Comparison of Absorption of Erythorbic Acid and Ascorbic Acid in Guinea Pig Small Intestine

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Summary Both the ascorbic acid (AsA) and erythorbic acid (ErA) absorption in the small intestine of guinea pigs were determined by the perfusion of the small intestine using isotonic phosphate buffer recycled in situ. The absorption rate of AsA in the small intestine of guinea pigs was higher than that of ErA; however, $K_m$ of AsA absorption was lower than that of ErA in normal guinea pigs. In AsA-deficient guinea pigs, the absorption rates of both AsA and ErA were higher than those in normal ones. The absorption of AsA and ErA in the small intestine of guinea pigs was inhibited by ouabain. Furthermore, AsA and ErA inhibited each other’s absorption. Based on the results, the net amount of the absorbed ErA in the small intestine may be lower than that of AsA, and ErA absorption mechanism seemed to be similar to that of AsA. The absorption rate of both AsA and ErA in the small intestine of guinea pig might be dependent on the AsA level in the tissues.

Key Words erythorbic acid, ascorbic acid, small intestine perfusion

Erythorbic acid (ErA), which is one of the epimers of ascorbic acid, is used as a food additive. In our previous paper, we reported that the content of ErA in the tissues of guinea pigs supplemented with ErA was much lower than that of ascorbic acid (AsA) in the guinea pigs supplemented with the same amount of AsA (1). This suggested that the rate of AsA absorption in the tissues might be different from that of ErA. So, it is important to get more information on the ErA absorption mechanism in the tissues.

As AsA is one of the essential vitamins for human and guinea pigs, the clarification of the absorption mechanism of AsA and its stereoisomers in gastrointestinal is necessary. Hornig et al. reported that the site of absorption in the guinea pig orally supplemented with $^{14}$C-AsA was located in the duodenum and the proximal part of the jejunum (2). Mellors et al. determined the unidirectional influx of $^{14}$C-AsA across the mucosal border of guinea pig ileum in vitro, and
reported that the maximal influx was 140 nmol/cm²·h and the AsA concentration which gave a half-maximal influx \( (K_m) \) was 1.0 mM \( (3) \). Siliprandi et al. reported that AsA was transported by a mobile carrier mechanism in the study using brush border vesicles from the guinea pig small intestine \( (4) \).

Rose and Nahrwold found that the influx of AsA using segment of the small intestine of the guinea pigs given high amount of AsA for 28 days was lower than that of the normal guinea pigs \( (5) \). AsA dose levels seemed to affect the AsA absorption in the small intestine. However, since there is little information on ErA absorption, it is important to determine whether the absorption rate of ErA in the guinea pig small intestine is dependent on the AsA level in the tissues or not.

In this paper, we determined the AsA and ErA absorption rate in the small intestine of the normal and AsA-deficient guinea pigs by perfusion using isotonic phosphate buffer recycled \textit{in situ}, in order to know the difference in the absorption of AsA and ErA, and to know the change in both AsA and ErA absorption rate in the small intestine of guinea pig with low AsA tissue level.

**MATERIALS AND METHODS**

The whole small intestine of the guinea pigs used was not removed from the live guinea pig during experiment, and thus the arterial, neural and lymphatic connections remained intact. Therefore, we would rather say that the results in this experiment were considered to be obtained from guinea pigs almost in normal physiological conditions compared with the results obtained from using brush border vesicle or segments of the guinea pig small intestine.

**Experiment 1.** Hartley male guinea pigs used in this experiment were divided into two groups: one group was supplemented with 5 mg/day AsA orally and another group was supplemented with no AsA for 10 days.

Isotonic phosphate buffer (pH 6.5) containing 0.5 mM AsA or ErA added with 15 mg/liter of phenol red was perfused in the small intestine of the AsA-supplemented and AsA-deficient guinea pigs which were fasted over night.

**Experiment 2.** Isotonic phosphate buffer (pH 7.4) containing AsA and/or ErA added with 15 mg/liter of phenol red was perfused in the small intestine of the guinea pigs supplemented with 5 mg/day AsA. Also, 0.01 mM ouabain was added to the recirculated buffer containing 0.5 mM AsA or ErA.

