Transgenic Mice Overexpressing Catalase Specifically in the Heart for Studying Cardiac Oxidative Injury Induced by Copper Deficiency

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Abstract

Copper deficiency causes more salient pathological changes in the heart than in the liver of rats. Although oxidative stress has been implicated in copper deficiency-induced pathogenesis, little is known about the selective toxicity to the heart. Therefore, in an effort to determine the selective cardiotoxicity of copper deficiency, we examined the relationship between the capacity of antioxidant defense system and the copper deficiency induced oxidative damage in the heart compared to that in the liver (Chen et al. 1994). Weanling rats were fed a purified diet deficient in copper (0.4 µg/g diet) or one containing adequate copper (6.0 µg/g diet) for 4 weeks. Copper deficiency induced a two-fold increase in lipid peroxidation in the heart (thiobarbituric assay), but did not alter peroxidation in the liver. The antioxidant enzymatic activities of superoxide dismutase, catalase and glutathione peroxidase were, respectively, 3-, 50- and 1.5-fold lower in the heart than in the liver, although these enzymatic activities were depressed in both organs by copper deficiency. In addition, the activity of glutathione reductase was 4 times lower in the heart than in the liver. The results suggested that a weak antioxidant defense system was responsible for the relatively high degree of oxidative damage in the copper-deficient heart.

Because catalase is a major enzyme involved in detoxification of hydrogen peroxide (H₂O₂) in mammalian cells, the present study was undertaken to determine whether elevation of catalase activity specifically in the heart of transgenic mice could provide protection against oxidative cardiotoxicity. A transgene for overexpression of catalase in the heart was constructed to contain fragments from the rat catalase cDNA ligated behind the alpha cardiac myosin heavy chain promoter. The transgenic mice were identified by using Southern and dot blot, and PCR procedures. Catalase activities and mRNA concentrations in the heart and other organs were measured.

Cardiac catalase activity was analyzed in 6 animals (3 males and 3 females) of each transgenic line and non-transgenic controls. As shown in Figure 1, catalase activity was markedly elevated in the transgenic hearts. This elevation ranged from 2-fold in line 786 to 630-fold in line 784. There was no significant difference (p > 0.10) in the catalase activity between males and females within the same transgenic line (data not shown). The level of mRNA for catalase was also increased in the transgenic heart (data not shown). This overexpression was rather stable as evidenced by consistent results obtained in several assays performed over one year. It is clear that the increased catalase activity results from expression of the transgene. We tested whether the catalase activity is elevated to the same extent in both atria and ventricles. The results showed that the elevated catalase activity was the same in atria and ventricles from 5 representative transgenic mouse lines. We also determined whether the elevated catalase expression was specific to the heart. Catalase activities in liver, kidneys, lungs, and skeletal muscles of the transgenic mice were measured. As shown in Figure 2, catalase activities in all these tissues were the same as controls.

Keywords: antioxidant, catalase, copper deficiency, transgenic mice

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We then determined whether other antioxidant enzyme activities in the transgenic heart were altered because of changes in catalase activity. The activities of superoxide dismutase (SOD), glutathione peroxidase (GSHpx), and glutathione reductase (GR) were measured in the catalase-enriched transgenic hearts. As shown in Table 1, none of these enzyme activities were changed. We also measured the concentrations of two important nonenzymatic antioxidant components, glutathione (GSH) and metallothionein (MT). There was no alteration in either of the two components in the catalase-enriched transgenic hearts (Table I).

These transgenic mice have been shown to be highly protective against oxidative injury in the heart induced by Adriamycin, an important anticancer drug (Kang et al. 1996). This model is thus ideal for the study of the role of catalase in protection against oxidative injury to the heart induced by copper deficiency.

Table 1. Enzyme activities of SOD, GSHpx and GR, and concentrations of GSH and MT in the hearts of control and MyCat transgenic mice.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MyCat</th>
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<tbody>
<tr>
<td>SOD (U/g · wet wt)</td>
<td>8.6 ± 3.7</td>
<td>108.3 ± 13.4</td>
</tr>
<tr>
<td>GSHpx (nmol NADPH/min · mg protein)</td>
<td>29.8 ± 4.4</td>
<td>26.2 ± 3.0</td>
</tr>
<tr>
<td>GR (nmol NADPH/min · mg protein)</td>
<td>14.2 ± 1.7</td>
<td>16.0 ± 1.4</td>
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<tr>
<td>GSH (µg/g · wet wt)</td>
<td>362.2 ± 11.4</td>
<td>356.0 ± 10.1</td>
</tr>
<tr>
<td>MT (µg/g · wet wt)</td>
<td>5.1 ± 0.6</td>
<td>4.3 ± 0.5</td>
</tr>
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</table>

The data were obtained from non-transgenic mice (Control, n = 6) and 5 representative transgenic mouse lines as shown in Figure 4 (line 738, 742, 782, 776, 777). Three mice were used from each of the 5 lines and the data were pooled together to give the average value (MyCat, n = 15).

References

Discussion

Q1. Paul Saltman, University of California at San Diego, CA, USA: I think that this is absolutely spectacular work, but I want to come back to the original problem. Why does copper deficiency increase the oxidative stress and the amount of the peroxide that is in the heart? You haven’t told us that yet. It seems almost counter-intuitive.
A. No, I haven’t told you that yet. I think this model will give you a valuable tool to test that.

Q2. Jim Kirkland, University of Guelph, ON, Canada: Is catalase restricted usually to the peroxisome in the heart, and if you over-express it to that extent, have you forced it out of the peroxisome?
A. That’s a good question. My post-doc right now is working on that localization. At this point we don’t know.