Note

Increase of Lipid Hydroperoxides in the Rat Liver and Kidney after Administering Ferric Nitrilotriacetate

Kazumi Ikeda,* Fang Sun,* Kyoko Tanaka,* Sadako Tokumaru,† and Shosuke Kojo***

*Department of Food Science and Nutrition, Nara Women's University, Nara 630-8506 Japan
†Joetsu University of Education, Joetsu, Niigata 943-8512, Japan

Received January 14, 1998

Two hours after an intraperitoneal injection of ferric nitrilotriacetate to rats at a dose of 15 mg of Fe/kg of body weight, the level of lipid hydroperoxides, which was determined by a specific method involving chemical conversion into 1-naphthylphenolphosphine oxide and HPLC, had increased significantly in the liver (from 190.1 ± 55.8 of the control to 467.1 ± 175.8 pmol/mg of protein) and kidney (from 181.8 ± 52.3 of the control to 405.9 ± 22.7 pmol/mg of protein). These results demonstrate that oxidative stress was transiently increased by an iron overload in these tissues.

Key words: ferric nitrilotriacetate; hydroperoxide; lipid peroxidation; radical reaction; iron overload

Although radical reactions receive much attention in relation to pathogenic disorders such as atherosclerosis, cancer, aging, and so on, the search for a reliable indicator of lipid peroxidation in animal tissues is still an important activity. Conventional indicators are classified in three main categories, which are (1) products of lipid peroxidation such as malondialdehyde (MDA), total aldehydes as thiobarbituric acid-reactive substances (TBARS), modified proteins, DNA, (2) decreased antioxidants like vitamin E and glutathione, and (3) activity change of antioxidative enzymes including superoxide dismutase and glutathione peroxidase. As another kind of index, the oxidative mediator, rather than the products of peroxidation, may be postulated. Lipid hydroperoxide is a probable candidate for such an oxidative mediator, because it is formed by radical reactions in the membrane, has a sufficient lifetime to migrate in the cell, and finally modifies protein and DNA.

We have recently developed a specific and sensitive method to determine the total level of lipid hydroperoxides in animal tissues involving quantitative chemical conversion of 1-naphthylphenolphosphine into its oxide with lipid hydroperoxides and subsequent measurement of the oxide by HPLC. The efficiency of lipid hydroperoxides as an index of oxidative stress has been confirmed by their increase in aged, vitamin C-deficient and vitamin E-deficient animal tissues. As an extension of this work, we now report a transient increase of lipid hydroperoxides in the liver and kidney of rats which have been administered with ferric nitrilotriacetate (Fe-NTA), which has been suggested to cause radical reactions in the liver and kidney, resulting in hemochromatosis and renal adenocarcinoma, by repeated injection.

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.

An Fe-NTA solution was prepared immediately before use by the reported method. The experimental group was given an intraperitoneal injection of Fe-NTA at dose of 15 mg of Fe/kg of body weight. The control group was injected with a solution which had been prepared in a similar manner to Fe-NTA solution, but without the use of ferric nitrate, and that contained an equivalent dose of nitrilotriacetate (NTA).

At 2 h or 12 h after the treatment with Fe-NTA or NTA, 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 and kidney were excised for measuring hydroperoxides according to the methods in the literature. Protein concentration was determined according to the reported method, using bovine serum albumin as the standard. Each data value is expressed as the mean ± SD of four rats for each group and was analyzed statistically by Welch's t-test.

The level of lipid hydroperoxides in the kidney of rats which had been treated with Fe-NTA 2 h before sacrifice was significantly higher than that of the control animals (Table 1). At 12 h after the injection, the content of lipid hydroperoxides in the kidney was not significantly different from that of the control (Table 1). These results suggest that radical reactions were transiently enhanced to increase the reactive hydroperoxides which mediated the peroxidation and/or modification of lipids, proteins.

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

Abbreviations: Fe-NTA, ferric nitrilotriacetate; MDA, malondialdehyde; NTA, nitrilotriacetate; TBARS, thiobarbituric acid-reactive substances
Hydroperoxide Formation by an Iron Overload

Table 1. Levels of Lipid Hydroperoxides in the Liver and Kidney of Rats 2 and 12 h after an Injection of Fe-NTA or NTA.

<table>
<thead>
<tr>
<th></th>
<th>Liver Hydroperoxides (pmol/mg of protein)</th>
<th>Kidney Hydroperoxides (pmol/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe-NTA</td>
<td>2 h</td>
<td>467.1 ± 175.8*</td>
</tr>
<tr>
<td>Control (NTA)</td>
<td>2 h</td>
<td>190.1 ± 58.8</td>
</tr>
<tr>
<td>Fe-NTA</td>
<td>12 h</td>
<td>372.2 ± 196.5</td>
</tr>
<tr>
<td>Control (NTA)</td>
<td>12 h</td>
<td>187.0 ± 46.0</td>
</tr>
</tbody>
</table>

Rats were intraperitoneally administered Fe-NTA at a dose of 15 mg of Fe/kg of body weight. The control group was injected with an equal dose of NTA. The levels of lipid hydroperoxides in the kidney and liver after 2 and 12 h were determined as described in the text. Each value is the mean ± SD for 4 rats. Asterisks indicate a significant difference from the corresponding control value (Welch's t-test; *P < 0.05 and **P < 0.01).

and DNA. This idea is supported by reports which have described the increase of radical-derived products such as TBARS, 18-22 4-hydroxy-2-nonenal-modified proteins, 20 8-hydroxy-2-deoxyguanosine, 23 and aldehyde-modified proteins 23) in the kidney within a few hours of the administration of Fe-NTA. These results also suggest that radical reactions were rapidly initiated after the administration of Fe-NTA and did not last for a long time. This assumption is supported by literature which has reported that renal levels of TBARS, 20-23 4-hydroxy-2-nonenal-modified proteins, 20 and aldehyde-modified proteins 23 reached a maximum 1-3 h after the administration of Fe-NTA and declined sharply thereafter. This may be the reason why repeated injections were necessary to cause renal adenocarcinoma.

At 2 h after the injection of Fe-NTA, the level of lipid hydroperoxides in the liver was elevated significantly compared to the control value and declined at 12 h (Table 1). This result demonstrates that Fe-NTA also augmented the oxidative stress in the liver shortly after the administration. This observation is consistent with previous work reporting that heavy iron deposits were found in liver parenchymal cells 23 and that Fe-NTA increased TBARS in rat liver. 24

In summary, 2 h after the intraperitoneal injection of ferric trinitroacetate (Fe-NTA) to rats, the level of lipid hydroperoxides, a reactive mediator of radical reactions, had increased transiently in the liver and kidney. These observations support the idea that Fe-NTA raised oxidative stress, which is a cause of hemochromatosis and renal adenocarcinoma.

References