**Perfusion procedure.** The guinea pigs were anesthetized with pentobarbital and the small intestine was cannulated at the duodenal and ileal ends \textit{in situ}, using the method of Koizumi et al. \( (6) \). The small intestine was first perfused with 100 ml of 0.9% NaCl solution and then 10 ml of buffer solution with temperature maintained at 37°C. The tubings attached to the inlet and outlet cannule were then connected to a flask containing 40 ml of the buffer solution which was circulated continuously through the small intestine for 1 h, at 37°C by a peristaltic pump adjusted at a flow rate of 5 ml/min. The buffer solution perfused was pipetted out at 0, 10, 20, 30, 45, and 60 min after the start of circulation.
**Determination of AsA and ErA concentration.** AsA and ErA concentrations of the buffer solution perfused were measured using a high-performance liquid chromatography (7).

**Determination of phenol red concentration.** Phenol red was added to buffer solution as an indicator for the correction of perfusate volume. Concentration of phenol red in perfused buffer solution was determined by measuring the optical absorption of alkalized solution at 550 nm after pH adjustment with 0.1 N NaOH.

**Determination of disappearance rate of AsA and ErA in the perfusate.** Forty milliliters of buffer solution containing AsA and/or ErA were circulated and periodically pipetted out in the same way as described above except that a silicon tube was used instead of the small intestine of guinea pig.

**Absorption rate constant of AsA and ErA.** The decrease in the concentration of AsA and ErA of the perfusate was considered to be caused by both their absorption in the small intestine and non enzymatic degradation which occurred simultaneously.

The decrease rate constant of AsA and ErA was calculated from the percentage of AsA or ErA that remained in the perfusate. So, absorption rate constant was numerically calculated from the decrease rate constant in both the small intestine perfusate and the perfusate recirculated in the silicon tube.

**RESULTS AND DISCUSSION**

Table 1 shows the absorption rate of AsA and ErA in the small intestine of the AsA-supplemented and AsA-deficient guinea pigs. The absorption rate of AsA was higher than that of ErA in both the AsA-supplemented guinea pigs and AsA-deficient guinea pigs. It seemed that AsA was absorbed more easily than ErA in the small intestine of both the normal and AsA-deficient guinea pigs.

The absorption rate of AsA in the AsA-deficient guinea pigs increased about 3 times that in the AsA-supplemented animals. Also, the absorption rate of ErA showed the same tendency as AsA; however, the increase of the absorption rate of AsA was higher than that of ErA. In the AsA-deficient guinea pigs, both AsA and ErA absorption rate increased. On the other hand, Siliprandi et al. concluded that ErA was considered to be uptaken by the AsA transporter, based on their observation that ErA inhibited AsA absorption (4). Our result also suggested that

<table>
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<th>Group</th>
<th>AsA (mmol/liter/h)</th>
<th>ErA (mmol/liter/h)</th>
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<tr>
<td>Normal guinea pigs(^1)</td>
<td>0.19±0.01*</td>
<td>0.09±0.03</td>
</tr>
<tr>
<td>AsA-deficient guinea pigs(^2)</td>
<td>0.57±0.16</td>
<td>0.14±0.06</td>
</tr>
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\(^1\)Five mg AsA-supplemented guinea pigs. \(^2\)Ten-day-AsA-deficient guinea pigs. *Values are means±SE; n=3–4.
ErA carrier mediate could possibly be the same as that of AsA.

The 10-day-AsA-deficient guinea pigs did not show body weight loss and the activity of liver acid phosphatase and the content of liver cytochrome P-450 were similar to the AsA-supplemented animals, but AsA hardly remained in their tissues as reported in our previous paper (7, 8). During this period, they did not show any scorbutic symptom, but the absorption rates of AsA and ErA were higher than those in normal guinea pigs. The absorption rate seemed to have been increased by AsA depletion. Our observation showed that the amount of AsA absorbed in the small intestine of the guinea pig seemed to depend on the AsA level in the tissues. However, it was not clear whether the increase of the AsA absorption rate was due to the increase in the amount of the carrier mediate and/or to the increase of the affinity of carrier mediate for AsA.

