Effect of Erythorbic Acid Administration on Ascorbic Acid Content in Guinea Pig Tissues

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Summary The effect of erythorbic acid (ErA) on ascorbic acid (AsA) content in the tissues of normal and AsA-deficient guinea pigs was studied. The animals were sacrificed at varying intervals during the experimental period, and the liver, adrenal glands, spleen and kidneys were removed. The amounts of AsA and ErA in the tissues were measured by HPLC. The content of AsA in the tissues of the animals administered both AsA and ErA was lower than that of the animals administered only AsA. But the disappearance rate of AsA from the tissues of the AsA-deficient animals was similar to that of the animals administered only ErA. The amount of AsA in the tissues of the animals administered both AsA and ErA during the repletion period was lower than that of the animals administered only AsA. These results suggest that ErA administration may affect the amount of AsA in the tissues by inhibiting its tissue uptake or its storage in the tissues, and not by accelerating the catabolism of AsA in the tissues.

Key Words ascorbic acid, erythorbic acid, guinea pigs, high-performance liquid chromatography (HPLC)

Erythorbic acid (ErA), also known as D-isoascorbic acid or D-araboascorbic acid, is a stereoisomer of L-ascorbic acid (AsA) and has been widely used in the food industry as a food additive, especially as an antioxidant. Although the antioxidant properties of ErA are very similar to those of AsA, the antiscorbutic activity of ErA has been reported to be only about one-twentieth that of AsA (1, 2). This difference in biological activities is thought to be due mainly to the structural difference in the position of the hydroxyl group at carbon 5 of ErA and AsA.

Many workers have investigated the biological properties of ErA (1–8, 12). Reiff and Free (3) reported that ErA had a protective effect on AsA and a tendency

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to slow down the development of acute vitamin C deficiency, but did not have significant antiscorbutic activity. Hughes and Hurley (4), who determined the total concentration of AsA and ErA in the tissues of guinea pigs administered AsA and/or ErA, concluded that AsA was better retained in the tissues than ErA. Hughes et al. (5) also indicated that ErA was lost more rapidly than AsA from the tissues of guinea pigs saturated with AsA or ErA. On the other hand, Hornig and Weiser (6) reported that the administration of ErA together with AsA reduced the bioavailability of AsA. The remaining amount of [1-14C]AsA in the tissues of the guinea pigs supplemented with ErA was reduced and the biological half-life for [1-14C]AsA was also found to be reduced. Pelletier (7) also indicated that the loss of [1-14C]AsA from the tissues of guinea pigs supplemented with ErA was higher than that of those supplemented with AsA.

In the above reports (4, 6), the concentrations of AsA and ErA in the tissues were not measured separately, because the usual chemical determination methods for these compounds, such as the 2,4-dinitrophenylhydrazine (9) and the 2,6-dichlorophenolindophenol methods (10), could not differentiate between these two stereoisomers due to their similar chemical reactivity. Recently Arakawa et al. (11) demonstrated an accurate method for simultaneous determination of both AsA and ErA by using high-performance liquid chromatography (HPLC) to estimate trace amounts of these compounds in animal tissues.

In this study, the effect of ErA on AsA content in the tissues of normal and AsA-deficient guinea pigs was evaluated and the effect of ErA on the bioavailability of AsA was investigated using the sensitive HPLC method (11).

MATERIALS AND METHODS

Animals and diet. Male albino guinea pigs (Hartley strain) with initial body weights of about 220 g were used in two experiments. All animals were housed individually in wire cages and maintained on the AsA-deficient diet shown in Table 1. Water and diets were offered ad libitum, and body weights were recorded daily. After prefeeding for 7 days, all animals were randomly divided into experimental groups.

Experimental procedures

Experiment I. The animals were divided into four groups. Group A (control animals) was orally supplemented with 5 mg AsA/day, group B with 100 mg ErA/day, group C with both 5 mg AsA and 100 mg ErA/day and group D with neither AsA nor ErA. AsA and/or ErA were dissolved in water immediately before use and administered orally. On days 1, 4, 10, 16 and 30 of the experimental period, selected animals were sacrificed after 24 h fasting. The liver, adrenal glands, spleen and kidneys were removed immediately to determine their amounts of AsA and ErA by HPLC.

