Therapeutic Role of Coenzyme Q10 in Brain Injury during Experimental Diabetes

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ABSTRACT
Diabetes mellitus is a complex disease associated with peripheral and central complications; these complications include retinopathy, nephropathy and neuropathy. Antioxidant therapies may be useful in decreasing the risk of diabetic complications. This study carried out to investigate the role of coenzyme Q10 (CoQ10) in decreasing oxidative stress as well as attenuating brain injury in diabetic rats. Sixty male albino rats were used in this study and classified into four groups (fifteen rats in each group) including: control, CoQ10, diabetic and treated groups. Fasting blood sugar was determined. Brain malondialdehyde (MDA), advanced oxidation protein products (AOPP), nitric oxide (NO) and superoxide dismutase (SOD) were estimated by colorimetric methods. In addition, brain CoQ10 was estimated by HPLC method using C18 column and UV detector at 275 nm. Brain oxidant parameters were significantly increased in diabetic group concomitant with a reduction in brain antioxidants, while CoQ10 supplementation in treated group attenuated them. We concluded that oral CoQ10 may be a viable antioxidant strategy for neurodegenerative disease in diabetes mellitus.

INTRODUCTION
Diabetes mellitus is a heterogeneous disease characterized by chronic hyperglycemia and requires long-term management. Chronic hyperglycemia in diabetes can lead to various complications, affecting the central nervous system (CNS) (Kumar et al., 2010).

The development of diabetes associated complications in the nervous system was found to be directly attributed to the increased glucose concentration (Ibrahim, 2008), this causes autoxidation of glucose; glycation of proteins and activation of polyol metabolism in brain (Osawa and Kato, 2005). These changes accelerate the generation of reactive oxygen species to increase oxidative modifications of lipids, DNA, and proteins in various tissues. Brain cells are particularly vulnerable to oxidative stress, resulting from increased production of reactive oxygen species as well as increased lipid peroxidation in diabetes (Kumar et al., 2010). Advanced oxidation protein products (AOPP) are the products of plasma protein oxidation, especially oxidation of albumin. Because of its rapid response to changes, it is thought to be suitable biochemical marker for measuring short-term changes in oxidative stress. This marker is increased in the inflammatory conditions such as diabetes, atherosclerosis and renal failure. It is formed during oxidative stress by myeloperoxidase in activated neutrophils and is accumulated in biological systems and thus causing damage to biological membranes (Sharada et al., 2012). Complications of diabetes include disease of the kidney, blood vessels, eyes (Elseweidy et al., 2009), that is a reason to hope that long term antioxidant therapies may be useful in decreasing the risk of diabetic complications (Modi et al., 2006).

Coenzyme Q10 is an endogenous antioxidant that scavenges free radicals directly, inhibits biomolecule oxidation and affects antioxidants in vivo (Modi et al., 2006). Although its structural characteristic allows it to diffuse into the membrane phospholipids bilayer, where it serves as an electron transfer intermediate in the mitochondrial respiratory chain, its reduced form is a powerful antioxidant. Coenzyme Q10 regulates oxidative phosphorylation and prevents lipid peroxidation (Modi et al., 2006).

Rauscher et al. (2001) observed various effects of coenzyme Q10 as antioxidant substance. In addition, the reduction of blood pressure and insulin resistance in hypertensive patients with coronary artery disease during CoQ10 treatment was mentioned (Modi et al., 2006).
In this light, this study carried out to investigate the role of coenzyme Q10 (CoQ10) in decreasing oxidative stress as well as attenuating brain injury in diabetic rats.

MATERIALS AND METHODS

Materials

Chemicals

Coenzyme Q10 HPLC standard and streptozotocin were purchased from Sigma Aldrich medical company St.Louis USA. CoQ10 capsules were purchased from Arab Company for Pharm. & Medicinal Plants (MEPACO-MEDIFOOD) Enshas-Sharkeya-Egypt. All other chemicals were of HPLC grade and purchased from Sigma.

