Liver and colon pro- and anti-oxidant enzyme activities in rats after long-term ethylnitrosourea exposure

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Liver and colon pro- and anti-oxidant enzyme activities were investigated in rats treated with ethylnitrosourea (ENU) (i.p.) (4 mg/kg body wt) for 6 months. The pro-oxidant enzymes (NADPH cytochrome c reductase, NADH cytochrome c reductase, NADH cytochrome b5 reductase and cytochrome P-4502E1 and the anti-oxidant enzyme, superoxide dismutase (SOD) exhibited significantly increased activity in liver and colon. Glucose-6-phosphate dehydrogenase (G6PDH) and glutathione-S-transferase (GST) showed enhanced activity in liver, but decreased activity in colon. Glutathione peroxidase (GP) and glutathione reductase (GR) activities were significantly increased in colon, but decreased in liver. Catalase (CAT) activity while showed a significant increase in liver, exhibited only marginal increase in colon. Malondialdehyde (MDA) level was significantly elevated in both tissues.

Nitrosamines are the carcinogenic and mutagenic compounds, which can either be taken from environmental sources or are formed from their precursors such as secondary amines and nitrite in mammalian stomach1. The secondary amines are also present in fish products, cereals, tea, tobacco etc. and also in some medicines2-4.

Ethylnitrosourea (ENU) has been reported to induce tumour in various organs of mammalian species5,6. It is a potent inducer of cellular stress leading to chromosomal aberrations such as point mutations, translocations, deletions, insertions and cell death7-9. It is also a potent cell mutagen due to its alkylating function and induces DNA damage in the cell9,10. Cytochrome P450 enzymes mainly catalyze the activation of nitrosamines. Distribution of various P450 forms in different tissues is important in determining the tissue specificity of different nitrosamines. Cytochrome P4502E1 has been shown to be a key enzyme in the activation of low molecular weight nitrosamines, especially with methyl and ethyl groups11.

Earlier, it has been shown that there is a relation between free radicals, lipid peroxidation, peroxidation products and carcinogenesis. Also, several carcinogenic compounds have been reported to cause cancer by increasing the oxidative stress in the cell12. In this study, we aimed to investigate the relation between cytochrome P-4502E1, pro- and anti-oxidant enzyme activities and lipid peroxidation levels in liver and colon of rats exposed to ENU (i.p.) (4 mg/kg body wt) for 6 months.

All chemicals used were from Sigma Chemical Co. USA. Wistar albino rats (wt 270-300 g each) were used. The experiments were done on two groups, control and experimental, including 10 rats each. The control group was given physiological saline injections, i.p., while the experimental group was given 4 mg/kg ENU (in 10 g/100 ml polyethylene glycol), i.p.13.

The animals were killed by cervical dislocation. The livers were perfused with ice-cold 0.9% saline, weighed and homogenized in 3 vols. at 140 mM NaCl, 40 mM sodium phosphate (pH 7.0) and centrifuged at 15,000 g for 30 min at +4°C. The supernatant obtained was centrifuged at 105,000 g for 60 min at +4°C. In order to obtain pure microsomal pellet, the pellet was resuspended in buffer, centrifuged again at 105,000 g and again resuspended in 0.1 M sodium citrate (pH 7.0), buffer containing 0.1 M KCl glycercol (30%) (v/v)14.

The activities of G6PDH15, GP16, GST17, GR18, CAT19 and SOD20 were determined in the cytosolic fraction and activities of cytochrome P4502E121, NADPH cytochrome c reductase22, NADH cytochrome b5 reductase23 and NADH cytochrome c reductase24 in the microsomal fraction and expressed as units/mg of protein. Liver tissues were used to measure MDA levels25. Protein content of each subcellular fraction was determined as described26. Bovine serum albumin was used as the protein standard. All numerical data are expressed as the
mean ± S.E. and the significance between means was assessed by student’s t test.

A significant increase was obtained in liver and colon pro-oxidant enzyme activities in ENU-treated rats. However, it was observed that microsomal cytochrome P4502E1, which metabolizes nitrosamines was induced (Table 1). Anti-oxidant enzyme activities behaved similarly in both tissues in ENU-treated rats. Liver G6PDH, SOD, CAT and GST activities were significantly increased, compared to control, although GP and GR activities were found to be lower (Table 2). Colon GP, GR and SOD activities were also significantly increased, while G6PDH and GST activities were found to be lower, compared to control. However, CAT activity was not different than the control. MDA level was significantly elevated in both tissues (Table 2).

