The Effects of Oral Vitamin E on Induction and Consequence of Experimental Diabetes Mellitus in Rats

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Abstract: Streptozotocin destroys the β-cells of pancreas by generation of reactive oxygen species and vitamin E has documented antioxidant properties. To evaluate the preventive effect of vitamin E on induction of diabetes by streptozotocin and effect of oral vitamin E consumption on carbohydrate and lipid metabolism, forty male Wistar rats divided randomly to control, E₁, E₂ and E₃ groups. The diet of E₁, E₂ and E₃ groups were supplemented with 1, 2 and 4 g kg⁻¹ of vitamin E, respectively. Four days later all rats were made diabetic by IP injection of 45 mg kg⁻¹ streptozotocin and blood glucose was measured 72 h later to determine the severity of blood glucose elevation. Glycosylated hemoglobin, triglyceride, total cholesterol and HDL-c were measured and LDL-c and VLDL-c calculated in plasma of 6 diabetic rats with glucose more than 200 mg dl⁻¹ in each groups 21 days after streptozotocin injection. Vitamin E had no effect on diabetes induction by streptozotocin, but elevation of glycosylated Hb and reduction of LDL-c in group E₁, were significant. Vitamin E also increased HDL-c although it was not statistically significant. We suggest that oral vitamin E consumption may have some beneficial effect on the correction of lipid metabolism disorders of diabetes, although it may worsen carbohydrate metabolism in mild diabetes.

Key words: Glucose, glycosylated Hb, lipid, HDL-c and LDL-c

INTRODUCTION

Diabetes mellitus is a disease characterized by hyperglycemia and its complications have been attributed to glucose metabolism. On the other hand hyperlipidemia has been frequently observed in diabetes mellitus (Hirano et al., 1991; Brownlee et al., 1988; Nikkila and Kekki, 1993).

Streptozotocin (STZ) is used to induce experimental diabetes by selectively destroying pancreatic β-cells (Szkudelski, 2001). STZ is taken up by pancreatic β-cells via glucose transporter GLUT2 (Schulze et al., 1994). STZ was found to generate Reactive Oxygen Species (ROS), which also contribute to DNA fragmentation and evoke other deleterious changes in the cells (Takasu et al., 1991). The inhibition of xanthine oxidase by allopurinol restricts the cytotoxic effect of STZ in vitro. Pretreatment of β-cells with this inhibitor prevented the STZ-induced decrease of insulin secretion (Nukatsuka et al., 1990). Therefore, intracellular antioxidants attenuate STZ toxicity (Szkudelski, 2001).

All tissues in the body contain adequate amounts of antioxidants to protect them against the toxic actions of free radicals. A deficiency of these antioxidants can result in tissue and organ damage (Krishna and Das, 2001). Roza et al. (1995) showed that pancreatic antioxidant enzymes such as SOD, glutathione peroxidase and catalase are low in normoglycemic, diabetic-prone BB rats compared with the low-risk group. This idea is supported by the observation that antioxidants such as vitamin E and probucol can prevent alloxan-induced diabetes in rats, furthermore superoxide dismutase and catalase protected β-cells of isolated pancreatic islets against alloxan cytotoxicity, as did the hydroxyl radical scavengers Dimethyl Sulfoxide (DMSO) and butanol (Jorns et al., 1999; Slozim et al., 1983; Tibaldi et al., 1979).

Diabetes produces myocardial dysfunction that accelerates cardiovascular morbidity and mortality (Wold et al., 2001). Hyperglycemia and dyslipidemia have been shown to affect physiological changes in the vasculature leading to atherosclerosis (Kaur et al., 2002). Diabetics and experimental animal models exhibit high oxidative stress due to persistent and chronic
hyperglycemia, thereby depleting the activity of the antioxidative defence system and promoting the generation of free radicals (Hong et al., 2004). There is evidence suggests that antioxidants, especially vitamin E, have potential benefits with respect to cardiovascular disease (Harris et al., 2002).

It is well known that vitamin E, a lipid soluble and antioxidant vitamin protect unsaturated fatty acid, a main component of cell membrane, from the attack of oxygen-derived free radicals. However, we do not know that consumption of antioxidant vitamins have any effect on induction of diabetes mellitus by STZ. On the other hand, the effect of vitamin E consumption on the carbohydrate and lipid metabolism of diabetics is controversial. We designed this study to evaluate the effects of different doses of vitamin E on the induction of diabetes mellitus in STZ-rats and to assess the effect of vitamin E on some lipid parameters important in cardiovascular disease.

