Vitamin C Activity of Erythorbic Acid in Ascorbic Acid-Deficient Guinea Pigs

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Summary The vitamin C activity of erythorbic acid (ErA) in ascorbic acid (AsA)-deficient guinea pigs was evaluated. The guinea pigs depleted AsA for 16 days were divided into two groups: one group (control group) was supplemented with 1, 5, or 100 mg/day AsA and the other group (experimental group) was supplemented with 1, 5, 20, or 100 mg/day ErA for 4 days. The contents of AsA and ErA in the tissues of guinea pigs were determined by using HPLC, and the activities of liver aniline hydroxylase, of serum alkaline phosphatase and the content of liver cytochrome P-450 were measured. The AsA tissue content of AsA-supplemented guinea pigs was much higher than the ErA tissue content of ErA-supplemented ones, and also, the activities of liver aniline hydroxylase, of serum alkaline phosphatase and the content of liver cytochrome P-450 of AsA-supplemented animals were much higher than those of ErA-supplemented animals, even when the supplemented amount of ErA was equal to that of AsA. Based on these results, the vitamin C activity of ErA seems to be much lower than that of AsA in the AsA-deficient guinea pigs. This suggested that the apparent vitamin C activity of ErA was dependent on the AsA tissue levels of guinea pigs.

Key Words ascorbic acid, erythorbic acid, ascorbic acid-deficient guinea pigs, liver aniline hydroxylase, serum alkaline phosphatase, liver cytochrome P-450

Erythorbic acid (ErA), one of the ascorbic acid (AsA) epimers, has the same chemical property with AsA and is used as a food additive in many processed foods. We described in our previous paper that supplementation of a considerably large amount of ErA to normal guinea pigs showed a similar vitamin C activity of ErA to AsA, by measuring enzyme activities which were considered to be closely associated with the AsA level in tissues (1). However, little information is available
about the vitamin C activity of ErA in scorbutic guinea pigs. For instance, Reiff and Free reported that even when 100mg ErA was supplemented to 2-week-AsA-deficient guinea pigs, the animals continued to lose weight and eventually died, and they concluded that ErA did not have antiscorbutic activity (2). On the contrary, Pelletier and Godin reported that the body weight of guinea pigs which were supplemented with ErA 40mg/day after 17 days of AsA depletion recovered (3). The information on vitamin C activity of ErA in scorbutic guinea pigs mentioned above seemed to be conflicting.

This study aimed to clarify the vitamin C activity of ErA in scorbutic guinea pigs. The activities of aniline hydroxylase, and of serum alkaline phosphatase and the content of liver cytochrome P-450, which are known to be influenced by the AsA level in tissues, were measured. Also, the contents of AsA and ErA in the liver and adrenal glands of guinea pigs which recovered from AsA-deficiency by supplementation of AsA or ErA were determined.

MATERIALS AND METHODS

Animals. Male Hartley guinea pigs with initial body weights of 220g individually placed in wire cages were fed an AsA-deficient diet (4) and water ad libitum.

Experimental design. After 16 days of AsA depletion, the guinea pigs were divided into two groups: 1) the control group supplemented with AsA had 3 subgroups (A, B, and C), composed of 5, 9, and 4 animals, respectively, and 2) the experimental group supplemented with ErA had 4 subgroups (D, E, F, and G), composed of 7, 6, 7, and 5 animals, respectively. Groups A, B, and C were orally supplemented with 1, 5, and 100mg AsA/day, respectively. Groups D, E, F, and G were supplemented with 1, 5, 20, and 100mg ErA/day, respectively. AsA or ErA was dissolved in water immediately before use. At day 4 of repletion, guinea pigs were sacrificed and the liver and adrenal glands were removed and blood was collected.

It is considered that the activity of vitamin C is associated with the rate of progress during convalescence in scorbutic guinea pigs. Based on our previous results, supplementation of 5mg AsA to 16-day-AsA-deficient guinea pigs showed little recovery from scurvy at day 4 of repletion (5) but showed a complete recovery at day 20 (5). We presumed that the animals were in a convalescing stage at day 4 of repletion, and, to compare the effect of AsA and ErA on the recovery of AsA-deficient-guinea pigs from scurvy, we decided to carry out this experiment using a 4-day repletion period.

