Changes in the Level of Sialic Acid in Plasma, Brain and Liver of Inherently Scorbutic Rats during Vitamin C and E Deficiencies

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The plasma level of sialic acid (NeuAc) in inherently scorbutic [Osteogenic Disorder Shionogi (ODS)] rats was increased by 21 days of vitamin C deficiency and simultaneous vitamins C and E deficiency. The brain content of NeuAc was decreased by deficiencies of these vitamins. The NeuAc level in the liver was not affected significantly by these deficiencies.

Key words: sialic acid; N-acetylmuraminic acid; vitamin C; vitamin E; ODS rat

Plasma or serum sialic acid (NeuAc) has been known to increase in diseases such as cancer, inflammation, diabetes mellitus, and myocardial infarction. The increase of NeuAc was partly ascribed to the elevation of hepatic secretion of acute phase proteins, which are secreted in inflammatory conditions that oxidative stress was suggested to augment. Recently Yamamoto et al. reported that mouse serum NeuAc was increased by skin inflammation caused by UV irradiation, a kind of oxidative stress.

In this report, we measured levels of NeuAc in another typical case of increased oxidative stress, i.e., deficiency of antioxidative vitamins such as C and E, using inherently scorbutic (ODS:Osteogenic Disorder Shionogi) rats. We demonstrated that the deficiency of these vitamins increased lipid hydroperoxides and thiobarbituric acid-reactive substances (TBARS) in tissues of ODS rats.

Guidelines of the Prime Minister's Office of Japan (No. 6 of 27 March 1980) for the care and use of laboratory animals were followed. The homozygous male ODS rats (od/od), 5 weeks old, were purchased from Clea Japan Inc. Tokyo, Japan). The animals were housed in a room with a temperature 24 ± 2°C, and a 12 h light-dark cycle. Animals were permitted free access to food and water. For the first week, all rats were fed the synthetic basal diet prepared by Funahashi Farm Co. Ltd. (Chiba, Japan) according to AIN 76(10) and were offered ion exchanged water containing 1 g vitamin C/l, which was sufficient to maintain normal growth. After the week of acclimation, rats were divided into 4 groups [the control, vitamin C-deficient (designated as C-), vitamin E-deficient (designated as E-), and simultaneously vitamins C and E-deficient (designated as C-E)]. The number of rats in each group was 4. The diet of the C- group was prepared by Funahashi Farm Co. Ltd. using stripped corn oil (5 g/100 g) as the fat. The control group received vitamin C (1 g/l) in drinking water and the synthetic basal diet which contained stripped corn oil (also 5 g/100 g) and all-rac-α-tocopherol (50 mg/kg). The C-E group was fed the synthetic basal diet and vitamin C-free water. The C-E was fed with vitamin C-free water and the vitamin E-free diet as described above.

Rats were anesthetized with diethyl ether and killed by collecting the blood from the inferior vena cava using a syringe containing sodium heparin as an anticoagulant. After perfusion of ice-cold saline from the portal vein, organs were removed. The liver and brain were homogenized in 5 volumes of 10 mM-phosphate buffered saline (pH 7.2) and plasma was diluted with 4 volumes of the same buffer under cooling in an ice bath. All measurements were made by duplicated experiments. Protein in these samples was measured by the method of Lowry et al. using bovine serum albumin as the standard.

NeuAc concentration was measured based on the method of Hara et al. One hundred μl of a tissue homogenate or diluted plasma was taken into a dark-colored vial with a Teflon-lined screw cap, which contained 4 ml of 25 mM sulfuric acid. The mixture was hydrolyzed at 80°C for 2 h. The reaction time was observed in separate experiments using diluted plasma and tissue homogenates to give a plateau value. After cooling in ice, 10 μl of the reaction mixture was taken out and mixed with 100 μl of 25 mM sulfuric acid and 100 μl of 7 mM of aqueous 1,2-diamino-4,5-methyleneoxy-benzene dihydrochloride (DMB) solution containing 1.0 mM β-mercaptoethanol and 18 mM sodium hydrosulfite. The resulting solution was incubated at 60°C for 2.5 h. The reaction mixture (10 μl) was directly analyzed by HPLC. The DMB derivatives of NeuAc were separated on a reversed phase column and detected by a fluorescence detector (type RF-535, manufactured by Shimadzu Co., Ltd., Kyoto, Japan) as in the literature. As a calibration, 100 μl of standard NeuAc solution (0, 10, 100, and 1000 μM) was treated similarly.