MATERIALS AND METHODS

In the laboratory animal research center of veterinary school in Shahid Chamran University forty male Wistar rats (approximately 195 g) were divided randomly to four groups, control, E_1, E_2 and E_3. The control group was fed with commercial rat diet and the diet of group E_1, E_2 and E_3 were supplemented with 1, 2 and 4 g kg^{-1} of vitamin E, respectively. The animal room temperature was 23±1°C and rats were subjected to 12 h dark/12 h light cycle. The study was done at last May and all of animal manipulation and sample collection were done from 8-10 am.

Rats were made diabetic by IP injection of 45 mg kg^{-1} body weight STZ, 4 days after the beginning of vitamin consumption. To compare the amount of blood glucose elevation in different groups, the blood glucose was measured by glucometer (Glucocare, Italy) in tail vein blood 72 h after the injection of STZ (n = 10 in each group). Six rats with blood glucose greater than 200 mg dL^{-1} were maintained in each group and others omitted from the remaining part of the study. Blood samples were collected by heart puncture, 21 days after STZ injection under deep inhalation anesthesia by chloroform. Blood were mixed with EDTA, as an anticoagulant and centrifuged at 3000 rpm for 10 min, to separate blood cells and plasma. Plasma was freeze at -20°C until biochemical assay. RBCs were washed immediately three times with normal saline and glycylated hemoglobin was measured by a commercial kit (Mahsaryan) by cyanomethemoglobin method one day later.

The Total Cholesterol (TC), HDL-cholesterol and triglycerides (TG), were measured by an enzymatic assay commercial kits (Zyst-chimi). LDL-c and VLDL-c were calculated by the Friedewald formula (Friedewald et al., 1972):

\[
\text{VLDL-c} = 7.5 \times \text{TG} \\
\text{LDL-c} = \text{TC} - \text{HDL-c} - \text{VLDL-c}
\]

Results obtained were expressed as the means±SEM. Data were analyzed by one way ANOVA with Tukey post hoc test. The analyses were performed using the Sigmathat software.

RESULTS AND DISCUSSION

Blood glucose of rats 72 h after STZ injection was not statistically different (Table 1). Glycylated hemoglobin increased by vitamin E consumption and it was statistically greater in group E_3 in comparison with control group (Table 1).

TC and LDL-c decreased and HDL-c increased in diabetic rats by vitamin E consumption, but only the reduction of LDL-c was statistically significant in group E_3 vs. control group (Table 2).

Table 1: Blood glucose level 72 h after streptozotocin injection and glycosylated haemoglobin 3 week after diabetes induction in control, E_1, E_2 and E_3 groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose (mg dL^{-1})</th>
<th>Glycosylated Hb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 10)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>Control</td>
<td>240.7±35.66</td>
<td>7.87±0.54*</td>
</tr>
<tr>
<td>E_1</td>
<td>224.9±35.77</td>
<td>8.05±0.40</td>
</tr>
<tr>
<td>E_2</td>
<td>239.3±45.63</td>
<td>8.62±0.11</td>
</tr>
<tr>
<td>E_3</td>
<td>258.5±44.30</td>
<td>9.88±0.66*</td>
</tr>
</tbody>
</table>

* p<0.05

Table 2: Triglyceride, total cholesterol, HDL-c, LDL-c and VLDL-c in control, E_1, E_2 and E_3 groups (n = 6 in each groups)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Triglyceride</th>
<th>Total cholesterol</th>
<th>HDL-c (mg dl^{-1})</th>
<th>LDL-c</th>
<th>VLDL-c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>94.5±14.07</td>
<td>266.3±13.5</td>
<td>8.85±3.0</td>
<td>238.6±12.47*</td>
<td>18.87±2.81</td>
</tr>
<tr>
<td>E_1</td>
<td>100.0±18.50</td>
<td>248.3±10.60</td>
<td>8.13±0.46</td>
<td>220.2±7.75</td>
<td>20.00±3.60</td>
</tr>
<tr>
<td>E_2</td>
<td>63.6±11.69</td>
<td>227.0±6.81</td>
<td>10.25±0.99</td>
<td>204.6±7.98</td>
<td>12.70±2.33</td>
</tr>
<tr>
<td>E_3</td>
<td>108.8±22.00</td>
<td>227.0±13.43</td>
<td>9.85±0.38</td>
<td>195.4±12.03*</td>
<td>21.70±4.51</td>
</tr>
</tbody>
</table>