Experimental Animals

Sixty male albino rats weighing 180-200 g were obtained from the animal house of National Research Center, Giza, Egypt., and fed a standard commercial diet (control diet) purchased from the Egyptian company of oils and soaps. Water was available ad libitum for acclimatization before starting the experiment; rats were kept under constant environmental conditions at room temperature. The guidelines of the ethical care and treatment of the animals followed the regulations of the ethical committee of the National Research Center.

Methods

Induction of Diabetes

Streptozotocin (STZ) was dissolved in 50 mM sodium citrate solution (pH adjusted at 4.5) containing 150 mM NaCl. The solution (6.0 mg/100g body weight) was subcutaneously administrated in rats; fasting blood sugar was estimated after 3 days to confirm the development of diabetes mellitus (Uchiyama & Yamaguchi 2003).

Experimental design

Animals were divided into four groups (15 rats in each group) as follows:

- **Group I**: healthy rats, received corn oil (10 mg /Kg b.w./day) orally.
- **Group II**: healthy rats, received Co Q10 (10 mg /Kg b.w./day) orally.
- **Group III**: diabetic rats, received corn oil (10 mg /Kg b.w./day) orally.
- **Group IV**: diabetic rats, received Co Q10 (10 mg /Kg b.w./day) orally (Rauscher et al., 2001).

After the experimental period (8 weeks), animals were kept fasting for 12 hours before blood sampling, blood was withdrawn from the retro-orbital venous plexus of the eye using capillary tubes and collected in tubes contain sodium fluoride for blood glucose estimation. Brain was removed quickly on ice, homogenized and prepared for estimation of other biochemical parameters.

Preparation of tissue homogenate

The frozen tissues were cut into small pieces and homogenized in 5 ml cold buffer (0.5 g of Na₂HPO₄ and 0.7 g of NaH₂PO₄ per 500 ml deionized water (pH 7.4) per gram tissue, then centrifuged at 4000 rpm for 15 minutes at 4°C and the supernatant was removed and used in estimation of chemical parameters (Manna et al., 2005).

Biochemical assays

Fasting blood sugar was estimated by colorimetric method using commercial kit purchased from Vitro Scient, Egypt based on the method described by Trinder (1969). Oxidant/antioxidant parameters were determined by commercial kits; brain malonenedialdehyde (MDA) was measured by the method described by Uchiyama and Mihara (1978), superoxide dismutase (SOD) was measured according to the method of Nishikimi et al. (1972) and nitric oxide (NO) was estimated according to the method described by Montgomery and Dymock (1961). All kits were purchased from BioMed.Diagnostics. Brain advanced oxidation protein products (AOPP) as a marker of oxidative stress was measured by ELISA kit as described by Deschamps-Latscha et al (2005).

Determination of brain coenzyme Q10

Brain CoQ10 was estimated by using high performance liquid chromatography (HPLC) system, Agilent technologies 1100 series, equipped with a quaternary pump (Quat. pump, G131A model).

Homogenate samples were treated with 2ml ethanol and CoQ10 was extracted with 5ml hexane, after vigorous shaking, 4ml of hexane layer were dried under nitrogen gas and the residue was dissolved in 400 µl ethanol.

HPLC condition

20 µl from the solution were injected in HPLC; separation was achieved on reversed phase column (C18, 25 9 0.46 cm i.d. 5 µm).

The mobile phase consisted of ethanol/methanol 70/30 (v/v) and was delivered at a flow rate of 2 ml/min. UV detection was performed at 275 nm. Serial dilutions of standards were injected, and their peak areas were determined. A linear standard curve was constructed by plotting peak areas versus the corresponding concentrations. The concentration in samples was obtained from the curve.

Statistical analysis

All data were expressed as mean ± standard error. Data were analyzed using one-way ANOVA using SPSS (Version 12). Duncan’s new multiple-range test was used to assess differences between means. Pearson's correlation test was used to assess correlations between means. A significant difference was considered at the level of P < 0.05.
RESULTS AND DISCUSSION

Diabetes mellitus is a complex disease associated with peripheral and central complications. These complications include retinopathy, nephropathy and neuropathy. Several investigations have confirmed the role of oxidative stress in developmental diabetic mediated disorders, possibly via the formation of free radicals (Hussein et al., 2012). In this study we aimed to investigate the role of coenzyme Q10 (CoQ10) in decreasing oxidative stress as well as attenuating brain injury in diabetic rats. In this study, there was a significant increase in blood glucose level in diabetic group compared to control. In addition, there was a marked increase in brain MDA, NO and AOPP levels in diabetic group concomitant with a reduction in SOD and CoQ10 compared to control group (fig.1-2& tab.1).

