Change in the Concentration of Vitamins C and E in Rat Tissues by Paraquat Administration

Kazumi Ikeda, Yumi Kumagai, Yuka Nagano, Naoko Matsuzawa, and Shosuke Kojo†

Department of Food Science and Nutrition, Nara Women’s University, Nara 630-8506, Japan

Received September 10, 2002; Accepted December 26, 2002

Paraquat causes lung injury by oxidative stress. After 48 h of intraperitoneal administration of paraquat (50 mg/kg of body weight) to rats, the vitamin C concentration in the lungs was significantly decreased, while lung vitamin E content was increased after 12 h. These results indicate that vitamin C directly reflected the oxidative stress in the lungs.

Key words: oxidative stress; paraquat; radical; vitamin C; vitamin E

Vitamins C and E are well-known antioxidants which rapidly react with radicals. Accordingly, it has been assumed that both vitamins C and E are consumed during radical reactions. However, in rat tissues, the change in the concentration of vitamin C caused by oxidative stress does not always match that of vitamin E. We have previously reported that the liver concentration of vitamin C decreased during the oxidative stress caused by streptozotocin-induced diabetes,1) and by the administration of such hepatotoxins as thioacetamide,2) carbon tetrachloride,3) and D-galactosamine.4) The decrease in the vitamin C concentration was always accompanied by an increase in lipid hydroperoxides,5–9) mediators of the radical reaction,5) while the liver concentration of vitamin E did not always decrease under these conditions because of mobilization from plasma6) or other tissues.1–4) In this work, we compare the changes in vitamins C and E in rat lungs during typical oxidative stress induced by paraquat (PQ, 1,1′-dimethyl-4,4′-bipyridinium) administration. It has been well established that PQ induced severe lung inflammation and interstitial fibrosis by reactive oxygen species.7–9)

Guidelines from the Prime Minister’s Office of Japan (No. 6 of 27 March 1980) for the care and use of laboratory animals were followed. Eight-week-old male rats (SLC: Wistar strain) were obtained from Japan SLC Co. (Hamamatsu, Shizuoka, Japan). The animals were housed in a room with a 24 ± 2°C temperature and a 12 h/12 h light-dark cycle. The animals were permitted free access to a commercially available diet (type MF, Oriental Yeast Co., Tokyo, Japan) and water.

The experimental group was given an intraperitoneal injection of PQ at a dose of 50 mg/kg of body weight in 400 μl of saline. This dose was similar to that used in previous studies.10,11) PQ being purchased from Sigma Aldrich Japan Co. The control group received 400 μl of saline alone. For all determinations, the control rats gave almost identical results independent of the time after injecting the saline (12, 24, or 48 h). Therefore, all data reported for the control group are those for rats that had been injected with saline and then fed for 48 h.

After 12, 24, or 48 h of being treated with PQ or saline, the rats were anesthetized with diethyl ether and killed by collecting the blood from the vena cava inferior by a heparinized syringe. After perfusing chilled isotonic saline through the portal vein, the liver, kidneys, and lungs were excised. Blood was centrifuged at 3,000 × g for 5 min at 4°C to separate the plasma. Vitamin C was determined by a specific and sensitive method12) involving chemical conversion and HPLC. The concentration of α-tocopherol (vitamin E) was determined by HPLC,13) and the protein concentration was determined by the method14) of Lowry et al., using BSA as the standard. Each data value is expressed as the mean ± S.D., and data were analysed by ANOVA with Stat View software (Abacus Concepts, Berkeley, CA, U.S.A.). Differences between group means are considered significant at P<0.05 by using the Bonferroni/Dunn procedure generated by this same program.

After 48 h of PQ injection, the level of vitamin C in the lungs had significantly decreased when compared with the control group and the rats treated with PQ for 12 h and 24 h (Table 1). The level of vitamins C and E in this work is expressed per mg of protein, and not per 1 g of tissue as in our previous studies.1–4) since the lung weight was doubled (P<0.05) and the protein content was decreased to 68 ± 9% (P<0.05) in comparison with the control rats due to the severe edema caused by PQ. This resulted in the lung concentrations of both vitamins C and E decreasing significantly when based on 1 g of tissue. PQ did not

† To whom correspondence should be addressed. Fax: +81-742-20-3459; E-mail: kojo@cc.nara-wu.ac.jp

Abbreviation: PQ, paraquat
vitamin E was mobilized during oxidative stress by those in the literature, which has reported that plasma to the lungs. These results are consistent with suggested that vitamin E was mobilized from the lung by PQ and extensively consumed vitamin C.

Table 1. Levels of Vitamin C in the Tissues of Rats Administered with PQ and of Control Rats

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 5)</th>
<th>12 h (n = 4)</th>
<th>24 h (n = 8)</th>
<th>48 h (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lungs</td>
<td>16.30 ± 2.13</td>
<td>18.02 ± 4.41</td>
<td>17.02 ± 2.27</td>
<td>8.13 ± 1.74</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.66 ± 0.17</td>
<td>0.73 ± 0.26</td>
<td>0.70 ± 0.25</td>
<td>0.60 ± 0.07</td>
</tr>
<tr>
<td>Liver</td>
<td>6.66 ± 0.17</td>
<td>7.90 ± 0.56</td>
<td>8.15 ± 2.77</td>
<td>7.39 ± 1.91</td>
</tr>
<tr>
<td>Kidneys</td>
<td>4.20 ± 0.35</td>
<td>4.11 ± 0.16</td>
<td>3.97 ± 0.60</td>
<td>4.19 ± 1.27</td>
</tr>
</tbody>
</table>

Units are nmol/ml for the plasma, and nmol/mg of protein for the liver, kidneys, and lungs.

Table 2. Levels of Vitamin E in the Tissues of Rats Administered with PQ and of Control Rats

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 5)</th>
<th>12 h (n = 5)</th>
<th>24 h (n = 8)</th>
<th>48 h (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lungs</td>
<td>0.12 ± 0.02</td>
<td>0.20 ± 0.05</td>
<td>0.14 ± 0.06</td>
<td>0.12 ± 0.03</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.21 ± 0.07</td>
<td>0.15 ± 0.03</td>
<td>0.13 ± 0.04</td>
<td>0.16 ± 0.03</td>
</tr>
<tr>
<td>Liver</td>
<td>0.09 ± 0.03</td>
<td>0.09 ± 0.02</td>
<td>0.12 ± 0.05</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.03 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>0.03 ± 0.01</td>
</tr>
</tbody>
</table>

Units are nmol/ml for the plasma, and nmol/mg of protein for the liver, kidneys, and lungs.

To evaluate the vitamin E concentrations in the plasma, liver, and kidneys (Table 1). These results suggest that oxidative stress was specifically enhanced in the lung by PQ and extensively consumed vitamin C.

The vitamin E concentration in the lungs had increased 12 h after the treatment with PQ was initiated and that in plasma had decreased 12, 24, and 48 h after the PQ treatment (Table 2). These observations suggested that vitamin E was mobilized from the plasma to the lungs. These results are consistent with those in the literature, which has reported that vitamin E was mobilized during oxidative stress by PQ.

In summary, the level of vitamin C was decreased in the lung by the intraperitoneal injection of PQ to rats, while the vitamin E level was initially increased. These results suggest that the vitamin C concentration reflects more directly the oxidative stress in the lungs than that of vitamin E, this being a similar effect to that in our studies made on the liver.

References