* p<0.05
Tsujinaka et al. (2005) showed that diet high in lipid hydroperoxide by vitamin E deficiency accelerate glucose intolerance through impairments of both sensitivity and secretion of insulin. Srinivasan et al. (2005) reported that combination of high fat diet-fed and low dose STZ-treated rats serves as an alternative animal model for type 2 diabetes. Pretreatment of pancreatic β-cells with allopurinol (xanthine oxidase inhibitor) prevented the STZ-induced decrease of insulin secretion in vitro (Nukatsuka et al., 1990). In present study, vitamin E had no protective effects against STZ-diabetes induction.

Prior administration of SOD, catalase, monomethyl, dimethyl, or monoethyl urea can block alloxan-induced cytotoxic action on pancreatic β-cells (Jorns et al., 1999; Gandy et al., 1982; Granovist et al., 1979; Tibaldi et al., 1979). According to Nishizono et al. (2000) pre-treatment with tert-butylhydroquinone, a synthetic antioxidant reduced the severity of STZ-diabetes in rats and Sloum et al. (1983) demonstrated that vitamin E prevent alloxan-induced diabetes in rats. All these agents can quench either a superoxide anion or a hydroxyl radical and thus are probably able to protect against alloxan-induced damage to the pancreatic β-cells both in vitro and in vivo. This suggests that alloxan-induced damage to the pancreatic B cells is a free-radical-dependent process. Oils rich in ω-3 and ω-6 long-chain fatty acids also can significantly attenuate alloxan-induced diabetes in these experimental animals (Krishna and Das, 2001).

Beside the results obtained from this study, vitamin E with current doses used in this study is not effectively potent to inhibit the toxicity of STZ in β-cells of pancreas.

In present study carbohydrate metabolism has been worsen by vitamin E consumption in diabetic rats. Studies on the effects of vitamin E on carbohydrate metabolism are controversial. Skrha et al. (1997) reported an increase in glycosylated hemoglobin after daily administration of 600 mg of vitamin E during 3 months in obese type 2 diabetes. However, in most of the studies vitamin E has no effect (Garg et al., 2005; Craven et al., 1997) or has beneficial effect (Maning et al., 2004; Al Shamsi et al., 2004) on carbohydrate metabolism. The severity of diabetes, dose, period and route of vitamin E consumption and different methodologies may be an explanation for these controversies. Signaling by insulin requires autophosphorylation of the insulin receptor kinase (Hubbard, 1997). In intact cells high concentrations of hydrogen peroxide on the order of 1 mM perversandate and thiol-reactive agents induce insulin-like effects in the absence of insulin (Heftuz et al., 1990). Lower and physiologically relevant concentrations (<0.1 mM) of hydrogen peroxide are not sufficient to trigger the autophosphorylation of the insulin receptor in the absence of insulin, but do enhance the response to 100 nM insulin (Schmid et al., 1999), indicating that the redox signal has a coregulatory signal in insulin receptor activation under physiologically relevant conditions (Droge, 2002). However, low dose of STZ injection produced a mild hyperglycemia in our study and may be vitamin E consumption (as an antioxidant) counteracted with low level of insulin in receptor autophosphorylation.

During the course of type 1 diabetes, there was a serum lipid profile disturbance characterized by an increase of blood cholesterol (Harris et al., 2002). According to Goikosu and Mostafazadeh (2003), plasma and tissue cholesterol concentrations in hypercholesterolemic animals did not appear to be affected by vitamin E treatment. However, Komarat et al. (1985) observed decreased total cholesterol, LDL-c and VLDL-c and an increase in HDL-c, with high doses of vitamin E in rabbits. Jeon et al. (2005) suppressed an increase in plasma total cholesterol and LDL-c in high cholesterol diet rabbits by vitamin E. Vieira da Costa and Vianna (2005) showed an increase of HDL-c and a decrease of LDL-c, but the concentrations of triglycerides and total cholesterol did not change.

Present data suggests that oral vitamin E consumption may have some beneficial effect on the correction of lipid metabolism disorders of diabetes although further studies should be taken on vitamin E supplementation as an alternative therapy to treat lipid disorder for diabetic patients and more attention on the effect of vitamin E on carbohydrate metabolism in mild diabetes.

REFERENCES


