Protective antioxidant effect of vitamins C and E in streptozotocin induced diabetic rats

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We have investigated the protective effect of vitamin C and E together supplementation on oxidative stress and antioxidant enzyme activities in the liver of streptozotocin-induced diabetic rats, unsupplemented diabetic and control rats. We also determined the levels of both the vitamins and oxidative stress in plasma. Vitamin supplementation in diabetic rats lowered plasma and liver lipid peroxidation, normalised plasma vitamin C levels and raised vitamin E above normal levels. In liver, the activity of glutathione peroxidase was raised significantly and that of glutathione-S-transferase was normalised by vitamin supplementation in diabetic rats. The levels of lipid peroxidation products in plasma and liver of vitamin-supplemented diabetic rats and activities of antioxidant enzymes in liver suggest that these vitamins reduce lipid peroxidation by quenching free radicals.

Reactive oxygen species are an important part of the defence mechanisms against infection, but excessive generation of free oxygen radicals may damage tissue. Formation of lipid peroxides by the action of free radicals on unsaturated fatty acids has been implicated in the pathogenesis of atherosclerosis and vascular diseases\textsuperscript{1,2}. Diabetic patients have an increased incidence of vascular disease and it has been suggested that free radical activity increased in diabetes\textsuperscript{3,4}. Glucose itself and hyperglycemia-related increased protein glycosylation are important sources of free radicals\textsuperscript{5,6}. Elevated glucose causes slow but significant non-enzymatic glycosylation of proteins in diabetes\textsuperscript{7}. Kumari and Sahib have shown glycosylation to increase linearly with duration of hyperglycemia\textsuperscript{8}.

Previous studies have examined the effect of intake of vitamin C and E, nutrients with antioxidant properties \textit{in vivo} on glycosylated hemoglobin concentrations in human subjects\textsuperscript{9,12}. Cieriello \textit{et al.}\textsuperscript{13} found that large doses of all-rac-\textgreek{t}ocopherol orally administered to individuals with diabetes, lowered glycosylated hemoglobin concentrations. Davie \textit{et al.}\textsuperscript{14} observed a similar pattern when large doses of ascorbic acid were orally administered to non-diabetic subjects. The mechanism for this reduction in non-enzymatic glycosylation of proteins may be related to antioxidant properties of these nutrients whereby they quench free radical intermediates formed during early stages of glycation. Our previous findings on the antioxidant status of diabetic rats supplemented with vitamin C and vitamin E\textsuperscript{5,16} prompted us to study the antioxidant status of diabetic rats supplemented with both of these vitamins (C and E) together.

Materials and Methods
Streptozotocin, vitamin E and vitamin C were from the Sigma Chemical Company, St. Louis Mo, USA (kindly supplied by professor Ronal R. MacGregor, Department of Anatomy and Cell Biology, University of Kansas, Medical Centre, Kansas City, Kansas, USA). All other reagents used were of analytical grade.

Female Sprague-Dawley rats of 100-125 g body weight were obtained from the Central Animal House of Panjab University of Chandigarh. Diabetics were induced by single intraperitoneal injection of 75 mg/kg body weight of streptozotocin in citrate buffer (pH 4.5). Induction of diabetes was confirmed by determining plasma glucose levels after one week of injection.

Diabetic animals were divided into two groups, streptozotocin (SZ) and streptozotocin + vitamins (SZ+vits.). The third control group consisted of animals injected with citrate buffer alone. Each group contained six animals. The SZ + vitamins group rats received vitamins for five weeks beginning after one week of streptozotocin injection. Ascorbic acid (60 mg) was given daily in drinking water and vitamin E (200 mg/kg body weight) in soya oil was given intraperitoneally twice a week. The dosages for vitamin