Experiment II. Animals which received the AsA-deficient diet for 16 days to
Table 1. Composition of AsA-deficient diet for guinea pigs.

| Ingredient                  | Amount (mg/100g diet) |
|-----------------------------|-----------------------|
| Corn                        | 20%                   |
| Sucrose                     | 15                    |
| Wheat bran                  | 20                    |
| Milk casein                 | 20                    |
| Soybean meal                | 5                     |
| Soybean oil                 | 5                     |
| Alfalfa meal                | 10                    |
| AsA-free vitamin mixture*   | 1                     |
| McCollum salt No. 185       | 4                     |

AsA content determined by Fujita's method (14) was 1.0 mg/100 g diet.

* AsA-free vitamin mixture (mg/100 g diet).

RESULTS

Figure 1 shows the average body weight response of the guinea pigs supplemented with AsA and/or ErA. The growth responses of the ErA-supplemented animals (group B and group C) were almost equal to those of the control animals (group A), however, the AsA-deficient animals (group D) showed a significant reduction in their body weight gain after 12 days and some characteristic symptoms.
Fig. 1. Body weight changes of guinea pigs. ●, group A, 5mg AsA-supplemented group; ■, group B, 100mg ErA-supplemented group; ▲, group C, 5mg AsA- and 100mg ErA-supplemented group; ○, group D, AsA-deficient group.

Table 2. Contents of AsA and ErA in liver of guinea pigs supplemented with AsA and/or ErA.

| Group         | A               | B               | C               | D               |
|---------------|-----------------|-----------------|-----------------|-----------------|
|               | AsA (mg/100 g)  | AsA (mg/100 g)  | ErA (mg/100 g)  | AsA (mg/100 g)  | ErA (mg/100 g)  | AsA (mg/100 g)  |
| Day of experiment |                 |                 |                 |                 |                 |                 |
| 1             | 2.08 ± 0.36     | 1.18 ± 0.42     | 0.29 ± 0.16     | 1.52 ± 0.49     | 0.13 ± 0.07     | 1.37 ± 0.53     |
| 4             | 3.11 ± 0.78     | 1.17 ± 0.46     | 0.31 ± 0.08b    | 0.91 ± 0.12a    | 0.11 ± 0.03b    | 1.07 ± 0.28     |
| 10            | 1.93 ± 0.18     | 0.31 ± 0.12     | 0.37 ± 0.13     | 1.54 ± 0.39     | 0.45 ± 0.11     | 0.28 ± 0.17     |
| 16            | 2.29 ± 0.42a    | 0.06 ± 0.04     | 0.45 ± 0.12     | 0.94 ± 0.30a    | 0.22 ± 0.07     | 0.06 ± 0.03     |
| 30            | 2.13 ± 0.63     | ND              | 0.38 ± 0.20     | 0.89 ± 0.28     | 0.34 ± 0.18     | —               |

A, 5mg AsA-supplemented group; B, 100mg ErA-supplemented group; C, 5mg AsA- and 100mg ErA-supplemented group; D, AsA-deficient group. * Values are means ± SE; n=5–9 (except day 1 of group A, n=10, day 16 of group A, n=12, and day 16 of group D, n=14). Means in the same horizontal row with a common superscript letter are significantly different. a,b p<0.05. ND, not detected.

of scurvy.

The contents of AsA and ErA in the liver are presented in Table 2, those in the adrenal glands are presented in Table 3, those in the spleen are presented in Table 4 and those in the kidneys are presented in Table 5. The AsA contents of the tissues of
Table 3. Contents of AsA and ErA in adrenal glands of guinea pigs supplemented with AsA and/or ErA.

| Group | A         | B         | C         | D         |
|-------|-----------|-----------|-----------|-----------|
|       | AsA       | AsA       | ErA       | AsA       | ErA       | AsA       |
| Day of experiment | (mg/100 g tissue) |
| 1     | 29.2 ± 4.8* | 33.3 ± 7.0 | 10.9 ± 1.7 | 29.9 ± 3.7 | 10.1 ± 2.6 | 31.6 ± 7.3 |
| 4     | 29.7 ± 1.3a | 11.8 ± 2.6 | 8.9 ± 1.3  | 17.3 ± 0.9a | 6.1 ± 1.8  | 18.5 ± 3.4  |
| 10    | 20.8 ± 3.5b | 4.3 ± 1.5  | 10.7 ± 3.1 | 7.2 ± 2.2b  | 6.8 ± 2.0  | Trace      |
| 16    | 23.6 ± 4.1c | 1.4 ± 0.5  | 8.9 ± 2.4  | 10.1 ± 2.2c | 6.1 ± 1.9  | 1.0 ± 0.3  |
| 30    | 21.9 ± 4.6  | 0.1 ± 0.1  | 20.3 ± 2.3 | 15.9 ± 2.4  | 14.0 ± 1.0 | —          |

A, 5 mg AsA-supplemented group; B, 100 mg ErA-supplemented group; C, 5 mg AsA- and 100 mg ErA-supplemented group; D, AsA-deficient group. * Values are means ± SE; n = 5–8 (except day 1 of group A, n = 10, day 16 of group A, n = 13, and day 16 of group D, n = 14). Means in the same horizontal row with a common superscript letter are significantly different. *p < 0.001, a p < 0.01, c p < 0.05.