Data were expressed as mean ± SD and analyzed by ANOVA using StatView software (Abacus Concepts, * To whom correspondence should be addressed. Tel. & Fax: +81-742-20-3459; E-mail: kojo@cc.nara-wu.ac.jp

Abbreviations: DMB, 1,2-Diamino-4,5-methyleneoxy-benzene dihydrochloride; LDL, low density lipoprotein; ODS, Osteogenic Disorder Shionogi; TBARS, thiobarbituric acid-reactive substances.
Table 1. NeuAc Level of Plasma, Brain and Liver in Inherently Scorbutic Rats Fed Control, Vitamin C-deficient, Vitamin E-deficient, and Simultaneously Vitamin C and E-deficient Diets for 21 d.

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>Brain</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol/mg protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>28.0±3.8a</td>
<td>35.4±3.8a</td>
<td>4.78±0.37</td>
</tr>
<tr>
<td>−C</td>
<td>38.4±3.0b</td>
<td>27.0±2.1b</td>
<td>5.04±0.67</td>
</tr>
<tr>
<td>−E</td>
<td>29.0±2.4a</td>
<td>28.7±1.7a</td>
<td>4.24±0.31</td>
</tr>
<tr>
<td>−C, −E</td>
<td>40.6±4.9a</td>
<td>28.9±3.5a</td>
<td>4.68±0.09</td>
</tr>
</tbody>
</table>

Inherently scorbutic rats were divided into four groups (control, vitamin C-deficient, vitamin E-deficient, and simultaneously vitamin C and E-deficient groups). After 21 d, NeuAc levels of plasma, brain, and liver were measured as described in the text. Values are means ±SD of 4 rats. Values within each column not sharing a common superscript letter are significantly different (P<0.05) by Fisher's protected least significant difference test. Berkeley, CA). Differences between group means were analyzed using Fisher's protected least significant difference test (PLSD). Differences were considered significant at P<0.05. After 21 d of feeding of each experimental diet, the plasma NeuAc levels of both the −C and the −C, −E groups were significantly higher than that of the control group (Table 1). The ascorbate levels in plasma, heart, lung, liver, kidney, and muscle of the −C and −C, −E groups were scarcely detectable.2,6 These results suggested that the increased oxidative stress induced by vitamin C deficiency increased the plasma level of NeuAc. It is possible that the increase of NeuAc is partly due to the elevated hepatic secretion of acute phase proteins such as C-reactive protein, α1-acid glycoprotein, α1-antitrypsin, α2-macroglobulin, and fibrinogen, as reported in other pathologic conditions involving oxidative stress.2,6 However, no significance was observed in the NeuAc level of the liver itself among these 4 groups (Table 1). No additive effect of vitamin E deficiency with vitamin C deficiency on the elevation of plasma NeuAc concentration was observed.

Vitamin E deficiency for 21 d when the concentrations of vitamins C and E were 85 and 8% of those of the control liver, respectively,9 did not affect the plasma concentration of NeuAc compared to that of the control group (Table 1). Usually symptoms of vitamin E deficiency are detected only after more prolonged deficiency. Therefore, vitamin E depletion for 21 d may not be sufficient to increase oxidative stress in the liver enough to cause induction of acute phase proteins resulting in the elevation of plasma NeuAc.

Recently we reported that NeuAc attached to LDL was converted to unknown compound(s) and decreased by radical reactions.10 It is conceivable that NeuAc in the tissue is decreased by the oxidative stress induced by deficiency of these vitamins. In fact, the NeuAc concentration in the brain, the content of NeuAc of which is high, is decreased by deficiencies of vitamins C and E (Table 1). Involvement of radical reactions in the brain is also suggested in these decreases of NeuAc as in the case of LDL.4 NeuAc in the brain decreased even in the −E group, where the vitamin E level of the brain was depleted to about 70% of that of the control group. These results suggest that the NeuAc level is a sensitive indicator of oxidative stress in the brain and/or that the oxidative stress of the environment of neuronal cells is increased more than that inside the cell by an unidentified mechanism to decrease brain NeuAc, which is assumed to exist on the cell surface.

Acknowledgments
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References