In agreement, Singh and Niaz (1999) indicated that STZ induced diabetes and oxidative stress due to the generation of free radicals which promotes lipid peroxidation; leading to a reduction in coenzyme Q10 content and inactivation of respiratory chain enzymes (Matthews et al., 1998).

The elevation of NO and the reduction in SOD level in diabetic rats were found by Ibrahim et al. (2008) who stated that NO level as a biomarker of oxidative stress showed significant increase in experimental diabetic rats as well as significant decrease in SOD activity.

Oxidative stress and changes in nitric oxide formation or action play major roles in the onset of diabetic complications. Nitric oxide synthase oxidizes arginine to citrulline in the presence of biopterin, NADPH and oxygen. Increased oxidative stress and subsequent activation of the transcription factor Necrotic Factor Kappa-B (NF-κB) have been linked to the development of late diabetic complications. NF-κB enhances nitric oxide production, which is believed to be a mediator of islet beta-cell damage. Nitric oxide may react with superoxide anion radical to form reactive peroxyl nitrite radicals (Maritim et al., 2003). In the current study, CoQ10 supplementation effectively decreased oxidative stress. Also, a negative correlation was found between blood glucose and brain CoQ10 (fig.3) indicating the beneficial effect of supplemented CoQ10 in increasing brain contents of CoQ10 in addition to the reduction of oxidative stress as well as blood glucose level. These results were in agreement with Rauscher et al. (2001) who indicated that coenzyme Q10 ameliorated some of the diabetes-induced changes in oxidative stress. Wadsworth et al. (2010) indicated that oxidative damage to brain proteins was attenuated by CoQ10 supplementation, so oral CoQ10 may be a viable antioxidant strategy for neurodegenerative disease, including Alzheimer’s disease. The suppression of oxidative damage in the brains of CoQ10-fed rats may be explained in a number of possible ways; one possibility is that the ratio of reduced to oxidized CoQ10 might be favorably altered by CoQ10 supplementation, resulting in an antioxidant effect. It is also possible that systemic CoQ10 is able to achieve antioxidant effects by an indirect mechanism for example by, restoration of other brain antioxidants (Wadsworth et al., 2010).

These findings derive importance from the fact that increased brain oxidative stress has been linked to the development of neurodegenerative diseases (Halliwell, 2001).

Table 1: Brain oxidant/antioxidant parameters in different studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>AOPP ng/g.tissue</th>
<th>MDA nmol/g.tissue</th>
<th>NO nmol/g.tissue</th>
<th>SOD U/g.tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>mean ± SE</td>
<td>56 ± 1.0</td>
<td>4.8 ± 1.3</td>
<td>69 ± 0.002</td>
</tr>
<tr>
<td>Coenzyme Q10</td>
<td>mean ± SE</td>
<td>56.5 ± 1.2</td>
<td>4.6 ± 0.9</td>
<td>68 ± 0.001</td>
</tr>
<tr>
<td>Diabetic</td>
<td>mean ± SE</td>
<td>60.5 ± 1.1</td>
<td>62.8 ± 4.7</td>
<td>88 ± 0.002</td>
</tr>
<tr>
<td>Treated</td>
<td>mean ± SE</td>
<td>58 ± 1.8</td>
<td>38.6 ± 1.1</td>
<td>74 ± 0.002</td>
</tr>
</tbody>
</table>

Significant p value < 0.05
a = significant difference compared to Control group
b = significant difference compared to Diabetic group
n = number of animals = 15

![Fig. 1: Blood glucose levels in different studied groups.](image-url)
CONCLUSION

Coenzyme Q10 seems to be a highly promising and beneficial compound in protecting the diabetic rats against oxidative stress induced brain dysfunction.

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