Note

Reduction of Dehydroerythorbic Acid in Vitamin C-Deficient Guinea Pigs

Yan CUI1, Megumi OTSUKA2 and Yoko FUJWARA2,*

1 Department of Nutrition and Food Science, Ochanomizu Graduate School of Humanities and Sciences, and
2 Department of Food and Nutrition, Ochanomizu University, 2–1–1 Otsuka, Bunkyo-ku,
Tokyo 112–8610, Japan

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Summary A reduction of dehydroerythorbic acid (DERA) to erythorbic acid (ERA) in vitamin C-deficient guinea pigs was evaluated and compared with that of dehydroascorbic acid (DASA). Thirty-six guinea pigs were fed with vitamin C-deficient diets for 18 days. On day 19, the guinea pigs were divided into four groups for the administration of 100mg of DERA, ERA, ascorbic acid (ASA), or DASA every day. After 12 days of oral administration, the concentration of DERA, ERA, ASA, and DASA in the liver, adrenal, spleen, kidney, and plasma of guinea pigs was determined by HPLC. A recovery from scurvy was measured in terms of weight gain and serum alkaline phosphatase activity. All four groups showed similar recovery, indicating that the oral administration of relatively high concentrations of DERA reversed the effects of scurvy in vitamin C-deficient guinea pigs. In spite of DERA or DASA administration, ERA or ASA was mainly detected in the tissues. The reduction ratios of DEAR and DASA were similar (approximately 80%) in all tissues except spleen. These results suggest that both DASA and DERA are taken up and reduced to ASA or ERA in vivo.

Key Words ASA, DASA, ERA, DERA, vitamin C-deficiency

ERA, also known as d-isoascorbic acid or d-araboascorbic acid, is the stereoisomer of ASA, differing the spatial configuration of the hydroxyl group at carbon 5. The endiol group of the lactone ring of ERA and ASA exhibits the same acidic and reducing properties. ERA is widely used as an antioxidant for food additive. The antiscorbutic activity of ERA has been reported to be much lower than that of ASA (1–3). The vitamin C activity of ERA is reported as 1/20 or less of ASA because of the poor tissue uptake of ERA (4).

On the other hand, DASA, an oxidized form of ASA, is known to display vitamin C activities following the reduction to ASA in animal tissues (5–7). DASA is transported via GLUT (8, 9), whereas ASA is transported via sodium-dependent ascorbic acid transporters (10). Following uptake, DASA is reduced to ASA either by the enzyme action (11–15) or by the direct chemical reaction with GSH (16). The antiscorbutic effect of DASA in vitamin C-deficient guinea pigs is reported to be less than that of ASA (5, 17).

The mechanism by which DERA is metabolized in vivo is not known. However, based on the similarities in chemical structure, it is proposed that DERA is taken up and reduced to ERA in a manner similar to DASA. The purpose of this study is to investigate the reduction of DERA to ERA in guinea pigs and to compare the vitamin C activity of DERA and DASA with that of ASA in scurbutic guinea pigs.

Materials and Methods

Materials. ERA and ASA were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All the reagents were analytical grade.

Preparation of DERA and DASA. Since the commercial grades of DERA and DASA were not pure enough for our experiments (18, 19), DERA and DASA were prepared by the method of Ohmori and Takagi (20). Briefly, activated charcoal (Norit “SX Plus” obtained from Wako Pure Chemical Industries, Ltd.) was added to ERA or ASA dissolved in methanol. Oxygen bubbled through the methanol solution for 4 h, and the solution was then passed through Whatman No. 2 filter paper. The filtrate was concentrated in an evaporator to obtain DASA or DERA syrups.

The purity of DASA or DERA syrup was determined by the thin-layer chromatography (21), HPLC (7), and the 2,4-dinitrophenylhydrazine method (22). The concentrations of DASA and DERA in the syrup were approximately 75%, and those of their reduced forms were less than 0.01%. To prevent the degradation, DERA and DASA were prepared immediately before administration to the guinea pigs.

