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Very Low Vitamin C Activity of Orally Administered L-Dehydroascorbic Acid

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The biological activity of L-dehydroascorbic acid (DHA), which is easily formed from L-ascorbic acid (ASC) during storage and cooking processes, has been considered to be equivalent to that of ASC on the basis of studies made several decades ago, when a specific method to determine ASC was not available. The nutritional activity of orally ingested DHA has now been evaluated by comparing ASC concentrations in 12 tissues of rats administered four different doses of ASC. Determinations were made by using the specific and sensitive method, which had been developed by us. Here it is shown that the efficiency of DHA was almost 10% of that of ASC on a molar basis, based on animal experiments using the inherently scorbutic ODS rat, which is a convenient human model animal to investigate the metabolism of vitamin C. On the basis of these findings, it is proposed that it is necessary to reevaluate the nutritional requirement of vitamin C based on both ASC and DHA contents of foods.

KEYWORDS: Ascorbic acid; dehydroascorbic acid; scurvy; ODS rat; vitamin C

INTRODUCTION

L-Ascorbic acid (ASC) is better known as vitamin C (*1*). The recommended daily intake for vitamin C, which is the most basic information to public health, is determined to be 60 mg for adults (*2*). The biological activity of L-dehydroascorbic acid (DHA), which is easily formed from ASC during storage and cooking processes, has been considered to be equivalent to that of ASC (*2*) on the basis of studies (*3*−*5*) made several decades ago, when a specific method to determine ASC was not available. Although elegant studies concerning the molecular mechanisms of DHA transport have been reported (*6*, *7*), the nutritional activity of DHA as vitamin C has not yet been determined by modern analytical methods.

We evaluated the nutritional activity of orally administered DHA on the basis of the tissue concentration of ASC using a specific and sensitive method (*8*) to determine ASC. The new method (*8*) has shown that the colorimetric method (*9*), which was developed over a half century ago and is still used conventionally, gives a level up to 3 times higher than the true level in the determination of vitamin C in rat plasma (*8*). This finding demonstrates that reevaluation of the most fundamental studies of the vitamin is urgently necessary. In this study, the inherently scorbutic [osteogenic disorder Shionogi (ODS)] rat (*10*) was used. Because this rat lacks the L-gulonolactone oxidase (*11*) necessary for the synthesis of ASC from glucose as with the human, monkey, and the guinea pig, it is a good model for ASC studies (*12*, *13*).

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MATERIALS AND METHODS

Animals and Treatments. The guidelines of the Prime Minister's Office of Japan (No. 6, March 27, 1980) for the care and use of laboratory animals were followed. Homozygous male ODS rats (od/od), 5-weeks-old, were purchased from Clear Japan Inc. (Tokyo, Japan). The animals were housed in a room with a temperature of 24 ± 2 °C and a 12 h light/dark cycle. Animals were permitted free access to food and water. For the first week, all rats were supplied with a synthetic basal diet prepared by Funahashi Farm Co. Ltd. (Chiba, Japan) according to AIN 76 (*14*) and fed with ion-exchanged water containing 0.1% of vitamin C, which was shown to be sufficient to maintain normal growth (*10*). The synthetic basal diet contained stripped corn oil as a fat (5% content) and *all-rac*-α-tocopherol at 50 mg/kg instead of corn oil. After a week of acclimation, the rats were divided into five groups designated the control, 0.03% ASC-fed, 0.01% ASC-fed, 0.1% DHA-fed, and C-deficient groups. The control group received drinking water containing 0.1% ASC and the synthetic basal diet. The 0.03% ASC- and 0.01% ASC-fed groups were fed with the synthetic basal diet and drinking water containing 0.03 and 0.01% of ASC, respectively. Because the 0.01% ASC decreased to 70% of the initial level after 12 h at room temperature, the drinking water containing 0.01% ascorbate was changed every 12 h. The 0.03 and 0.1% ASC solutions were relatively stable, and they were changed every 24 h. The 0.1% DHA-fed group was given the synthetic basal diet and drinking water containing 0.1% DHA. DHA is immediately converted into 2,3-diketogulonate in a neutral solution, so DHA was dissolved in 1 mM hydrochloric acid. The pH of this solution was 3.0, which was similar to that of 0.1% ASC (pH 3.3). The pH values of 0.03 and 0.01% ASC were 3.5 and 3.8, respectively. Because DHA decreased to 85% after 24 h at room temperature in the 1 mM HCl solution, the DHA solution was changed every 24 h.

Analytical Methods. On the 21st day, the rats were sacrificed and all determinations were made on that day. Rats were anesthetized with diethyl ether, and the blood was collected from the inferior vena cava

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using a syringe containing sodium heparin as an anticoagulant. After perfusion of ice-cooled saline from the portal vein, organs were removed. The excised tissue was homogenized in 5 volumes of 10 mM phosphate-buffered saline (pH 7.2) under ice cooling. All determinations were made by duplicated experiments. The determination of vitamin C was made as described (*8, 12*). Data were expressed as means ± SE and analyzed by analysis of variance (ANOVA) using StatView software (Abacus Concepts, Berkeley, CA). Differences between the group means were considered to be significant at $P < 0.05$ using the Bonferroni/Dunn procedure generated by this program.