Enzyme assay. The activity of liver aniline hydroxylase was assayed according to the method of Imai et al. (6). The activity of serum alkaline phosphatase was measured using Alkaline Phospha B-Test (Wako Pure Chemical, Osaka, Japan). The content of liver microsome cytochrome P-450 was determined by the method of Omura and Sato (7).

Protein was measured by the method of Lowry et al. (8), using bovine serum
albumin as a standard.

**Determination of AsA and ErA contents.** The contents of AsA and ErA in the liver and adrenal glands were simultaneously determined by using HPLC, as described in our previous paper (4).

**Statistical analysis.** Analysis of variance and Tukey’s method were performed using the SYSTAT/SYGRAPH software (SYSTAT/SYGRAPH, 1992, Statistics, Version 5.2, Systat Inc. Evanston, IL).

**RESULTS**

Figure 1 shows body weight changes of guinea pigs during the depletion and repletion periods. Guinea pigs supplemented with 5 and 100 mg AsA (groups B and C, respectively) showed an apparent body weight recovery, but supplementation of 1 mg AsA was not enough to recover body weight loss. In ErA-supplemented groups, only the 100 mg ErA-supplemented guinea pigs (group G) showed body weight gain during the repletion period, and the body weight gain was similar to those of groups B and C. Guinea pigs supplemented with 20 and 5 mg ErA (groups F and E, respectively) did not show gains and those supplemented with 1 mg ErA (group D) continued to lose body weight.

Table 1 shows the contents of AsA and ErA in the liver and the adrenal glands of guinea pigs supplemented with AsA or ErA at day 4 of repletion. The content of AsA in the liver increased with the amount of AsA supplementation. On the other hand, the content of ErA in the liver of guinea pigs supplemented with 100
Table 1. Contents of AsA and ErA in liver and adrenal glands of guinea pigs supplemented with AsA or ErA at day 4 of repletion.

| Group | Liver AsA | ErA | Adrenal glands AsA | ErA |
|-------|-----------|-----|-------------------|-----|
| A     | 0.35±0.22a¹ | —   | 5.7±1.7a¹ | —   |
| B     | 1.54±0.74a² | —   | 13.0±4.0a   | —   |
| C     | 5.71±1.65b  | —   | 44.1±9.0b    | —   |
| D     | ND         | ND  | trace          | trace|
| E     | ND         | ND  | trace          | 3.8±1.4a |
| F     | ND         | ND  | 0.4±0.2a      | 4.3±2.0a² |
| G     | ND         | 0.35±0.22 | ND           | 10.3±2.4a² |
| H²    | 0.06±0.03  | —   | 1.0±0.3       | —   |

* A: 1 mg AsA-supplemented group; B: 5 mg AsA-supplemented group; C: 100 mg AsA-supplemented group; D: 1 mg ErA-supplemented group; E: 5 mg ErA-supplemented group; F: 20 mg ErA-supplemented group; G: 100 mg ErA-supplemented group.
* Values (mg/100 g tissue) are means±SE; n=4-6. Means in the same column not sharing a common superscript letter are significantly different (p<0.05).
* Sixteen-day-AsA-deficient group (data from Arakawa et al. (4)).

Table 2. Activity of liver aniline hydroxylase in guinea pigs supplemented with AsA or ErA at day 4 of repletion.

| Group | Activity (nmol p-aminophenol formed/(mg prot·min)) |
|-------|--------------------------------------------------|
| A     | 0.157±0.054¹ |
| B     | 0.169±0.020 |
| C     | 0.219±0.092 |
| D     | 0.089±0.025 |
| E     | 0.123±0.029 |
| F     | 0.157±0.030 |
| G     | 0.173±0.016 |
| H²    | 0.109±0.015 |

* A-G: see Table 1 legend. *¹ Values are means±SE; n=4-7. *²Sixteen-day-AsA-deficient group (data from Suzuki et al. (5)).

mg ErA (group G) which was 0.35±0.22 mg/100 g and that of AsA in the guinea pigs supplemented with 1 mg AsA (group A) which was 0.35±0.22 mg/100 g showed no difference. The AsA content in the adrenal glands of the AsA-supplemented guinea pigs increased with the dose of AsA. A small amount of AsA was retained in the adrenal glands in groups D, E, and F in the ErA-supplemented groups. ErA was found in the ErA-supplemented guinea pigs and the content increased with the dose of ErA. However, the amount of ErA in the adrenal glands of ErA-supplemented guinea pigs was much lower than that of AsA in the AsA-supplemented guinea pigs, when the dose of ErA was equal to that of AsA.

