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Original article

Alpha lipoic acid attenuates high-fructose-induced pancreatic toxicity

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ABSTRACT

Objectives: Chronic consumption of high-fructose corn syrup (HFCS) causes several problems such as insulin resistance. The goal of the study was to investigate pancreatic damage induced by chronic HFCS consumption and the protective effects of alpha lipoic acid (ALA) on pancreatic cells.
Methods: Wistar Albino, 4-month-old, female rats weighing 250–300 g were randomly distributed into three groups, each containing eight rats. The study included an HFCS group, an HFCS + ALA-administered group and a control group (CON). The prepared 30% solution of HFCS (F30) (24% fructose, 28% dextrose) was added to the drinking water for 10 weeks. ALA treatment was begun 4 weeks after the first HFCS administration (100 mg/kg/oral, last 6 weeks). Rats were anaesthetised and euthanised by cervical dislocation 24 h after the last ALA administration. Blood samples for biochemical tests (amylase, lipase, malondialdehyde (MDA) and catalase (CAT)) and tissue samples for histopathological and immunohistochemical examinations (caspase-3, insulin and glucagon) were collected.
Results: Comparing the control and HFCS groups, serum glucose (150.92 ± 39.77 and 236.50 ± 18.28, respectively, $p < 0.05$), amylase (2165.00 ± 150.76 and 3027.66 ± 729.19, respectively, $p < 0.01$), lipase (5.58 ± 2.22 and 11.51 ± 2.74, respectively, $p < 0.01$) and pancreatic tissue MDA (0.0167 ± 0.004 and 0.0193 ± 0.006, respectively, $p < 0.05$) levels were increased, whereas tissue CAT (0.0924 ± 0.029 and 0.0359 ± 0.023, respectively, $p < 0.05$) activity decreased in the HFCS group significantly. Histopathological examination revealed degenerative and necrotic changes in Langerhans islet cells and slight inflammatory cell infiltration in pancreatic tissue in the HFCS group. Immunohistochemically there was a significant decrease in insulin (2.85 ± 0.37 and 0.87 ± 0.64, respectively, $p < 0.001$) and glucagon (2.71 ± 0.48 and 1.00 ± 0.75, respectively, $p < 0.001$) secreting cell scores, whereas a greater increase in caspase-3 (0.14 ± 0.37 and 1.00 ± 0.75, respectively, $p < 0.05$) expression was seen in this group compared with the controls. In the ALA-treated group, all of these pathologic conditions were improved.
Conclusions: This study indicated HFCS induced pancreatic lesions, but ALA had ameliorative effects.

Introduction

Dessert manufacturers prefer using high-fructose corn syrup (HFCS) rather than glucose and sucrose because of many advantages: it is sweet, it does not mask the original taste, it is cheaper and it retards satiety. HFCS commercially is named F30, F40 and F55, according to the ratio (30%, 40% and 55%) of fructose content. Prepared foods and soft drinks include these forms of HFCS in several countries [1].

Fructose is rapidly converted to triglycerides and stored in adipose tissue, and this causes obesity [2]. Some studies have shown that glucose tolerance and increased insulin resistance may occur by excessive consumption of sugary and fatty foods, which increases the risk of type 2 diabetes or metabolic syndrome [3].

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Hyperuricaemia, insulin resistance and elevated advanced glycation end product (AGE) levels are the harmful effects of chronic HFCS consumption. All of these metabolic disorders induce oxidative stress and inflammation. Serious tissue damage is shown in the pancreas because of oxidative stress. Oxidative stress as a result of lipid peroxidation causes apoptosis, inducing of malondialdehyde (MDA), formation of Mallory bodies, neutrophil chemotaxis, collagen production and fibrosis [4]. In addition, HFCS increases AGE, which causes several complications such as reduction in the antioxidant mechanism [5]. Decrease of antioxidant enzymes activity and increase of oxidative stress parameters are responsible for decrease in hormone secretion because of pancreatic damage [6].

Release of glucose from the liver to the bloodstream is important for regulation of plasma glucose levels via hormonal mechanisms. Insulin and glucagon may show opposite effects on the same target organs according to the ratio of insulin/glucagon. Further, hyperglycaemia inhibits or down-regulates glucagon release, and increases insulin release from excitable pancreatic α and β cells, plasma lipid and amino acid levels regulate the hormone-releasing effect of these cells at the same time [7–10]. The above-mentioned processes impaired in type 2 diabetes are due to hormonal secretory abnormalities. Moreover, losses of β-cell mass, peripheral insulin resistance, altered glucagon release and α-cell mass changes are the other causative factors [11].

Alpha lipoic acid (ALA) is a strong antioxidant that is used for the prevention of diabetes complications against AGE-induced oxidative stress and inflammation [12]. In addition, ALA reduces AGE to improve insulin sensitivity in skeletal muscle and the liver [13]. However, there are few studies about its use as an antioxidant against pancreatic damage.