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C and E were arrived at as described previously. Animals were housed in plastic cages with free access to pellet diet. After six weeks of diabetes induction animals were bled under light anaesthesia from retroliberal plexus and then killed by cervical dislocation to obtain liver tissues. Plasma lipid peroxidation, vitamin C and vitamin E levels were determined. The levels of lipid peroxidation in liver mitochondrial fraction and activities of enzymes, superoxide dismutase (SOD) (EC 1.5.1.1), catalase (CAT) (EC 1.11.1.6), glutathione peroxidase (GPx) (EC 1.11.1.9), glutathione reductase (GR) (EC 1.6.4.2), glutathione-S-transferase (GST) (EC 2.5.1.18) and glucose-6-phosphate dehydrogenase (G6PD) (EC 1.1.1.49) were determined in liver postmitochondrial supernatant. The liver homogenates were prepared in respective buffers used for each enzyme assay and then centrifuged at 1,000 g for 10 min at 4°C to remove nuclei and cell debris. Supernatants were again centrifuged at 10,000 g for 15 min. The mitochondrial pellet was used to estimate lipid peroxidation and the post mitochondrial supernatants were used to assay activity of various enzymes.

Statistical analyses of data were done using Student’s t-test. Results are expressed as means ± SD. Significance was chosen as P < 0.05.

**Results**

The levels of plasma vitamin C were significantly (P<0.05) higher in diabetic rats than in control rats, the increment was non-significant in vitamin supplemented diabetic rats (Table 1) as compared with control rats. The levels of plasma vitamin E were significantly lower (P<0.05) in diabetic rats as compared with control rats whereas in supplemented diabetic rats the levels were significantly higher (P<0.001) as compared with unsupplemented rats; P<0.01 as compared with control rats (Table 1). The level of blood glucose was not significantly different in supplemented diabetic rats as compared to unsupplemented diabetic rats (Table 1).

Lipid peroxidation in liver was (non-significantly) higher as in the terms of thiobarbituric acid reactive substances (TBARS) in diabetic rats (0.71 ± 0.08) as compared with control rats (0.62 ± 0.16). The levels of lipid peroxidation were significantly lower in plasma (P<0.001) (Table 1) and liver (0.49 ± 0.17) (P<0.05) of supplemented diabetic rats as compared with unsupplemented diabetic rats (0.71 ± 0.08).

The activities of superoxide dismutase, catalase and glutathione reductase were not significantly different in the liver of either diabetic group (Table 2). The activity of liver glutathione peroxidase was significantly (P<0.001) lower in both the diabetic groups as compared with control group. However, supplemented diabetic rats showed significantly (P<0.01) higher activity as compared to unsupplemented diabetic rats. The activity of liver glutathione-S-transferase, which was significantly (P<0.05) higher in unsupplemented diabetic rats than in control rats, was normalised in supplemented diabetic rats. The activity of liver glucose-6-phosphate dehydrogenase was significantly (P<0.05) lower in supplemented diabetic rats as compared with both control and unsupplemented diabetic rats.

**Discussion**

The levels of plasma and liver lipid peroxidation in the vitamin supplemented diabetic rats indicate that these vitamins can reduce lipid peroxidation significantly. Both vitamin C and E are known to prevent detectable lipid peroxidation. Our previous results...

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**Table 1** - Levels of plasma TBARS, vitamin C and vitamin E in control, diabetic (SZ) and diabetic supplemented with vitamins (SZ + Vits.) rats after six weeks of diabetes and five weeks of vitamin supplementation (n=6)

<table>
<thead>
<tr>
<th>Assay</th>
<th>Control</th>
<th>SZ</th>
<th>SZ + Vits.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>85.7±9.6</td>
<td>242±28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>208±24&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>TBARS (nmol/mL)</td>
<td>3.8±2.1</td>
<td>4.8±1.1</td>
<td>2.2±0.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin C (mg/dL)</td>
<td>0.28±0.02</td>
<td>0.68±0.12</td>
<td>0.40±0.06</td>
</tr>
<tr>
<td>Vitamin E (mg/dL)</td>
<td>0.16±0.07</td>
<td>0.07±0.04</td>
<td>0.03±0.02</td>
</tr>
</tbody>
</table>

