11.1.6) was measured by the protocol of Aebi [25]. The enzyme activity was calculated as nanomoles of hydrogen peroxide consumed per mg of protein per minute.

GR activity

The assay of this enzyme was carried out by the method of Carlberg and Mannervik [26]. The reaction is based on the NADPH-dependent reduction of glutathione disulfide into glutathione. The specific activity was reported in nanomoles of NADPH oxidized per g of protein per minute.

Estimation of MDA level

MDA levels were estimated by the procedure of Buege and Aust [27]. The reaction mixture contained equal volumes of tissue homogenate, thiobarbituric acid (0.67%), and tricyclic antidepressant (30%). After incubation in a boiling-water bath for 20 min, the tubes were centrifuged at 5000*g* for 15 min. The absorbance of the supernatant was determined at 530 nm and the amount of thiobarbituric-reactive substances present was determined using the molar extinction coefficient of 1.56×10^{-5} M^{-1} cm^{-1} for MDA-thiobarbituric acid colored complex.

GLUT4 estimation

Quantitative determination of GLUT4 was done by sandwich enzyme-linked immunosorbent assay (ELISA) in adipocyte and skeletal muscle homogenates (adipocytes and skeletal muscles) using the ELISA anti–glucose transporter 4, Mouse anti Glucose transporter 4 antibody kit (EIAab, Wuhan, China), following the manufacturer's instructions. The concentration of GLUT4 in the samples was determined by comparing the absorbance of the samples to the standard curve supplied with the kit.

Comet assay

The assay was performed in alkaline conditions in accordance with the protocol of Singh, et al., with some modifications [28]. Slides precoated with 1% normal-melting agarose (as base layer) were layered with pancreatic cell suspension in combination with 1% low-melting-point agarose. Following solidification on ice packs, a third layer of 0.5% low-melting-point agarose was applied. After lysis for up to 3 h and unwinding for 30 min in alkaline electrophoretic running buffer, electrophoresis was performed for 35 min at 4°C with constant field strength of 0.74 V/cm. The slides were then washed, neutralized, and stained with ethidium bromide. The slides were scored using a CX41 fluorescent microscope (Olympus, Tokyo, Japan) coupled with the Komet 5.5 imaging system (Kinetic Imaging Ltd., Liverpool, UK), attached with integrated 4910 CCD camera (Cohu, Poway, CA, USA) (equipped with 510–560 nm excitation and 590 nm barrier filters). The comets were scored at the magnification of 100× and tail length (migration of DNA from its nucleus in μm) was chosen as the parameter to assess the cellular DNA damage.

Preparation of samples for histopathological study

Liver, kidney, and pancreas of the animals were kept in 10% formalin in separate glass vials for histopathology after washing with chilled saline. Tissue blocks (sized at 10 × 5 × 3 mm) of the organs were processed for paraffin embedding. Sections measuring 5 μm thick were obtained and stained with hematoxylin and eosin. The sections were observed under a trinocular light BX40 Research Microscope (Olympus, Tokyo, Japan) and the photomicrographs depicting important features were snapped at a final magnification of 400×.

Statistical analyses

Results are expressed as mean ± SD for all continuous variables. Differences between the C, D, and D + A groups are assessed using analysis of variance followed by Student's *t* test. For all the tests, a *P* value of ≤ 0.05 was considered to be statistically significant. Analysis was performed using SPSS software, version 19 (IBM, Armonk, NY, USA) and Origin software, version 6.1 (OriginLab, Northampton, MA, USA).

Results and Discussion

The present study deals with investigation of dietary vitamin A supplementation in female mice suffering from T2-D. The prevalence for diabetes is only slightly greater in males than females, yet most studies on experimental diabetes have been carried out among males [29,30]. This study, therefore, explores the effect of supplementation of vitamin A, a potent dietary antioxidant, in female mice.

Hyperglycemia enhances free radical generation, causing damage to macromolecules like lipids, carbohydrates, proteins, and nucleic acids, thereby disrupting cell structure and function [13,31]. Antioxidants have therefore been ascribed a protective role in T2-D biology [14,32].

The therapeutic potential of vitamin A supplementation was analyzed on three broad parameters: glucose metabolism, oxidative stress, and DNA damage [33].

Effect on glucose metabolism

FBG levels are a direct indicator of diabetes. The levels were severely elevated in group D, while vitamin A treatment reduced the levels in group D + A to a small extent (Table 1). To assess

Download English Version:

https://daneshyari.com/en/article/6089147

Download Persian Version:

https://daneshyari.com/article/6089147

Daneshyari.com