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Research paper

Efficacy of azelaic acid on hepatic key enzymes of carbohydrate metabolism in high fat diet induced type 2 diabetic mice

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ABSTRACT

Azelaic acid (AzA), a C9 linear α,ω-dicarboxylic acid, is found in whole grains namely wheat, rye, barley, oat seeds and sorghum. The study was performed to investigate whether AzA exerts beneficial effect on hepatic key enzymes of carbohydrate metabolism in high fat diet (HFD) induced type 2 diabetic C57BL/6J mice. C57BL/6J mice were fed high fat diet for 10 weeks and subjected to intragastric administration of various doses (20 mg, 40 mg and 80 mg/kg BW) of AzA daily for the subsequent 5 weeks. Rosiglitazone (RSG) was used as reference drug. Body weight, food intake, plasma glucose, plasma insulin, blood haemoglobin (Hb), blood glycosylated haemoglobin (HbA1c), liver glycolytic enzyme (hexokinase), hepatic shunt enzyme (glucose-6-phosphate dehydrogenase), gluconeogenic enzymes(glucose-6-phosphatase and fructose-1,6-bisphosphatase), liver glycogen, plasma and liver triglycerides were examined in mice fed with normal standard diet (NC), high fat diet (HFD), HFD with AzA (HFD + AzA) and HFD with rosiglitazone (HFD + RSG). Among the three doses, 80 mg/kg BW of AzA was able to positively regulate plasma glucose, insulin, blood HbA1c and haemoglobin levels by significantly increasing the activity of hexokinase and glucose-6-phosphate dehydrogenase and significantly decreasing the activity of glucose-6-phosphatase and fructose-1,6-bisphosphatase thereby increasing the glycogen content in the liver. From this study, we put forward that AzA could significantly restore the levels of plasma glucose, insulin, HbA1c, Hb, liver glycogen and carbohydrate metabolic key enzymes to near normal in diabetic mice and hence, AzA may be useful as a biomaterial in the development of therapeutic agents against high fat diet induced T2DM.

1. Introduction

Metabolic syndrome is a combination of related disorders, including impaired glucose tolerance, abdominal obesity, high cholesterol and high blood pressure which increase the risk of cardiovascular disease and diabetes. According to International Diabetes Foundation (IDF), Type 2 diabetes (T2DM) currently affects 246 million people worldwide and is expected to increase to 380 million by 2025. Insulin resistance is a well recognized pathophysiologic feature of metabolic syndrome and T2DM [1] and is strongly associated with co-existing cardiovascular risk factors and accelerated atherosclerosis. Insulin resistance is assumed to disturb both glucose and lipid metabolism and accelerate obesity through stimulating insulin secretion [2–4].

Carbohydrate metabolism in liver is largely influenced by efficient insulin sensitivity (for uptaking glucose) and mitochondrial energy transduction (for scavenging free radicals). An increase in delivery of fatty acids or a decrease in intracellular metabolism of fatty acids leads to an increase in intracellular fatty acid metabolites such as diacylglycerol and fatty acyl CoA. These metabolites activate a serine/threonine kinase cascade leading to phosphorylation of serine/threonine sites on insulin receptor substrates (IRS-1 and IRS-2), which in turn reduces the ability of the insulin receptor substrates to activate PI 3-kinase. As a consequence, glucose transport activity and other events downstream of insulin receptor signalling are diminished [5]. Mitochondrial energy transduction is regulated by a mitochondrial inner membrane protein, Nicotinamide nucleotide transhydrogenase (Nnt) which produces high concentrations of NADPH, to be used in free radical detoxification. C57BL/6J mice possess a spontaneous in-frame five exon deletion (a loss-of-function mutation) in the Nnt gene in chromosome 13 [6,7]. Furthermore, a combination of high fat diet and Nnt mutation in C57BL/6J mice may develop Insulin resistance and mitochondrial dysfunction, leading to T2DM.

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Currently, the antidiabetic drugs in use for long-term therapy are found to be associated with various toxicities owing to which the developmental process in antidiabetic drug discovery has shifted its focus on natural plant-derived phytocompounds having minimal side effects [8]. Phytochemicals and antioxidants in whole grains have not received as much attention as those in fruits and vegetables although the increased consumption of whole grains and whole grain products has been associated with reduced risk of developing chronic diseases such as cardiovascular disease and type 2 diabetes [9–13]. Azelaic acid (AzA), a C9 linear α,ω-dicarboxylic acid is found in whole grains namely wheat, rye, barley, oat seeds and sorghum [14]. The molecular structure of AzA is shown in Fig. 1. Studies on AzA proved that it exhibits antiacne [15], radical scavenging [16], antimicrobial [17] and antitumour potentials [18]. Studies of Mastrofrancesco et al. revealed that azelaic acid modulates the inflammatory response in normal human keratinocytes and acts as peroxisome proliferator activator receptor (PPAR) agonist [19]. So, we compared the activity of AzA with a well characterized PPAR agonist, rosiglitazone (RSG). Many animal and human studies have demonstrated that AzA exhibits no systemic toxicity after either oral or topical administration [20,21]. These pharmacological properties of AzA may provide a healthy platform for the significant restoration of altered hepatic key enzymes profile (involved in carbohydrate metabolism) in high fat diet induced type 2 diabetic mice. As no detailed scientific investigation has been reported yet in this context, we investigated the efficacy of AzA on hepatic key enzymes of carbohydrate metabolism and other related parameters in high fat diet induced type 2 diabetic C57BL/6J mice.