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The contents of ErA in the adrenal glands of groups F and G were 4.3 ± 2.0 and 10.3 ± 2.4 mg/100 g, respectively, and those of AsA in groups A and B were 5.7 ± 1.7 and 13.0 ± 4.0 mg/100 g, respectively. It seemed that the difference between the contents of ErA and AsA was negligible.

Table 2 shows the activity of liver aniline hydroxylase of guinea pigs supplemented with AsA or ErA. The activity of guinea pigs supplemented with AsA or ErA increased with the increased amount of AsA or ErA supplementation. The activity of group A was higher than those of groups D and E. Group D showed the lowest activity in all supplemented groups, which was similar to that of 16-day-AsA-deficient guinea pigs (Group H). The activities of groups G and F were similar to those of groups B and A, respectively.

As shown in Table 3, the content of liver cytochrome P-450 of guinea pigs

| Group | Content (nmol/mg prot) |
|-------|------------------------|
| A     | 0.64 ± 0.07a¹         |
| B     | 0.67 ± 0.04a           |
| C     | 1.13 ± 0.18b           |
| D     | 0.51 ± 0.07a           |
| E     | 0.55 ± 0.07a           |
| F     | 0.67 ± 0.03a           |
| G     | 0.70 ± 0.06a           |
| H²    | 0.50 ± 0.05            |

A–G: see Table 1 legend. ¹Values are means ± SE; n=4–9. Means in the column not sharing a common superscript letter are significantly different (p<0.05).
²Sixteen-day-AsA-deficient group (data from Suzuki et al. (5)).

| Group | Activity (mmol p-nitrophenol liberated/(liter·h)) |
|-------|-------------------------------------------------|
| A     | 5.41 ± 0.60b,c¹                                 |
| B     | 9.41 ± 0.93c,d                                 |
| C     | 10.99 ± 1.17d                                  |
| D     | 1.56 ± 0.26a                                   |
| E     | 3.56 ± 0.74b,h                                 |
| F     | 6.61 ± 1.14b,c                                 |
| G     | 9.33 ± 1.09c,d                                 |
| H²    | 1.21 ± 0.09                                    |

A–G: see Table 1 legend. ¹Values are means ± SE; n=4–9. Means in the column not sharing a common superscript letter are significantly different (p<0.05).
²Sixteen-day-AsA-deficient group (data from Suzuki et al. (5)).
supplemented with AsA or ErA seemed to increase with the increased amount of AsA or ErA supplementation, and the content of cytochrome P-450 in group C was highest in all supplemented groups. Statistically, no significant difference in the contents of cytochrome P-450 among groups A, B, D, E, F, and G was observed.

Table 4 shows the activity of serum alkaline phosphatase of guinea pigs supplemented with AsA or ErA. This activity increased with the amount of AsA or ErA supplementation. The Activity of group B was similar to those of groups C and G. On the other hand, the activity of group A was similar to that of group F and was higher than those of groups D and E. Group D showed the lowest activity which was of similar level to 16-day-AsA-deficient guinea pigs (Group H).

DISCUSSION

In this study, guinea pigs supplemented with 5 and 100 mg AsA and 100 mg ErA (groups B, C, and G, respectively) showed recovery in body weight at day 4 of repletion. This result suggested that oral supplementation of 5 mg or more of AsA to scorbutic guinea pigs was enough for recovery from scurvy, but it needed 100 mg ErA or more to recover from scurvy. Thus, based on the result of recovery in body weight, vitamin C activity of ErA was lower than that of AsA.

