Net Vitamin C Activity of Erythorbic Acid in Guinea Pigs

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Summary The enzyme activities, which are influenced by the vitamin C level in tissues, were measured to evaluate the vitamin C activity of erythorbic acid (ErA) in guinea pigs administered ErA. Guinea pigs were divided into two groups: animals in one group (control group) were administered 1, 5, and 100mg/day ascorbic acid (AsA) and those in the other group (supplemented group) were administered 1, 5, 20, and 100 mg/day ErA for 16 days. At the end of the experimental period, they were sacrificed, blood was collected, and their livers were removed. The activities of liver aniline hydroxylase, of liver acid phosphatase, and of serum alkaline phosphatase, and the content of liver cytochrome P-450 were assayed. The activities of aniline hydroxylase and serum alkaline phosphatase and the content of liver cytochrome P-450 of the guinea pigs administered 1mg ErA were lower than those of the guinea pigs administered 1mg AsA. However, the enzyme activities and liver cytochrome P-450 content in the guinea pigs administered 5mg or more of ErA were similar in level to those in the guinea pigs administered 5mg AsA. These results suggested that administration of a considerably high amount of ErA to guinea pigs showed a similar vitamin C activity to that of AsA, which might suggest that vitamin C activity of ErA may be more than one-twentieth that of AsA, as has been generally believed.

Key Words erythorbic acid, ascorbic acid, aniline hydroxylase, serum alkaline phosphatase, cytochrome P-450, acid phosphatase, vitamin C activity

It was reported that in vitamin C-deficient guinea pigs, the activities of liver aniline hydroxylase, of serum alkaline phosphatase, and the content of liver cytochrome P-450 decreased and the activity of liver acid phosphatase increased (1–4). These enzyme activities were considered to be closely associated with

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vitamin C level in tissues of guinea pigs. Several workers reported the antiscorbutic activity of erythorbic acid (ErA), but since they could not determine separately at a time the contents of ascorbic acid (AsA) and ErA, they measured only the sum of ErA and AsA contents in the tissues of the guinea pigs and/or their body weight gains. For example, Yourga et al. reported that ErA had one-twentieth the vitamin C activity of AsA, determined on the basis of a 25-day weight response of guinea pigs (5). Zilva described that the sum of ErA and AsA contents in tissues of guinea pigs injected ErA was less than that of guinea pigs injected AsA (6); however, Reiff and Free concluded that ErA had no vitamin C activity in guinea pigs, by comparing the weight response between the ErA- and the AsA-supplemented guinea pigs (7). So far, only a few workers have evaluated vitamin C activity of ErA by measuring enzyme activity influenced by vitamin C level in tissues. Therefore, the activities of these enzymes in guinea pigs administered ErA or AsA were measured and compared to evaluate the vitamin C activity of ErA.

MATERIALS AND METHOD

Animals and diet. Male guinea pigs (Hartley strain) with initial body weight of about 220 g were used. All animals, individually kept in wire cages, were fed AsA-deficient diet and given water ad libitum. The same composition of the diet described in our previous paper was used (8). Their body weights were recorded daily.

Experimental plan. All animals were randomly divided into two groups: animals in one group were orally supplemented with AsA and those in the other group were supplemented with ErA. Group A was orally supplemented with 1 mg AsA/day, group B with 5 mg AsA/day, group C with 100 mg AsA/day (groups A, B, and C were used as control). Group D was supplemented with 1 mg ErA/day, group E with 5 mg ErA/day, group F with 20 mg ErA/day, and group G with 100 mg ErA/day. AsA or ErA was dissolved in water immediately before use.

At day 16 of the experimental period, these animals were sacrificed after 24-h fasting. The blood was collected from the abdominal aorta under light ether anesthesia and the liver was removed.

Enzyme assays. The activities of liver aniline hydroxylase, liver acid phosphatase, and the content of liver cytochrome P-450 were measured by using the methods of Imai et al. (8), Desai (9), and Omura and Sato (10), respectively, and the activity of serum alkaline phosphatase was determined by using Alkaline Phospha B-Test (Wako Pure Chemical Industries, Osaka, Japan). The same methods used in our previous paper (11) were employed.

Protein was measured by the method of Lowry (12), using bovine serum albumin as standard.

Statistical tests. Test of significance on the means of two groups were done using by Student’s t-test or the Cochran-Cox test, depending on whether variances were equal or different.
RESULTS

The aniline hydroxylase activity of the guinea pigs administered AsA or ErA is presented in Table 1. This activity seemed to increase with the increased amount of AsA or ErA administered. However, in AsA-supplemented groups the enzyme activity of 100 mg AsA-supplemented guinea pigs (group C) was not significantly different from that of the guinea pigs administered 5 mg AsA (group B). In ErA-supplemented groups, the enzyme activity of 100 mg ErA-supplemented guinea pigs (group G) was not significantly different from the guinea pigs administered 5 mg (group E) or 20 mg ErA (group F). Moreover, the enzyme activity of group B was similar to that of groups E, F, and G. The activity of the guinea pigs administered 1 mg ErA (group D) was lowest in all supplemented groups and was close to that of AsA-deficient guinea pigs. The aniline hydroxylase activity of AsA-supplemented guinea pigs was higher than that of ErA-supplemented guinea pigs where the amount of AsA administered was equal to that of ErA administered.

