The Tissue Distribution of L-Ascorbic Acid and Dehydro-L-Ascorbic Acid in the Guinea Pigs Injected Intravenously with Dehydro-L-Ascorbic Acid

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Summary The tissue distribution of L-ascorbic acid (AsA) and dehydro-L-ascorbic acid (DAsA) in guinea pigs injected with DAsA intravenously was examined using high-performance liquid chromatography. DAsA injected into guinea pigs fed normal diets containing AsA (control group) was readily taken into erythrocytes, and AsA contents of plasma and other tissues rapidly increased after DAsA injection. In animals fed vitamin C-deficient diets, DAsA was also detected in erythrocytes; however, the increase of AsA in their tissues was considerably less than that of control group. From these results, it was suggested that utilization of DAsA as AsA in vitamin C-deficient guinea pigs was less than that of control animals, and the reduction mechanism of DAsA to AsA in vitamin C-deficient guinea pigs may have differed from that of control groups.

Key Words L-ascorbic acid, dehydro-L-ascorbic acid, intravenous injection, glutathione, tissue distribution, vitamin C-deficient guinea pigs

In previous reports (1), the antiscorbutic effect of dehydro-L-ascorbic acid (DAsA) in vitamin C-deficient guinea pigs was less than that of L-ascorbic acid (AsA). Hornig et al. (2) had reported the distribution of radioisotopes in tissues of healthy guinea pigs injected intravenously with [1-14C]DAsA. Since qualitative analysis of AsA was carried out using thin layer chromatography (TLC), AsA derived from injected DAsA was not detected in liver. This was compatible with other reports (3, 4). The utilization of DAsA is highly dependent on its reduction in the animal body. The rates of conversion of DAsA to AsA in vitamin C-deficient guinea pigs should be compared with those in control animals in order to clarify the reduction mechanism. In this study, the distribution of AsA and DAsA in guinea pigs injected intravenously with DAsA was investigated.
EXPERIMENTAL

Reagent. AsA was obtained from Wako Pure Chemical Industries, Ltd. DAsA was prepared by the method described in a previous paper (1).

Animals and diets. Male albino Hartley strain guinea pigs weighing about 250 g were housed in individual cages placed in a temperature- and humidity-controlled room. The animals were divided into two groups. The animals of the control group (group C) were fed for 16 days on a commercial diet purchased from Oriental Yeast Co., Ltd., which contained 0.9 g AsA/kg diet. The daily intake of AsA was approximately 5 mg per animal. The animals of the vitamin C-deficient group (group D) were fed for 16 days on a vitamin C-deficient diet described in a previous report (5). Each group contained 14–15 animals.

On day 16 of feeding, each animal was injected intravenously with 5 mg of DAsA, using pentobarbital sodium (Abbott Laboratories, North Chicago, Ill, U.S.A.) as an anesthetic. Blood (0.2–0.4 ml) was collected from the jugular vein in a heparinized syringe every 5 min for the first 30 min following injection and every 15 min thereafter. Four to six animals were sacrificed from each group prior to injection and 30 and 90 min after injection (groups C-0, D-0, C-30, D-30, C-90 and D-90, respectively) and their tissues were removed.

Blood analysis. The blood samples were centrifuged at 1,000 × g for 10 min. The plasma was extracted with 2% metaphosphoric acid, and further centrifuged at 3,000 × g for 5 min. The erythrocytes were prepared in the following way: the erythrocytes were washed 3 times with an ice-cold saline solution, prepared as a 50% suspension in saline solution and saturated with carbon monoxide, and then centrifuged at 1,000 × g for 10 min. The resulting segment was extracted with 5% metaphosphoric acid, and further centrifuged at 3,000 × g for 5 min. The supernatant was used for determination of AsA and DAsA. The red blood cell count in the erythrocyte suspension was determined using a blood cell counter (Celltac, 4300: Nihon Koden, Co., Ltd.).

AsA and DAsA analysis in tissues. The preparation of tissue samples for determination of AsA and DAsA was described in a previous report (1).

High-performance liquid chromatography (HPLC). HPLC conditions were also described in a previous report (1).

RESULTS

AsA and DAsA in plasma and erythrocyte

Figure 1 shows the changes of total AsA (DAsA+AsA) and AsA concentrations in plasma of group C after injection of DAsA. Both concentrations apparently increased within 90 min after DAsA injection. DAsA concentrations in plasma remained almost within 0.1 mg% and corresponds to 3–5% AsA concentrations in plasma. No DAsA was detected in the erythrocytes of group C prior to injection of DAsA, but following injection DAsA was absorbed by erythrocytes.