<sup>a</sup>P<0.05; <sup>b</sup>P<0.01 as compared to control
<sup>c</sup>P<0.01 as compared to SZ

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**Table 2** - Activities of enzymes. SOD, CAT, GPx, GR, GST and G6PD in liver of control, diabetic (SZ) and diabetics supplemented with vitamins (SZ + Vits.) rats after six weeks of diabetes and five weeks of vitamin supplementation (n=6)

<table>
<thead>
<tr>
<th>Enzymes (units/mg protein)</th>
<th>Control</th>
<th>SZ</th>
<th>SZ + Vits.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD</td>
<td>5.0±1.58</td>
<td>5.19±1.67</td>
<td>4.46±1.59</td>
</tr>
<tr>
<td>CAT</td>
<td>4.58±1.16</td>
<td>4.79±1.63</td>
<td>4.81±1.07</td>
</tr>
<tr>
<td>GPx</td>
<td>1.6±0.62</td>
<td>1.00±0.12</td>
<td>0.96±0.07</td>
</tr>
<tr>
<td>GR</td>
<td>0.46±0.07</td>
<td>0.36±0.08</td>
<td>0.43±0.07</td>
</tr>
<tr>
<td>GST</td>
<td>15.35±3.32</td>
<td>27.8±6.6</td>
<td>26.4±4.02</td>
</tr>
<tr>
<td>G6PD</td>
<td>0.32±0.14</td>
<td>0.25±0.10</td>
<td>0.13±0.04</td>
</tr>
</tbody>
</table>

<sup>a</sup>units/mg protein
<sup>b</sup>P<0.05; <sup>c</sup>P<0.01 as compared to control
<sup>d</sup>P<0.05; <sup>e</sup>P<0.01 as compared to SZ
ports have shown that animals separately supplemented with vitamin C and vitamin E had lowered hepatic lipid peroxidation. However, vitamin C alone was unable to reduce plasma lipid peroxidation significantly.

Similarly, neither vitamin supplemented alone prevented depletion of plasma vitamin E. However, our results show that a combination of the vitamins raised plasma vitamin E levels in diabetic rats above control levels. This indicates that vitamin E is used in combating free radicals and if vitamin C is present, vitamin E levels are preserved. Frei has previously shown the ability of vitamin C to preserve the levels of other antioxidants in human plasma. Also vitamin C regenerates vitamin E from its oxidized form.

The significantly elevated levels of plasma vitamin C in the unsupplemented diabetic rats indicate impaired uptake of ascorbic acid, by cells. Hypoinsulinemia and/or hyperglycemia inhibit ascorbic acid cellular transport. As the chemical structure of ascorbic acid is similar to that of glucose, it shares the membrane transport system with glucose and hence competes with it for transport. The non-significant decrease in plasma ascorbic acid in the vitamin-supplemented diabetic group in our study indicates that supplemented vitamin C can raise circulating levels of ascorbic acid to high concentrations to compete with glucose for transport.

The lower levels of lipid peroxidation products and higher activity of liver glutathione peroxidase in supplemented diabetic rats as compared with unsupplemented diabetic rats indicate that these vitamins prevent a decrease of enzyme activities by some mechanism. Inhibition of protein glycosylation by vitamin C and E appears to be one of the possibilities. The lower activity of glucose-6-phosphate dehydrogenase in liver, in supplemented diabetic rats as compared with unsupplemented diabetic rats indicates the inhibition of activities of antioxidant enzymes in the presence of these vitamins.

The significantly lower levels of lipid peroxidation products in plasma and liver of vitamin C plus vitamin E-supplemented diabetic rats and activities of antioxidant enzymes in liver suggest that these vitamins reduce oxidative stress by quenching free radicals.

Acknowledgment
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References
20. Roe J H & Kuehler C A. The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenyl hydrazine derivative of dehydroascorbic acid. J Biol Chem, 147 (1943) 399.