The goal of the study was to investigate the protective effects of ALA on pancreatic damage induced by chronic HFCS consumption.

Materials and methods

The protocol was carried out according to the Animal Care and Use Committee guidelines of Suleyman Demirel University (22/08/2013-03) and was performed in accordance with the National Institutes of Health Guidelines for the Care and Handling of Animals.

Animals

In the study, 4-month-old, 24 Wistar Albino female rats ($n = 24$), each weighing 250–300 g, were included. Rats were separated into two groups, and there was no significant difference between the average weights of the groups. Rats were housed individually in stainless-steel cages in pathogen-free conditions in our laboratory at a temperature of 21–22 °C. All of the rats were fed standard commercial chow diet (Korkuteli yem, Turkey) composed of 88% dry material (mostly oat crust), 23% protein, 7% cellulose, 8% ash that does not dissolve in 2% HCl, 1–1.8% Ca^{+2}, 0.9% PO-4, 0.5–0.8% Na, 1% NaCl, 0.3% methionine and 1% lysine.

F30 HFCS was obtained from Toposmanoglu (Isparta, Turkey), which contains approximately 24% fructose and 28% dextrose in the syrup of 73% total solids. Previous research has shown that subjects served meals with either 30% glucose beverages or 30% fructose beverages had differing hormonal and metabolic responses. Glycaemic excursions and insulin responses were reduced by 66% and 65%, respectively in the fructose-consuming subjects [14–16]. For that reason in this study, the prepared 30% solution of F30 was added to drinking water for 10 weeks.

Thioctacid 600 mg tablets (Meda Pharma, Turkey), a commercial form of ALA, were used for treatment. ALA was dissolved in distilled water. The single daily dose of 100 mg/kg was given orally for last 6 weeks of the experiment.

Experimental design

Before the consumption, all the rats were randomly divided into three equal groups consisting of eight rats in each.

1 Control group (given only standard commercial diet and tap water).
2 HFCS group (given 30% F30 solution for 10 weeks).
3 HFCS + ALA group (given 30% of F30 solution for 10 weeks and 100 mg/kg ALA by oral gavage for the last 6 weeks of the experiment).

Water intake was investigated daily, and weight gain was recorded weekly. F30 was prepared as 30% fructose syrup solution and given to rats in drinking water for 10 weeks, in either the presence or absence of ALA. Ventura et al. examined many soft drinks with varied concentrations of HFCS and determined sugar ranges from 7% to 15% according to the sugar content [16–18]. ALA was given orally for the last 6 weeks of the experiment because reduction of insulin sensitivity occurred at approximately the fourth week during corn syrup consumption [19,20]. At the end of the experiment, 24 h after the last ALA administration, blood samples were collected from the tail vein to determine the serum glucose, amylase, and lipase levels, and then rats were euthanized by cervical dislocation. After the abdominal incision, pancreatic tissue samples were taken. One half of partitioned pancreatic tissues were placed in formaldehyde solution for histopathological and immunohistochemical examinations. The other half of the tissues were homogenised and kept at −80 °C for biochemical studies.

Biochemical analysis

Pancreas samples were collected for biochemical analyses. Tissues were homogenised in a motor-driven tissue homogeniser (IKA Ultra-Turrax T25 Basic; Labortechnic, Staufen, Germany) and sonicator (UW–2070 Bandelin Electronic, Germany) with phosphate buffer (pH 7.4). Unbroken cells, cell debris and nuclei were sedimented by centrifugation at 10,000 *g* for 10 min. Catalase (CAT) activity, protein and MDA levels were measured using the method described by Aebi [21], Bradford et al. [22] and Draper and Hadley [23], respectively. An autoanalyser (Beckman Coulter AU680, Brea, California, USA) was used for analysing the serum glucose, amylase and lipase levels.

Histopathological analysis

Tissue samples were collected during the necropsy and fixed in 10% buffered formalin. After routine processing, tissues were embedded in paraffin, sectioned into 5-μm thickness, stained with haematoxylin–eosin (HE) and examined microscopically. All of these preparations were performed on blinded samples. Microscopic changes were examined in 10 different areas in a rat. The histopathological changes were scored as (0) no structural damage; (+1) minimal damage; (+2) moderate damage; and (+3) severe damage according to research article of Abdel-Wahhab et al. [24].

Immunohistochemical examination

All antibodies were purchased from Abcam, Cambridge, UK, and used in 1/100 dilution. Selected tissue sections were immunostained by active caspase-3 [Anti-Caspase 3 antibody (ab4051)], insulin [Anti-insulin + Proinsulin antibody (D6C4) ab8304] and glucagon [Anti-glucagon antibody, ab8055] expression in pancreatic tissue sections according to the manufacturer's instructions.

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