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Fig. 1. Total AsA and AsA concentrations of plasma in control and vitamin C-deficient guinea pigs injected intravenously with DAsA. Each point of total AsA (DAsA + AsA, ●) and AsA (○) of the control group is a mean of four samples. The calculated regression equations of total AsA and AsA in the control group were $Y = 0.018X + 0.811$ (---, $r=0.978$) and $Y = 0.014X + 0.771$ (-----, $r=0.958$), respectively. The points of total AsA (■) and AsA (□) of the vitamin C-deficient group were the actual values.

(Fig. 2). The concentration tended to decrease slightly with time. In group D, however, AsA and total AsA concentrations in plasma were much lower than those of group C (<0.3 mg% in Fig. 1). DAsA also appeared in the erythrocytes, although no relationship with time was obtained by the analysis of first regression (Fig. 2).

**Total AsA and AsA contents in tissues**

Table 1 shows the total AsA and AsA contents in the whole tissues of experimental animals in groups C and D. Total AsA and AsA contents in the tissues of animals in group C were significantly higher 30 and 90 min after injection of DAsA (groups C-30 and C-90) than they were immediately prior to injection (group C-0). Total AsA and AsA contents in the tissues of animals in group D were also significantly higher 30 and 90 min after injection of DAsA (groups D-30 and D-90) than those observed immediately prior to injection (group D-0). They reached almost the same levels as those of group C-0, except for the liver content which was about 4 times that of group C-0.

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There was no significant difference in the total AsA contents of the tissues of the following groups: C-30 vs. C-90 and D-30 vs. D-90. Although AsA contents in kidneys of the animals in each group were higher 90 min after injection than 30 min after ($p < 0.05$), the differences between 30 and 90 min of AsA in other tissues in each group cannot be regarded as significant.

On the other hand, total AsA and AsA contents in the tissues of groups D-30 and D-90 were lower than those of groups C-30 and C-90, respectively.

**DISCUSSION**

The increase of AsA concentrations in plasma of control guinea pigs fed a normal diet (Fig. 1) indicated that the injected DAsA was rapidly converted to AsA which filled the tissues, and surplus AsA appeared in the plasma. The failure of DAsA and AsA to appear in the plasma of vitamin C-deficient guinea pigs may be due to absorption into their tissues, further degradation of injected DAsA or excretion in the urine. From the analysis of the urine in the bladder, AsA and DAsA were not detected 30 min after injection (data not shown). Although the reduction of DAsA had been previously clarified to be closely related to glutathione (GSH), Christine et al. (6) suggested that in erythrocytes an enzyme other than glutathione reductase was involved in reduction of DAsA. The presence of DAsA reductase in human erythrocytes and guinea pig liver was reported by Hughes (7, 8) and Bigley (9). DAsA reductase, however, has been isolated only from carp hepatopan-
Table 1. Contents of total AsA and AsA in tissues of control and vitamin C-deficient guinea pigs injected intravenously with DAsA. Total means contents of DAsA plus AsA. Values are means ± SEM. The animals of groups C-0, C-30 and C-90 fed a normal diet were dissected at 0, 30 and 90 min after injection of DAsA, respectively. The animals of groups D-0, D-30 and D-90 fed a vitamin C-deficient diet were dissected at 0, 30 and 90 min after injection of DAsA, respectively. Numbers of animals are in parentheses. (×10⁻² mg/whole tissue)

| Tissues   | C-0 (6) | C-30* (4) | C-90b (4) |
|-----------|---------|-----------|-----------|
|           | Total   | AsA       | Total     | AsA       | Total     | AsA       |
| Adrenals  | 2.7 ± 0.2 | (2.5 ± 0.5) | 12.9 ± 1.6** | (11.6 ± 1.6)* | 18.0 ± 3.4* | (16.9 ± 4.0)* |
| Spleen    | 3.3 ± 0.2 | (1.7 ± 0.4) | 16.2 ± 4.1 | (13.4 ± 4.1) | 16.4 ± 5.8 | (13.8 ± 5.4) |
| Kidneys   | 3.2 ± 0.3 | (2.7 ± 0.5) | 22.5 ± 2.3** | (20.5 ± 3.2)* | 65.4 ± 15.4* | (47.1 ± 5.8)**a |
| Liver     | 28 ± 4   | (24 ± 3)   | 262 ± 48*  | (186 ± 89)   | 292 ± 40**  | (250 ± 30)**  |

| Tissues | D-0 (3) | D-30c (6) | D-90 (6) |
|---------|---------|-----------|----------|
|         | Total   | AsA       | Total     | AsA       | Total     | AsA       |
| Adrenals | 0.5 ± 0.1 | (0.3 ± 0.1) | 0.9 ± 0.2a' | (0.6 ± 0.2)a' | 1.3 ± 0.2ab | (1.1 ± 0.2)ab |
| Spleen  | N.D.    | (N.D.)    | 1.6 ± 0.3a | (0.5 ± 0.2)  | 1.9 ± 0.6   | (1.1 ± 0.5)  |
| Kidneys | 0.5 ± 0.4 | (0.3 ± 0.1) | 4.0 ± 0.6ab | (0.7 ± 0.3)a | 4.2 ± 0.3ab | (3.2 ± 0.3)ab |
| Liver   | 9 ± 6   | (4.0 ± 0.1) | 104 ± 16ab | (96 ± 13)ab  | 135 ± 8ab   | (128 ± 6)ab   |