Maximum rate of absorption ($V_{\text{max}}$) and Michaelis constant ($K_m$) of both AsA and ErA absorption were calculated using Lineweaver-Burk plot, Figs. 1 and 2, respectively. $V_{\text{max}}$ of AsA was 0.6 mmol·liter$^{-1}$·h$^{-1}$ and that of ErA was 0.3 mmol·liter$^{-1}$·h$^{-1}$. $K_m$ of AsA was 0.6 mM and that of ErA was 1.4 mM. $V_{\text{max}}$ of AsA was higher than that of ErA; on the contrary, $K_m$ of AsA was lower than that of ErA. These results show that AsA has stronger affinity for carrier mediate than ErA has. So, these observations suggested that AsA have been absorbed more effectively than ErA. Because the amount of AsA absorbed from the small intestine of guinea pig seemed to be higher than that of ErA, AsA content in tissues of guinea pigs might be higher than ErA on the same dose level.

![Fig. 1. Lineweaver-Burk plot of AsA absorption in guinea pig small intestine perfused with AsA.](image-url)
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Fig. 2. Lineweaver-Burk plot of ErA absorption in guinea pig small intestine perfused with ErA. ■ represents means and bars represent SE. n=4–6.

Table 2. Effect of addition of ouabain on AsA and ErA absorption in small intestine of guinea pig.

Table 2 shows that the addition of ouabain, one of the inhibitors of glucose active transport system, reduced the absorption rate of AsA in the small intestine of guinea pig. Also, the absorption rate of ErA was reduced by addition of ouabain. The percentage of decrease of the AsA absorption rate was similar to that of ErA. Stevenson and Brush concluded that guinea pig has an active transport mechanism for the intestinal absorption of AsA, using the small intestine rings of guinea pigs (9). Our result also suggested that both AsA and ErA were absorbed in the small intestine by active transport mechanism. Moreover, since ErA inhibited the AsA absorption in the small intestine (Fig. 3), the possibility of ErA being transported by the same carrier mediate as AsA remained.

In our previous paper we reported that the tissue AsA content of guinea pigs administered both 5 mg AsA and 100 mg ErA was lower than that of AsA in an
only 5 mg AsA-supplemented ones (10). Due to this observation, it was assumed that ErA inhibited AsA absorption in the guinea pig small intestine. Therefore, inhibitory effect of ErA on the AsA absorption in the small intestine was determined in this paper.

Inhibitory constant \((K_i)\) of ErA for the AsA absorption obtained using Dixon plot, shown as Fig. 3, was about 1.2 mM and that of AsA in an unpublished data was about 0.2 mM, showing that \(K_i\) of AsA was smaller than ErA. This shows that AsA is more affinitive to carrier mediate than ErA. The result indicated that, when the same amounts of AsA and ErA were simultaneously supplemented to guinea pigs, the amount of the absorbed AsA did not significantly decrease. This observation would explain partly our previous results that the AsA content in the tissues of the guinea pigs administered both 5 mg ErA and 5 mg AsA was almost the same as that of the guinea pigs administered only 5 mg AsA (10).

Based on our results, the absorption rate of both AsA and ErA might depend on the AsA level in the tissues of guinea pig. This might suggest that the amount of absorbed AsA was higher in low-AsA-supplemented guinea pigs than in normal ones. Because the absorption of AsA and ErA in the small intestine of guinea pig was inhibited by ouabain and both AsA and ErA inhibited each other's absorption, AsA could be absorbed in the guinea pig small intestine by active transport system, and also ErA seemed to be absorbed by the same mechanism as AsA. However, AsA has stronger affinity for the carrier mediate than ErA has and the absorption rate of AsA is higher than that of ErA. Our result suggested that the amount of the absorbed ErA in the small intestine of guinea pig could be lower than that of AsA. Therefore, this suggestion could partly explain the phenomena that the ErA content in the tissues of the guinea pig was lower than the AsA tissue content when the dose...
level of AsA was equal to that of ErA.

REFERENCES