Table 4. Contents of AsA and ErA in spleen of guinea pigs supplemented with AsA and/or ErA.

| Group | A         | B         | C         | D         |
|-------|-----------|-----------|-----------|-----------|
|       | AsA       | AsA       | ErA       | AsA       | ErA       | AsA       |
| Day of experiment | (mg/100 g tissue) |
| 1     | 18.7 ± 4.6* | 14.3 ± 2.4 | 2.8 ± 0.6  | 17.4 ± 3.2 | 3.2 ± 0.9  | 13.8 ± 2.3 |
| 4     | 12.2 ± 0.7a | 5.4 ± 0.7  | 1.1 ± 0.4  | 8.7 ± 1.2a | 1.0 ± 0.4  | 4.3 ± 1.0  |
| 10    | 8.0 ± 0.5  | 0.5 ± 0.3  | 1.0 ± 0.3  | 5.3 ± 1.2  | 1.6 ± 0.9  | ND         |
| 16    | 10.6 ± 1.1b | 0.3 ± 0.2  | 1.7 ± 0.7  | 4.1 ± 0.9b | 1.5 ± 0.4  | ND         |
| 30    | 9.8 ± 2.3  | 0.7 ± 0.4  | 4.3 ± 0.6a | 6.2 ± 1.2  | 2.0 ± 0.7a | —          |

A, 5 mg AsA-supplemented group; B, 100 mg ErA-supplemented group; C, 5 mg AsA- and 100 mg ErA-supplemented group; D, AsA-deficient group. * Values are mean ± SE; n = 5–8 (except day 1 of group A, n = 10, day 16 of group A, n = 13, and day 16 of group D, n = 14). Means in the same horizontal row with a common superscript letter are significantly different. *p < 0.05, b p < 0.001. ND, not detected.

animals administered 5 mg AsA/day showed little fluctuation throughout the experimental period. On the other hand, the AsA contents of the tissues of the AsA-deficient animals apparently decreased with time. The amount of AsA in the liver of the ErA-supplemented animals (group B) was not significantly different from that of the AsA-deficient animals (group D), and furthermore, the disappearance rate of
Table 5. Contents of AsA and ErA in kidneys of guinea pigs supplemented with AsA and/or ErA.

| Group       | A            | B            | C            | D            |
|-------------|--------------|--------------|--------------|--------------|
|             | AsA (mg/100 g tissue) | ErA (mg/100 g tissue) | AsA (mg/100 g tissue) | ErA (mg/100 g tissue) |
| Day of experiment |               |               |               |               |
| 1           | 2.28 ± 0.41* | 1.50 ± 0.30  | 0.15 ± 0.06  | 1.55 ± 0.27  | 0.18 ± 0.08  | 0.81 ± 0.23  |
| 4           | 2.38 ± 0.36  | 1.04 ± 0.33  | 1.11 ± 0.31* | 1.44 ± 0.24  | 0.10 ± 0.06* | 0.50 ± 0.25  |
| 10          | 1.68 ± 0.24* | 0.61 ± 0.33  | 0.47 ± 0.23  | 0.59 ± 0.25* | 0.25 ± 0.09  | ND           |
| 16          | 1.69 ± 0.32  | 0.08 ± 0.05  | 0.19 ± 0.14  | 0.95 ± 0.29  | 0.32 ± 0.10  | ND           |
| 30          | 1.63 ± 0.54  | ND           | 0.18 ± 0.09  | 1.15 ± 0.50  | 0.24 ± 0.16  | —            |

A, 5 mg AsA-supplemented group; B, 100 mg ErA-supplemented group; C, 5 mg AsA- and 100 mg ErA-supplemented group; D, AsA-deficient group. * Values are means ± SE; n = 5–8 (except day 1 of group A, n = 11, day 16 of group A, n = 13, and day 16 of group D, n = 14). Means in the same horizontal row with a common superscript letter are significantly different. * p<0.05. ND, not detected.