Significantly different from the value of group C-0 at *p < 0.05 and **p < 0.01. Significantly different from the value of group D-0 at 'p < 0.05 and †p < 0.01. Significantly different from the value of group C-30 at a, p < 0.05 or a', p < 0.01. Significantly different from the value of group C-90 at b, p < 0.05 or b', p < 0.01. Significantly different from the value of group D-30c at c, p < 0.05.
creas (10) and spinach leaves (11), and its distribution in the animal body is obscure.

Basu et al. (12) postulated that the reduction of DAsA in erythrocytes was coupled with the GSH-regenerating system. If the reduction of DAsA mainly takes place in erythrocytes, two possible reasons why the vitamin C-deficient guinea pigs produced less AsA derived from injected DAsA are: 1) a reduced amount of available GSH; and 2) loss of the GSH-regenerating system. It has been reported that the level of GSH decreases in vitamin C-deficient guinea pigs (13). The GSH level in an animal body is strongly dependent on food intake (14). In this experiment the actual amount of feed consumed by animals in group D gradually decreased. Since GSH did not disappear during the reduction of DAsA in healthy human erythrocytes (6), the GSH-regenerating system is thought to be partially missing in vitamin C-deficient animals.

In this study, the detection of DAsA alone in erythrocytes was in accordance with the results of Stankova et al. (15). They explained this phenomenon as being caused by catalytic effects of hemoglobin on the oxidation of AsA. The infusion of carbon monoxide as the reducing medium in erythrocytes during sample preparation had no effect on determination of AsA, suggesting that other factors are concerned in the oxidation of AsA. They also reported the reduction was closely coupled in erythrocytes. In this study, injected DAsA was apparently incorporated into erythrocytes of groups C and D, but the difference of DAsA concentrations in erythrocytes between groups C and D observed 90 min after DAsA injection was not statistically significant. Consequently, the reduction of DAsA in erythrocytes cannot be positively supported by these data and further investigation is necessary to elucidate the reduction mechanisms of DAsA.

The total AsA contents of the tissues in each group increased 30 min after injecting DAsA and showed no significant change with time, indicating that the injected DAsA was rapidly absorbed into the tissues. AsA contents in all tissues except the kidney also showed the same tendency to increase after DAsA injection, indicating that the injected DAsA was rapidly reduced to AsA. The increases of AsA in the kidney of group C were closely related to those in plasma, suggesting the presence of a reabsorption mechanism of AsA in the kidney.

Total AsA of the tissues in groups C-30 and C-90 was mainly composed of AsA with only a little DAsA. On the other hand, in comparing groups D-30 and D-90, the AsA content in all tissues except the liver showed a tendency to increase with time in relation to total AsA, although the total AsA values were not significantly different. In the case of the liver, AsA contents were almost constant. Therefore, this indicated that the reduction of AsA in vitamin C-deficient animals was slower than that in normal animals.

The total AsA and AsA tissue contents after intraperitoneal injection of DAsA in normal guinea pigs has been reported by others (3, 4, 16). Dayton et al. (3) and Tsujimura et al. (4) reported about 20% of intraperitoneally injected DAsA was found as AsA in liver 24 h and 120 min after injection. Our results show approximately the same recovery rate. Based on the initial amounts of AsA in the liver of
groups C-0 and D-0, AsA in the livers of groups D-30 and D-90 increased less than that of groups C-30 and C-90 (Table 1). The lower detection of AsA in the vitamin C-deficient group may be due to the loss of the GSH-regenerating system or the changes in the membrane permeability of DAsA in liver.

The fact that scarcely any DAsA was found in the tissues other than erythrocytes revealed that DAsA is not a suitable form for storage (2) and may possibly be changed to other forms besides AsA in both control and vitamin C-deficient guinea pigs. The other possible degradation product, 2,3-diketogulonic acid, was not detected in liver.

From the results obtained in this experiment, it was suggested that the ratio of DAsA converted to AsA in vitamin C-deficient guinea pigs was much lower than that in the control group, presumably due to the low utilization of DAsA.

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