2. Materials and methods

2.1. Chemicals

Azelaic acid was purchased from Sigma–Aldrich (St. Louis, Missouri, USA). All other chemicals used in this study were of analytical grade obtained from HIMEDIA, Mumbai, India.

2.2. Experimental animals

Healthy adult male C57BL/6J mice were obtained from Sri Ramachandra University, Chennai and reared in Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University. Weight matched animals (18–20 g) were selected and housed in polypropylene cages layered with husk and kept in a semi-natural light/dark condition (12 h light/12 h dark). The animals were allowed free access to water and standard pellet diet. Animals were cared in accordance with the "Guide for the care and use of Laboratory Animals" (NIH, 1985). All studies were conducted in accordance with Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), "CPCSEA Guidelines for Laboratory Animal Facility". The study protocols were approved by the Institutional Animal Ethics Committee of Rajah Muthiah Medical College and Hospital (Reg No. 160/1999/CPCSEA, Proposal number: 850), Annamalainagar.

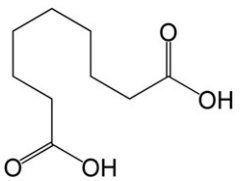

Fig. 1. Molecular structure of AzA.

2.3. Experimental induction of diabetes

The induction of type 2 diabetes was through high fat diet. The standard diet which is commercially obtained from Sai Enterprises, Chennai, had a fat composition of 4.2%. The beef tallow based high fat diet was composed of protein – 17.7 g, fat – 35.2 g, carbohydrate – 34.5 g, fibre – 3.4 g, minerals – 6.8 g and vitamins – 1.8 g. Mice (6 nos.) from normal control group (group I) were fed standard diet for a period of 15 weeks. Mice from rest of the groups (group II–VI) were fed high fat diet for a period of 15 weeks. At the end of 10th week, the mice from all the groups were tested for blood glucose levels. Mice with blood glucose level of 220 mg/dL and above were considered to have developed diabetes and were subjected to intragastric administration of various doses of AzA and RSG (as mentioned in the experimental design) during 11th to 15th weeks.

2.4. Experimental design

36 nos. of male C57BL/6J mice (body weight 20–22 g each) were segregated into 6 groups with 6 mice each and used for the study. The study design is portrayed in Fig. 2. Body weight was measured biweekly throughout the study. At the end of the experimental period (15 weeks), all the mice were fasted overnight, anesthetized by injecting ketamine (24 mg/kg/BW) intramuscularly and sacrificed by cervical decapitation during the early hours (between 8:00–9:00 am). Blood was collected in tubes containing potassium oxalate and sodium fluoride (3:1) mixture for the estimation of haemoglobin and glycosylated haemoglobin. Plasma was immediately isolated from blood to estimate the levels of glucose and insulin. Liver was surgically removed, washed with cold physiological saline, cleared off adherent lipids and immediately used for the measurement of various parameters.

2.5. Biochemical analysis

Plasma glucose was estimated by the method of Trinder, 1969. Haemoglobin (Hb) and glycosylated haemoglobin (HbA1c) were estimated by the method of Drabkin and Austin, 1932 and Sudhakar and Pattabiraman, 1981 [22] respectively. Plasma insulin was measured by the method of Burgi et al., 1998 [23]. Hepatic hexokinase activity was assayed by the method of Brandstrup et al., 1957

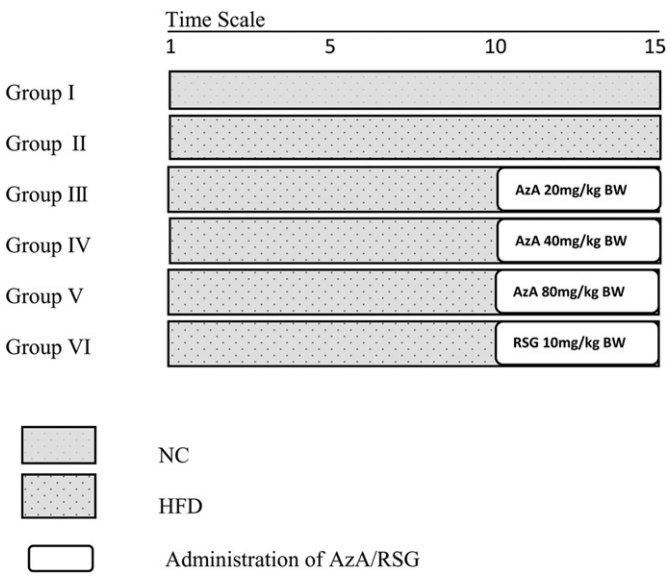

Fig. 2. Study design.

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