AsA from the tissues of the AsA-deficient animals was very similar to that of the ErA-supplemented animals. At the early stage of the experimental period, the AsA contents of the tissues of the animals administered both AsA and ErA decreased considerably, and then showed almost constant values after 10 days, though the values were apparently lower than those of the control animals. The content of ErA in all tissues except the adrenal glands of group B, was lower than the AsA content of group A throughout the experimental period. Also, within group C the amount of ErA in all tissues except the adrenal glands was lower than that of AsA. The ErA tissue content of group B showed a tendency to be higher than that of group C. In the case of the adrenal glands, the content of ErA in group B was not significantly different from that of AsA in group A at the end of the experimental period. Also, within group C the amount of ErA in the adrenal glands was not significantly different from that of AsA at the end of experimental period.

Figure 2 shows the body weight curves of the animals during depletion and repletion in experiment II. After 10 days’ depletion the animals started to lose weight. As soon as AsA and/or ErA were administered, the animals started to gain weight. No difference in body weight gain was observed among the three groups.

Table 6 shows the contents of AsA and ErA in the liver during repletion of the depleted animals. Table 7 shows those in the adrenal glands, Table 8 shows those in the spleen and Table 9 shows those in the kidneys. It appears that the AsA repletion pattern in all tissues was similar and the ErA repletion pattern in all tissues was similar, but the two repletion patterns were slightly different from each other. The AsA contents of all tissues tended to increase gradually with administration of AsA and both AsA and ErA, but recovery of the AsA content was slower in animals.
Fig. 2. Body weight changes of guinea pigs during depletion and repletion period. ●, group E, 5 mg AsA-supplemented group; ■, group F, 100 mg ErA-supplemented group; ▲, group G, 5 mg AsA- and 100 mg ErA-supplemented group.

Table 6. Contents of AsA and ErA in liver of guinea pigs supplemented with AsA and/or ErA during repletion period.

| Group | E          | F            | G          |
|-------|------------|--------------|------------|
|       | AsA (mg/100 g tissue) | AsA (mg/100 g tissue) | ErA (mg/100 g tissue) | AsA (mg/100 g tissue) | ErA (mg/100 g tissue) |
| Day of repletion period | 0  | 0.06 ± 0.03abc | ND | 0.68 ± 0.10 | 0.42 ± 0.15 | 0.55 ± 0.21 |
|                  | 1  | 0.56 ± 0.12ad | ND | 0.35 ± 0.22 | 0.51 ± 0.22 | 0.38 ± 0.02 |
|                  | 4  | 1.54 ± 0.74 | ND | 0.48 ± 0.09 | 0.73 ± 0.06ce | 0.42 ± 0.09 |
|                  | 20 | 1.60 ± 0.28bcde | ND | 0.73 ± 0.06ce | 0.42 ± 0.09 |

E, 5 mg AsA-supplemented group; F, 100 mg ErA-supplemented group; G, 5 mg AsA- and 100 mg ErA-supplemented group. * Values are means ± SE; n = 4–6 (except day 0, n = 14). Means with a common superscript letter are significantly different. a–e p < 0.05, b–d p < 0.01, c–e p < 0.001. ND, not detected.

administered both AsA and ErA (group G) than that in animals administered only AsA (group E). On the other hand, the ErA content of the tissues increased as soon as administration of ErA began. For instance, on the first day of the repletion period, the amount of ErA in the adrenal glands, spleen and kidneys of the ErA-
supplemented animals (group F) was apparently higher than that of AsA in group E, and in the adrenal glands, spleen and kidneys of group G the amount of ErA was higher than that of AsA. However, in groups F and G the ErA content in the tissues except the adrenal glands was almost the same on day 1 and day 20. In the adrenal glands, by the end of the repletion period the ErA content in the animals administered 100mg ErA (group F), seemed to be nearly similar to the AsA level in animals administered 5mg AsA (group E). Also, among animals ad-
Table 9. Contents of AsA and ErA in kidneys of guinea pigs supplemented with AsA and/or ErA during repletion period.

| Group | AsA | ErA | AsA | ErA |
|-------|-----|-----|-----|-----|
|       | (mg/100 g tissue) |       |       |       |
| Day of repletion period | E | F | G | E | F | G |
| 0 | ND | ND | 0.26±0.10 | 0.59±0.20 |   |   |
| 1 | 0.12±0.08 | 2.21±1.07 |   |   |   |   |
| 4 | 0.87±0.27 | 0.05±0.03 | 0.21±0.10 | 0.12±0.06 |   |   |
| 20 | 1.43±0.30 | 0.94±0.39 | 0.52±0.18 | 0.52±0.10 |   |   |

E, 5 mg AsA-supplemented group; F, 100 mg ErA-supplemented group; G, 5 mg AsA- and 100 mg ErA-supplemented group. * Values are means±SE; n=4-6 (except day 0, n=14). Means with a common superscript letter are significantly different. a,c,d p<0.05, b p<0.01.