Animals and diets. Male Hartley guinea pigs (CLEA Japan Inc., Tokyo, Japan) weighing 220 g were allowed free access to the vitamin C-deficient diet and water. Thirty-six guinea pigs were fed vitamin C-deficient diets (CLEA Japan Inc.) throughout the experimental period. On day 19 the guinea pigs were divided into four groups...
Reduction of Dehydroerythorbic Acid in Vitamin C-Deficiency

and orally given 100 mg per day of DERA, ERA, DASA, or ascorbic acid (ASA) for 12 d. On day 13 of the repletion period, the animals were anesthetized with pentobarbital; the liver, spleen, kidneys, and adrenals were removed, and blood was collected. The animal Experiment Ethics Committee of Ochanomizu University approved all experiments.

**Determination of DERA, ERA, DASA, and ASA.** The concentration of DERA, ERA, DASA, or ASA in tissues was determined by HPLC as previously described (7). The concentration of DERA or DASA was estimated by subtracting the values of ERA or ASA from those of total ERA or total ASA determined after a reduction by dithioerythritol.

**Statistics.** The student’s t-test was used to compare the experimental groups with the control group.

**Results and Discussion**

Figure 1 shows the relative changes in body weight of the animals during the experimental periods. The body weight of the guinea pigs fed with vitamin C-deficient diets decreased after 14 days of depletion. They showed typical scorbutic symptoms, including weakening and stiffening of the lower limbs. The average body weight at the beginning of the depletion period expressed as 100% was 287±23.3 g. The body weights of animals increased gradually after 5 days of repletion (after 3 days in the ASA group), and all animals had recovered from scurvy at the end of the repletion period. The weight of body and tissues showed no significant differences among the four groups (Table 1).

The activities of serum alkaline phosphatase, a marker of scorbutic conditions, of the group DERA, DASA, ERA, and ASA were 125±0.56, 113±0.54, 118±0.72, and 125±0.67 IU/L, respectively. All the values were in the normal range (1), and no significant differences appeared among the four groups. These data indicated that the guinea pigs supplemented with DERA also recovered from scurvy.

In this study, we administered 100 mg of ASA, DASA, ERA, or DERA to guinea pigs. DASA is reported to have less antiscorbutic activity than ASA in vitamin C-deficient guinea pigs (7). The biological activity of ERA approximated that of ASA in normal guinea pigs fed large amounts of ERA (4). Scorbutic guinea pigs require 100 mg or more ERA to recover from scurvy compared with only 5 mg of ASA (23). Our results suggest that a large amount of DERA is required to supply the same vitamin C activity as ASA.

Table 2 shows the concentrations of ERA, total ERA,
Table 2. Concentrations of ASA, ERA, total ASA, and total ERA in plasma and tissues.

### Adrenal

| Group | Total ASA (mg/100 g tissue) | ASA (mg/100 g tissue) | ratio ASA/total ASA | Total ERA (mg/100 g tissue) | ERA (mg/100 g tissue) | ratio ERA/total ERA |
|-------|-----------------------------|------------------------|--------------------|-----------------------------|------------------------|--------------------|
| ASA   | 57.4±5.37                  | 44.9±5.87              | 0.76               | ND                          | ND                     | ND                 |
| DASA  | 82.2±4.30*                 | 68.9±4.63*             | 0.84               | ND                          | ND                     | ND                 |
| ERA   | ND                          | ND                     | —                  | 19.0±3.40**                | 15.7±2.60**           | 0.81               |
| DERA  | ND                          | ND                     | —                  | 11.9±1.63**                | 10.4±1.31**           | 0.88               |

Values are mean±SE (n=9). Significant differences from group ASA. *, p<0.05; **, p<0.01.

### Spleen

| Group | Total ASA (mg/100 g tissue) | ASA (mg/100 g tissue) | ratio ASA/total ASA | Total ERA (mg/100 g tissue) | ERA (mg/100 g tissue) | ratio ERA/total ERA |
|-------|-----------------------------|------------------------|--------------------|-----------------------------|------------------------|--------------------|
| ASA   | 15.5±1.26                   | 9.45±0.63              | 0.64               | ND                          | ND                     | ND                 |
| DASA  | 16.8±1.05                   | 12.5±0.96              | 0.75               | ND                          | ND                     | ND                 |
| ERA   | ND                          | ND                     | —                  | 3.58±0.56**                | 1.16±0.37**           | 0.31               |
| DERA  | ND                          | ND                     | —                  | 3.00±0.28**                | 0.70±0.16**           | 0.23               |

