Note

2-O-Octadecylascorbic Acid with No Vitamin C Activity in ODS-od/od Rats

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L-Ascorbic acid (AsA, Fig. 1a) is as biologically active as vitamin C. Nearly all species of animals synthesize AsA and do not require it as an essential nutrient. However, humans, other primates, guinea pigs and ODS-od/od (ODS) rats cannot synthesize the vitamin due to lack of hepatic L-gulono-γ-lactone oxidase. AsA has many biological properties such as acting as a cofactor and antioxidant.

2-O-Octadecyl ascorbic acid (CV-3611, Fig. 1b), which is a newly synthesized derivative of AsA, is a specific free radical scavenger. The biological activity of CV-3611 has not been examined in comparison with that of AsA, although it is presumed that CV-3611 might be as beneficial a compound as vitamin C. In this study, the antiscorbutic activity of CV-3611 was examined in ODS rats unable to synthesize AsA.

Four-week old male ODS rats generously supplied by the Aburah Laboratory of Shionogi & Co., Ltd. were used. The dietary requirement of AsA to maintain normal growth and prevent any sign of scurvy is about 300 mg of AsA/kg diet. Thirty ODS rats (125–130 g) were divided into five groups of six rats each. Each group was fed on a different test diet ad libitum for 22 days, ascorbic acid and/or CV-3611 being added to the basal diet* (AsA-free) at different concentrations. CV-3611 was generously supplied by Takeda Chemical Industries Ltd. The group designation and experimental diet fed to each group were as follows: group A, the basal diet supplemented with 300 mg of AsA/kg of diet; group B, the basal diet supplemented with 730 mg of CV-3611/kg of diet; group C, the basal diet supplemented with 2190 mg of CV-3611/kg of diet; group D, the basal diet supplemented with 3650 mg of CV-3611/kg of diet; group E, the basal diet supplemented with 300 mg of AsA and 500 mg of CV-3611/kg of diet. 730 mg of CV-3611 is equimolar to 300 mg of AsA. All data obtained in this study are shown in Table I.

The body-weight gain in groups B, C and D was lower than that in group A, while the body weight gain in group C or D was slightly higher than that in group B. On the last few days of the 22-day course, hemorrhaging around the eyes and nose was observed in groups B, C and D. Moreover, hemorrhaging in the muscles and joints of the legs was observed when the rats in these three groups were killed. We regard these phenomena as signs of scurvy. The combined supplementation with AsA and CV-3611 in group E did not affect the growth when compared with that in group A.

The food intake during days 6–7 in each group did not differ significantly. However, the food intake during days 16–17 in groups B, C and D was lower than that in group A. The liver weight (g/100 g of body weight) in groups B, C and D was lower than that in group A, probably due to the reduction of food intake. The combined supplementation with AsA and CV-3611 (group E) did not affect the liver weight when compared with that in group A.

In the ascorbic acid-deficient ODS rats, high levels of corticosterone in the serum and adrenal glands have been observed. Corticosterone in the serum and adrenal glands was measured fluorometrically according to the method described previously. The serum level of corticosterone in groups B, C and D was higher than that in group A (Table I). The level of adrenal corticosterone in groups B and D was also higher than that in group A, while the serum and adrenal levels of corticosterone in groups A and E did not differ significantly.

It has been reported that ascorbic acid deficiency causes hypercholesterolemia and an accumulation of cholesterol in the liver due to the depression of bile acid synthesis in guinea pigs. The levels of serum and hepatic cholesterol were determined by the method described previously. The serum level of cholesterol in groups C and D was higher than that in group A, while the levels in groups A, B and

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Fig. 1. Chemical Structure of (a) Ascorbic Acid and (b) 2-O-Octadecyl Ascorbic Acid (CV-3611) (b).

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* The basal diet (AsA free) contained 30% casein, 55.3% carbohydrate (sucrose-starch, 1:2), 5% corn oil, 5% salt mixture, 0.2% choline chloride, 0.5% vitamin mixture (AsA free), 6.67 mg/kg of retinyl palmitate, 100 μg/kg of ergocalciferol, and 100 mg/kg of dl-α-tocopheryl acetate.
Table 1. Effect of Dietary Supplementation of Ascorbic Acid (AsA) and 2-O-Octadecyl Ascorbic Acid (CV-361) on the Growth, Food Intake, Liver Weight, Serum and Adrenal Level of Corticosterone, Serum and Hepatic Level of Cholesterol, and Hepatic Level of Cytochrome P-450