Table 2 shows the liver cytochrome P-450 content of the guinea pigs administered AsA or ErA. No significant difference in the content of liver cytochrome P-450 was found among groups B, C, D, E, F, and G and the content of AsA-deficient guinea pigs was apparently lower than those of all supplemented groups.

Table 3 shows the liver acid phosphatase activity of the guinea pigs administered AsA or ErA. The acid phosphatase activity of group A was similar to that of group D and was significantly higher than that of groups B, C, E, F, and G. Also,

Table 1. Liver aniline hydroxylase activity of guinea pigs supplemented with AsA or ErA.

| Group | Activity (nmol p-aminophenol formed/min/mg prot) |
|-------|------------------------------------------------|
| A     | 0.197±0.019*ab                                 |
| B     | 0.240±0.014c                                   |
| C     | 0.287±0.018^gdef                               |
| D     | 0.131±0.022bghi                                |
| E     | 0.206±0.021*g                                  |
| F     | 0.203±0.017th                                  |
| G     | 0.246±0.025i                                   |
| H**   | 0.109±0.015                                    |

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 are means±SE, n=5–13. Means with a common superscript letter are significantly different. ^ p<0.05, _ p<0.01, * p<0.001. **H, 16-day AsA-deficient group (data from Suzuki et al. (1989) J. Nutr. Sci. Vitaminol., 35, 123–131).
Table 2. Liver cytochrome P-450 content of guinea pigs supplemented with AsA or ErA.

| Group | Content (nmol/mg prot) |
|-------|------------------------|
| A     | 1.02 ± 0.05*a,b,c      |
| B     | 0.86 ± 0.04*a          |
| C     | 0.90 ± 0.04            |
| D     | 0.75 ± 0.06*c          |
| E     | 0.83 ± 0.11            |
| F     | 0.86 ± 0.07            |
| G     | 0.83 ± 0.06*b          |
| H**   | 0.50 ± 0.05            |

A–G, see Table 1 legend. *Values are means ± SE, n = 6–13. Means with a common superscript letter are significantly different. a,b,c;p < 0.05, c;p < 0.01. **H, 16-day AsA-deficient group (data from Suzuki et al. (1989) J. Nutr. Sci. Vitaminol., 35, 123–131).

Table 3. Liver acid phosphatase activity of guinea pigs supplemented with AsA or ErA.

| Group | Activity (nmol p-nitrophenol liberated/min/mg prot) |
|-------|--------------------------------------------------|
| A     | 55.7 ± 2.5*a,b,c,d,e                            |
| B     | 42.1 ± 2.3df                                      |
| C     | 40.8 ± 1.5*g                                      |
| D     | 52.9 ± 2.5g                                      |
| E     | 43.9 ± 3.3*a                                      |
| F     | 46.7 ± 3.1b                                      |
| G     | 44.9 ± 3.8*c                                      |
| H**   | 58.9 ± 3.2                                       |

A–G, see Table 1 legend. *Values are means ± SE, n = 5–13. Means with a common superscript letter are significantly different. a,b,c,d,e;p < 0.05, f;p < 0.01, g,h,i;p < 0.001. **H, 16-day AsA-deficient group (data from Suzuki et al. (1989) J. Nutr. Sci. Vitaminol., 35, 123–131).

the enzyme activity of group D was significantly higher than that of groups B and C. However, the enzyme activity of group B was not significantly different from that of groups C, E, F, and G.

As shown in Table 4, the serum alkaline phosphatase activity was similar in all AsA-supplemented groups. In the ErA-supplemented groups, this enzyme activity was also similar in groups E, F, and G, but the activity of group D was lowest among all supplemented groups and was close to that of AsA-deficient animals. There was no significant difference in the activity among groups A, B, C, F, and G. When the amount of AsA administered was equal to that of ErA, the activity of the AsA-supplemented guinea pigs was higher than that of the ErA-supplemented...
Table 4. Serum alkaline phosphatase activity of guinea pigs supplemented with AsA or ErA.

| Group | Activity (mmol p-nitrophenol liberated/h/liter) |
|-------|-----------------------------------------------|
| A     | 6.02±0.42<sup>*,<sup>b</sup></sup>            |
| B     | 5.70±0.49<sup>d</sup>                           |
| C     | 5.28±0.66<sup>e</sup>                           |
| D     | 2.49±0.39<sup>ace</sup>                         |
| E     | 4.24±0.37<sup>bdf</sup>                         |
| F     | 5.76±0.99<sup>g</sup>                           |
| G     | 5.04±0.22<sup>h</sup>                           |
| H**   | 1.21±0.09                                      |

A–G, see Table 1 legend. *Values are means±SE, n=4–13. Means with a common superscript letter are significantly different. <sup>a</sup><sup>,<sup>p<0.05, <sup>b</sup><sup>,<sup>c</sup><sup>,<sup>d</sup><sup>,<sup>e</sup><sup>,<sup>f</sup><sup>,<sup>g</sup><sup>,<sup>h</sup><sup>,<sup>p<0.01. **H, 16-day AsA-deficient group (data from Suzuki et al. (1989) J. Nutr. Sci. Vitaminol., 35, 123–131).