DISCUSSION

Reiff and Free (3) concluded that ErA given orally to guinea pigs had no significant antiscorbutic activity, because the body weights of the animals administered 10, 100 or 250 mg ErA/day decreased during the experiment, and some typical symptoms of AsA deficiency were observed in these animals. Moreover, no recovery in body weight was observed when a considerable amount of ErA was given to vitamin C-deficient animals. Hornig and Weiser (6) reported that ErA administration reduced the body weight gain to about 50% that of guinea pigs administered only AsA.

In our study, guinea pigs administered 100 mg ErA/day showed weight gain similar to that of control animals. In the repletion experiment, the animals administered ErA or both AsA and ErA showed almost the same pattern of weight gain recoveries as those observed in the AsA-supplemented animals. These observations were similar to those reported by Goldman et al. (12) who stated that the administration of 100 mg ErA/day produced a comparable recovery of body weight gain. Our results indicated that ErA had antiscorbutic activity and did not decrease AsA utilization in animals simultaneously administered AsA and ErA.

The amount of AsA in the tissues of the animals administered both AsA and ErA (group G) the ErA content of the adrenal glands seemed to be nearly similar to the AsA level by the end of the repletion period.
contrary, Pelletier (7) reported that the disappearance rate of [1-14C]AsA from the tissues of ErA-supplemented animals was faster than that in AsA-deficient animals. The results of this study indicated that ErA may neither reduce the AsA body pools nor accelerate the catabolism of AsA, since the administration of ErA did not stimulate the loss of AsA from the tissues. In animals administered both AsA and ErA, the decrease of AsA content of the tissues may have been caused by ErA inhibition of AsA absorption from the gastrointestinal or ErA inhibition of AsA transport through the tissue membranes, or both. The amount of AsA plus ErA in the tissues of the animals administered both AsA and ErA was lower than the amount of AsA in animals administered only AsA. This suggests that AsA is not replaced by ErA in the tissues, and at the same time ErA may inhibit AsA storage in the tissues. By the end of the experiment, the amounts of AsA and ErA in the adrenals of the AsA- and ErA-supplemented animals were closely similar, and the adrenal ErA content in the ErA-supplemented animals was almost the same as the AsA content in the AsA-supplemented animals. This might be attributed to the characteristic adrenal need for high levels of vitamin C for physiological function.

When ErA was given in a dose 20 times larger than the AsA dosage to both normal and AsA-deficient animals, the amount of ErA retained in the tissues was still lower than AsA levels. Therefore, the tissues may retain AsA selectively, and the mechanism of ErA storage in the tissues may be different from that of AsA. The transport system of ErA through the membrane may also be different from that of AsA (13). In this study the amounts of AsA and ErA in the tissues were determined 24h after the last administration. If the assay had been made within a few hours after the final administration, it might have been helpful in clarifying the above assumption.

As shown in Tables 7–9, the ErA content in the adrenals, spleen and kidneys of the animals in groups F and G was higher than the AsA content in those of the animals in groups E and G on the 1st day of the repletion period. Since AsA was scarcely detected in the tissues after 16 days of depletion, it appears that these tissues would be in a vitamin C-requiring state. This suggests that larger amounts of ErA may be absorbed by scurvy tissues than by normal ones. Moreover, in scurvy animals, AsA may be catabolized faster than in normal animals, and it might also be postulated that scurvy animals catabolized AsA faster than ErA. AsA catabolism in the scurvy state is still uncertain and needs further investigation.

Our observations suggest that the mechanism of absorption and storage of ErA might be different from that of AsA; however, if a considerable amount of ErA was present in the tissues, it might exhibit almost the same vitamin C activity as AsA.

The AsA content in the tissues of the animals administered both AsA and ErA was lower than that of the animals administered AsA alone. The disappearance rate of AsA from the tissues of the ErA-supplemented animals was similar to that of the AsA-deficient animals. This indicates that ErA administration may have an effect on AsA content by inhibiting the uptake of AsA to the tissues or the storage of AsA.
in the tissues, or both, and not by accelerating the AsA catabolism.

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