The activities of liver aniline hydroxylase and of serum alkaline phosphatase and the content of liver cytochrome P-450 in guinea pigs supplemented with AsA or ErA increased with the increased amount of AsA or ErA supplementation at day 4 of repletion. However, in the case of guinea pigs supplemented with 1 mg ErA, the enzyme activities and the cytochrome P-450 content were similar to those of 16-day-AsA-deficient guinea pigs, and their body weight continued to decrease. Based on these results, oral supplementation of 1 mg ErA was not enough to recover from scurvy. Supplementation of 5 mg or more of ErA to scorbutic guinea pigs showed recovery in both the enzyme activities and the cytochrome P-450 content. The enzyme activities and the cytochrome P-450 content in ErA-supplemented guinea pigs were similar to those of AsA-supplemented guinea pigs when the supplemented amount of ErA was 20 times as much as that of AsA. Thus, the vitamin C activity of ErA seemed to be much lower than that of AsA. Conversely, we reported that the vitamin C activity of ErA was similar to that of AsA, when a considerably large amount of ErA was supplemented to normal guinea pigs (I). However, our results showed that the vitamin C activity of ErA in AsA-deficient guinea pigs was lower than that in normal guinea pigs, suggesting an association between the vitamin C activity of ErA with AsA level in tissues of guinea pigs.

In a previous paper, we found that the amount of ErA absorbed in the small intestine of guinea pigs was much lower than that of AsA at the same dose level, determined by perfusion of the small intestine (9). Moreover, the amount of AsA excreted in urine 8 h after intraperitoneal supplementation of 5 mg AsA was lower than that of ErA in the guinea pigs supplemented intraperitoneally with the same dose of ErA (unpublished data). On the other hand, ErA and AsA were found to
have the same stimulating function in proline hydroxylation reaction in vitro, suggesting that there might be no difference in their vitamin C activity (10). Oral supplementation of a small amount of ErA showed less vitamin C activity than AsA in normal guinea pigs, but the supplementation of a considerably large amount of ErA resulted in similar vitamin C activity with AsA (1). The results suggested that ErA was less absorbed and retained in tissues than AsA, therefore, a large amount of ErA is necessary to supply the same vitamin C activity provided by AsA.

Since the amount of vitamin C required in the tissues of AsA-deficient guinea pigs is higher than that of normal ones, a higher amount of ErA supplementation is necessary to meet the vitamin C requirements in the tissues of AsA-deficient guinea pigs. ErA, therefore, showed lower vitamin C activity in AsA-deficient guinea pigs than in normal ones.

At day 4 of repletion, the amount of ErA in 100 mg ErA-supplemented guinea pigs was similar compared to that of AsA in 1 mg AsA-supplemented guinea pigs. On the contrary, the enzyme activities and the cytochrome P-450 content of 100 mg ErA-supplemented guinea pigs were higher than those of 1 mg AsA-supplemented guinea pigs and were similar to those of 5 mg AsA-supplemented animals. The enzyme activities and the cytochrome P-450 content per milligram of ErA retained in the liver of 100 mg ErA-supplemented animals were higher than those per milligram of AsA retained in the liver of 5 mg AsA-supplemented animals. The results suggested that the tissue content of ErA which was measured 24 h after the last supplementation in this study could not absolutely reflect the vitamin C activity. On the other hand, it was also reported that equal concentrations of ErA and AsA showed the same effect on prolylhydroxylase activity in vitro (10), therefore, it is presumed that the same amount of AsA and ErA would show the same vitamin C activity in the tissues. Thus, since in this study 100 mg ErA-supplemented guinea pigs showed similar recovery in the enzyme activities and the cytochrome P-450 content to those of 5 mg AsA-supplemented guinea pigs, it might be considered that the content of ErA in tissues was similar to that of AsA just after oral supplementation. Further study is necessary to measure the content of ErA in tissues of guinea pig as a function of time to estimate the vitamin C activity of ErA.

Our observations in this study showed that vitamin C activity of ErA in scorbutic guinea pigs might be lower than that in normal guinea pigs and that it might be dependent on the AsA level in animal tissues.

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