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dietary AsA (mg/kg)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary CV-361 (mg/kg)</td>
<td>0</td>
<td>730</td>
<td>2190</td>
<td>3650</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>129.1 ± 2.0*</td>
<td>129.5 ± 2.2</td>
<td>127.3 ± 3.4</td>
<td>128.2 ± 3.4</td>
<td>128.2 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>Body-weight gain for 22 days (g)</td>
<td>108.3 ± 3.3c</td>
<td>30.3 ± 4.8a</td>
<td>50.7 ± 6.5b</td>
<td>55.3 ± 4.4b</td>
<td>98.2 ± 8.4c</td>
<td></td>
</tr>
<tr>
<td>Food intake (g/day/rat) during days 6-7</td>
<td>17.7 ± 1.0</td>
<td>17.6 ± 0.9</td>
<td>16.4 ± 0.5</td>
<td>18.0 ± 1.1</td>
<td>18.0 ± 1.0</td>
<td></td>
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<tr>
<td>Liver weight (g/100 g of body weight)</td>
<td>16.9 ± 0.1c</td>
<td>9.3 ± 1.1a</td>
<td>11.4 ± 1.2b</td>
<td>12.9 ± 0.6b</td>
<td>20.2 ± 1.7d</td>
<td></td>
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<tr>
<td>Serum corticosterone (µg/dl)</td>
<td>3.5 ± 0.5a</td>
<td>36.8 ± 10.3d</td>
<td>17.5 ± 1.8a</td>
<td>20.6 ± 4.1c</td>
<td>5.6 ± 1.3ab</td>
<td></td>
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<tr>
<td>Adrenal corticosterone (µg/g)</td>
<td>11.4 ± 0.7ab</td>
<td>27.5 ± 4.3b</td>
<td>14.7 ± 2.9ab</td>
<td>19.3 ± 2.7b</td>
<td>8.4 ± 1.6a</td>
<td></td>
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<tr>
<td>Serum cholesterol (mg/dl)</td>
<td>83.5 ± 1.6a</td>
<td>95.1 ± 2.1ab</td>
<td>109.6 ± 11.3b</td>
<td>104.5 ± 4.7g</td>
<td>84.3 ± 5.8a</td>
<td></td>
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<tr>
<td>Liver cholesterol (mg/dl)</td>
<td>2.53 ± 0.04b</td>
<td>3.33 ± 0.11b</td>
<td>2.99 ± 0.08b</td>
<td>3.03 ± 0.11b</td>
<td>2.41 ± 0.06a</td>
<td></td>
</tr>
<tr>
<td>Hepatic level of cytochrome P-450 (nmol/g)</td>
<td>9.62 ± 0.44bc</td>
<td>7.21 ± 0.59b</td>
<td>6.98 ± 0.27a</td>
<td>7.78 ± 0.29ab</td>
<td>11.31 ± 2.6c</td>
<td></td>
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</table>

* Means ± SEM for 6 rats. Data were subjected to an analysis of variance (ANOVA) and Duncan’s multiple range test.10 Means within a line not followed by the same superscript letter are significantly different (p < 0.05).

E did not differ significantly from each other. The hepatic level of cholesterol in groups B, C and D was higher than that in group A, while that in group E showed no difference in comparison with that in group A.

The hepatic level of cytochrome P-450 was determined by the dithionite difference method of Omura and Sato.7 Ascobic acid deficiency caused a lower level of hepatic cytochrome P-450 in ODS rats.6 The hepatic level of cytochrome P-450 in groups B, C and D was lower than that in group A, although the level of cytochrome P-450 did not change as the dietary level of CV-361 was increased.

From these results, the antiscorbutic effect of CV-361 was not observed in maintaining normal growth and preventing biological signs of scurvy. CV-361 itself seemed to be ineffective for maintaining normal levels of serum and adrenal corticosterone, serum and hepatic cholesterol, and hepatic cytochrome P-450. We speculate that CV-361 could not be converted to AsA in vivo. These results, however, suggest that CV-361 did not have a competitive or inhibitory effect on the biological utilization of AsA.

CV-361 is lipophilic and a potent free radical oxygen (FRO) scavenger.2,20 It is supposed that this compound may act in or on the biological membrane as a FRO scavenger. Recently, it has been reported that CV-361 exerted beneficial effects in alleviating tissue injury due to ischemia and reperfusion in rats9 and dogs.6 It is expected that CV-361 can be utilized as a protecting agent against FRO-mediated tissue damage without affecting AsA utilization.

References