Earlier, it has been reported that the carcinogenesis is developed by oxidative stress caused carcinogenic agents. Oxidative stress is developed by the accumulation of free oxygen radicals, thus pro-oxidant enzyme systems are induced. However, anti-oxidant enzyme activities behave differently. In lymphoblastic leukemia, prostatic hyperplasia, human cervical carcinoma, laryngeal cancer and renal cell carcinoma, anti-oxidant enzyme activities were found decreased. On the other hand, CAT and GP activities increased in bone metastatic prostate cancer, SOD and GP increased in mammary cancer, whereas CAT decreased in this situation, and mitochondria SOD, GP, GST activities increased in colorectal tumours. It has been suggested that oxidative stress dependent DNA base lesions were responsible for the low anti-oxidant enzyme activities in acute lymphoblastic leukemia and prostate hyperplasia. Therefore, the increased lipid peroxidation and pro-oxidant enzyme activities found in the present study could indicate an increased oxidative stress. The decreased anti-oxidant activities supported the hypothesis of the role of oxidative stress in carcinogenesis. High anti-oxidant enzyme activities in liver and colon may be due to the

<table>
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<tr>
<th>Enzyme activity</th>
<th>Liver</th>
<th>Colorectum</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>ENU</td>
</tr>
<tr>
<td>Cytochrome P4502E1 (nmol/mg protein/min)</td>
<td>0.695±0.042</td>
<td>1.12±0.32*</td>
</tr>
<tr>
<td>NADPH cytochrome c reductase</td>
<td>0.185±0.15</td>
<td>0.219±0.015*</td>
</tr>
<tr>
<td>NADH cytochrome c reductase</td>
<td>0.192±0.013</td>
<td>0.308±0.007*</td>
</tr>
<tr>
<td>NADH cytochrome b5 reductase</td>
<td>9.24±1.02</td>
<td>36.85±6.86*</td>
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*Values statistically significant \( P<0.05 \)
ND, Not determined

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<tr>
<td></td>
<td>Control</td>
<td>ENU</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydrogenase</td>
<td>0.073±0.010</td>
<td>0.086±0.011*</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>3.405±0.0223</td>
<td>2.854±0.215*</td>
</tr>
<tr>
<td>Glutathione reductase</td>
<td>0.271±0.045</td>
<td>0.244±0.031*</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>328±24</td>
<td>456±33*</td>
</tr>
<tr>
<td>Catalase</td>
<td>418±42</td>
<td>734±59*</td>
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<tr>
<td>Glutathione-S-transferase</td>
<td>1.19±0.06</td>
<td>1.67±0.11*</td>
</tr>
<tr>
<td>MDA (nmol/g)</td>
<td>1.52±0.12</td>
<td>2.05±0.18*</td>
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*Values statistically significant \( P<0.05 \)
response of increased reactive metabolite production. High GP, SOD and GR and unchanged CAT in colon may be inadequate to detoxify high levels of H$_2$O$_2$ into H$_2$O. Formation of hydroxyl radicals lead to high MDA concentration.

In a hexachlorobenzene-induced oxidative stress, pro-oxidant activities increased, and anti-oxidant activities decreased, with the exception of GR, which was increased. Also, CAT was found unchanged. It could be seen that anti-oxidant enzyme activities give different response to oxidative stress. In different cancer types, the behaviours of anti-oxidant enzymes could be related to their inhibition effect by DNA damage caused by reactive oxygen metabolites, or their stimulating effect via an increase of the enzyme activities as a response to these metabolites.

In conclusion, it could be suggested that ENU causes elevated levels of free oxygen radicals by inducing pro-oxidant enzymes in both the tissues. Possibly, the free radicals might induce anti-oxidant enzymes, however, this induction cannot prevent cells from lipid peroxidation due to insufficient scavenging of free radicals. These results indicate that the anti-oxidant enzymes are incapable of scavenging the free radicals.

References
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