### Kidney

| Group | Total ASA (mg/100 g tissue) | ASA (mg/100 g tissue) | ratio ASA/total ASA | Total ERA (mg/100 g tissue) | ERA (mg/100 g tissue) | ratio ERA/total ERA |
|-------|-----------------------------|------------------------|--------------------|-----------------------------|------------------------|--------------------|
| ASA   | 2.20±0.12                   | 1.59±0.19              | 0.71               | ND                          | ND                     | ND                 |
| DASA  | 4.28±0.14                   | 3.54±0.14              | 0.83               | ND                          | ND                     | ND                 |
| ERA   | ND                          | ND                     | —                  | 0.77±0.11**                | 0.43±0.07**           | 0.61               |
| DERA  | ND                          | ND                     | —                  | 0.41±0.05**                | 0.31±0.03**           | 0.76               |

### Liver

| Group | Total ASA (mg/100 g tissue) | ASA (mg/100 g tissue) | ratio ASA/total ASA | Total ERA (mg/100 g tissue) | ERA (mg/100 g tissue) | ratio ERA/total ERA |
|-------|-----------------------------|------------------------|--------------------|-----------------------------|------------------------|--------------------|
| ASA   | 6.49±0.75                   | 5.08±0.81              | 0.76               | ND                          | ND                     | ND                 |
| DASA  | 10.9±1.45*                  | 9.59±1.36*             | 0.86               | ND                          | ND                     | ND                 |
| ERA   | ND                          | ND                     | —                  | 0.62±0.07**                | 0.46±0.07**           | 0.73               |
| DERA  | ND                          | ND                     | —                  | 0.38±0.06**                | 0.30±0.06**           | 0.79               |

### Plasma

| Group | Total ASA (<×10⁻² mg/100 mL) | ASA (×10⁻² mg/100 mL) | ratio ASA/total ASA | Total ERA (×10⁻² mg/100 mL) | ERA (×10⁻² mg/100 mL) | ratio ERA/total ERA |
|-------|-----------------------------|------------------------|--------------------|-----------------------------|------------------------|--------------------|
| ASA   | 43.6±15.9                   | 38.4±15.7              | 0.77               | ND                          | ND                     | ND                 |
| DASA  | 53.0±11.9                   | 45.6±10.2              | 0.84               | ND                          | ND                     | ND                 |
| ERA   | ND                          | ND                     | —                  | 19.0±4.80*                 | 12.3±3.44*            | 0.65               |
| DERA  | ND                          | ND                     | —                  | 9.93±1.95*                 | 6.84±1.36*            | 0.69               |

ASA, and total ASA, and the ratio of the reduced form of ERA or ASA to total compound in plasma and various tissues. Although the same amount of DERA, DASA, ERA, and ASA was supplemented, the uptakes of DERA and ERA in the tissues of the DERA and ERA groups were significantly lower than those of the DASA and ASA groups. The results showed that DASA and ASA accumulated in the tissues of vitamin C-deficient guinea pigs more effectively than DERA and ERA did. Recently, the mechanism of ASA uptake has been clarified. ASA is transported via sodium-dependent ASA transporters (9–11), which display exquisite substrate selectivity, greatly favoring ASA over its isomers ERA and DASA. DASA is incorporated via glucose trans-
porters, such as the GLUT family, which are known to transport DASA, but not ASA, in vitro (10, 24). However, it is not known if DERA can be transported through the same transport system as DASA.

Reduced forms of ERA and ASA were detected in tissues of groups supplemented with the oxidized form of ERA and ASA (DERA and DASA groups). This finding suggests that DERA and DASA are reduced to ERA and ASA, respectively, in vitamin C-deficient guinea pigs. The reduction ratios in the plasma and tissues of the four groups were almost equal and approximately 60–80% in all samples except the spleen. In the spleen, the reduction ratio of the DASA group was similar to that of the ASA group. This agreed with the previous result (7). However, the ratio of ERA to total ERA was much lower than that of ASA to total ASA (Table 2). It is reported that GSH (25, 26), DASA reductase (27, 28), and thioredoxin reductase (TR) (29) are responsible for the reduction of DASA to ASA in erythrocytes. Although the mechanism by which DERA is reduced is not known, the difference seen in the spleen may be derived from the substrate specificity between DASA and DERA to reduction enzymes in erythrocytes in the spleen.

This study is the first report of a reduction of DERA in vivo. Further study is needed to clarify the transport of DERA and the reduction mechanism of DERA to ERA in vivo.

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