Table 5. Final body weight gains of guinea pigs supplemented with AsA or ErA.

| Group | Relative body weight gain |
|-------|---------------------------|
| A     | 118±3<sup>a</sup>        |
| B     | 123±4                     |
| C     | 124±3<sup>b</sup>        |
| D     | 115±3<sup>bc</sup>       |
| E     | 119±4                     |
| F     | 123±6                     |
| G     | 127±3<sup>bc</sup>       |
| H**   | 101±4                     |

A–G, see Table 1 legend. *Values are means±SE, n=4–18. Means with a common superscript letter are significantly different. <sup>a</sup><sup>,<sup>p<0.05, <sup>b</sup><sup>,<sup>c</sup><sup>,<sup>p<0.01. **H, 16-day AsA-deficient group (data from Arakawa et al. (1986) J. Nutr. Sci. Vitaminol., 32, 171–181).

guinea pigs.

The relative body weight gains of the guinea pigs administered AsA or ErA are presented in Table 5. Groups A, B, C, E, F, and G showed almost similar body weight gains but group D showed the lowest gain, which was significantly lower than in groups C and G, in particular. On the contrary, AsA-deficient animals did not show any increase in body weight gain for comparative purposes, this information was excerpted from a previous study, as indicated in the table footnote.

DISCUSSION

Since the activities of aniline hydroxylase, of acid phosphatase, and of serum
alkaline phosphatase, and the content of liver cytochrome P-450 were not significantly different between the guinea pigs administered 5 mg AsA and those administered 100 mg AsA, the administration of 5 mg AsA could be concluded to be sufficient to keep the enzyme activities at a normal level. Also, Boyle et al. reported that administration of 5 mg AsA showed normal growth in dentine of guinea pigs (13).

The enzyme activities and the content of cytochrome P-450 in groups administered 5 mg or more of ErA (groups E, F, and G) were similar to those of the guinea pigs administered 5 mg AsA (Tables 1-4). It seemed that an administration of 5 mg or more of ErA could maintain the enzyme activities in guinea pigs in level with those administered 5 mg AsA. Although the content of ErA in the tissues was much lower than that of AsA (14), the guinea pigs administered 5 mg or more of ErA were not in a vitamin C-deficient state. Also, the body weight gains in both ErA- and AsA-supplemented groups were similar when the amount of ErA administered was equal to that of AsA. These observations suggested that administration of a considerable amount of ErA might have a similar enzyme activity effect with AsA in guinea pigs.

On the contrary, in the guinea pigs administered 1 mg AsA, the aniline hydroxylase activity was lower than that of the guinea pigs administered 5 mg AsA, but their acid phosphatase activity was higher than that of the guinea pigs administered 5 mg AsA. This shows that the administration of only 1 mg AsA seemed not enough to maintain a normal vitamin C state in guinea pigs. The activities of aniline hydroxylase and serum alkaline phosphatase and the content of cytochrome P-450 of the guinea pigs administered 1 mg ErA were significantly lower than those of the guinea pigs administered 1 mg AsA. This suggested that the antiscorbutic activity of 1 mg ErA was much lower than that of 1 mg AsA in guinea pigs. When a small amount of ErA was administered, it could not maintain the levels of enzyme activities that AsA of the same dose could. Our previous results showed that there was a remarkable difference in the AsA and ErA uptake in the tissues of guinea pigs, and the absorption mechanism of AsA in the tissues may be different from that of ErA (14). Moreover, since the retention mechanism of ErA seemed to be different from that of AsA, ErA content might be lower than AsA in the tissues (14). As the amount of ErA retained in tissues was smaller than that of AsA, the enzyme activities of the guinea pigs administered 1 mg ErA was lower than those of the guinea pigs administered 1 mg AsA. On the other hand, the experimental result conducted on the activity of prolylhydroxylase prepared from chick embryo, showed that when the concentration of ErA was equal to that of AsA, ErA could show the same prolylhydroxylase activity effect as that of AsA in vitro (15). So, if the content of ErA in the tissues is equal to that of AsA required, ErA might show the same vitamin C activity as AsA.

Based on the above results, it was concluded that, if a considerably high amount of ErA was administered to guinea pigs, its vitamin C activity was similar to that of AsA, which suggested that vitamin C activity of ErA might be higher.

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than one-twentieth that of AsA, as has been generally believed (5). However, in the case of the administration of small dose of ErA, there was a slight difference in their vitamin C activity. This difference might be partly attributed to the difference in the absorption and retention mechanisms of ErA and AsA.

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