RESULTS AND DISCUSSION

We evaluated quantitatively the nutritional activity of orally ingested DHA for the first time by comparing ASC concentrations in 12 tissues of rats administered four different doses of ASC, because tissue ASC concentrations, which directly reflect absorption, distribution, and utilization of DHA, are the most fundamental information in evaluating the nutritional activity of DHA. The rats were divided into five groups designated the control, 0.03% ASC-fed, 0.01% ASC-fed, 0.1% DHA-fed, and C-deficient groups. The control group received the synthetic basal diet and drinking water containing 0.1% ASC, which was shown to be sufficient to maintain normal growth (*10*). The 0.03% ASC- and 0.01% ASC-fed groups were fed the synthetic basal diet and drinking water containing 0.03 and 0.01% of ASC, respectively. The 0.1% DHA-fed group was given the synthetic basal diet and drinking water containing 0.1% DHA. To evaluate the activity of DHA, determinations were made on the 21st day, when the ASC level in all tissues except the brain, adrenal gland, and spleen of the C-deficient group decreased to null level based on our previous studies (*12*, *13*), and the findings in this experiment are shown in Table 1. The 21st day was also selected because ASC deficiency for >25 days resulted in high mortality of ODS rats.

The changes in body weight of all groups are shown in Figure 1 and show that the body weight of only the C-deficient group was significantly lower than that of the control group after the 16th day, which demonstrates that DHA has a definite anti-scorbutic activity.

The tissue level of the oxidized form of ASC, namely, the sum of DHA and 2,3-diketogulonate, was always <5% of the total ASC in all groups (data not shown), in agreement with our previous works (*12, 13*). Therefore, the total ASC concentration was defined as the ASC level. These results indicate that absorbed DHA is reduced and utilized as ASC.

Although the tissue level of ASC in 0.1% DHA-fed group was significantly higher than that of the C-deficient group in all tissues except plasma and muscle, the tissue concentration of ASC in 0.1% DHA-fed rats was significantly lower than that of the control (0.1% ASC-fed) group in all tissues and still significantly lower than that of 0.03% ASC-fed groups in all tissues except plasma, heart, and muscle (Table 1). These findings demonstrate that DHA has a definite activity as ASC but that the efficiency of DHA is significantly lower than 30% of ASC on weight and molar bases. No significant difference was observed between the tissue ASC concentration in the 0.1% DHA-fed group and that of the 0.01% ASC-fed group in all 12 tissues (Table 1). These findings clearly show that the activity of an orally ingested 0.1% DHA solution is equivalent to that of a 0.01% ASC solution; the efficiency of DHA as ASC is almost equivalent to that of 10% of ASC.

On the basis of these observations, we conclude that the anti-scorbutic activity of DHA is almost 10% of that of ASC, and we propose that it is necessary to reevaluate the nutritional

Table 1. Tissue Total Vitamin C in the Control [0.1% ASC-Fed {1}], 0.03% ASC-Fed {2}, 0.01% ASC-Fed {3}, 0.1% DHA-Fed {4}, and Vitamin C-Deficient {5} Rats after 21 Days[a]

group	plasma	liver	heart	kidney	stomach	small intestine	large intestine	brain	lung	adrenal gland	spleen	muscle
1 (5)	31.1 ± 4.0^{a}	1023 ± 54^{a}	284.4 ± 10.9^{a}	477.8 ± 21.0^{a}	636.3 ± 26.0^{a}	1138 ± 69.7^{a}	796.5 ± 48.8^{a}	1851 ± 18.4^{a}	1476 ± 35^{a}	15970 ± 650^{a}	2497 ± 156^{a}	188.0 ± 24.7^{a}
2 (4)	0 ± 0^{b}	577.8 ± 74.6^{b}	147.7 ± 36.5^{b}	330.4 ± 40.7^{b}	441.6 ± 46.4^{b}	938.5 ± 124.5^{ab}	610.0 ± 66.0^{a}	1817 ± 118^{a}	1038 ± 138^{b}	13170 ± 1170^{a}	1568 ± 155^{b}	0 ± 0^{b}
3 (4)	0 ± 0^{b}	296.3 ± 32.2^{c}	157.8 ± 31.9^{b}	172.6 ± 27.3^{c}	277.3 ± 21.7^{c}	611.4 ± 99.5^{bc}	316.3 ± 40.1^{b}	1381 ± 27^{ab}	692.4 ± 88.0^{bc}	7612 ± 609^{b}	963.1 ± 96.3^{c}	0 ± 0^{b}
4 (4)	0 ± 0^{b}	259.5 ± 26.6^{c}	174.0 ± 40.9^{b}	192.3 ± 30.6^{c}	228.7 ± 32.2^{c}	403.0 ± 63.3^{c}	324.9 ± 63.2^{b}	1068 ± 177^{b}	599.9 ± 85.6^{c}	6630 ± 811^{b}	927.7 ± 118.9^{c}	0 ± 0^{b}
5 (4)	0 ± 0^{b}	0 ± 0^{d}	0 ± 0^{c}	0 ± 0^{d}	0 ± 0^{d}	0 ± 0^{d}	0 ± 0^{c}	561.5 ± 36.2^{c}	0 ± 0^{d}	731.8 ± 53.4^{c}	179.5 ± 37.1^{d}	0 ± 0^{b}

[a] Total concentration of tissue ascorbate is shown as mean ± SE. The number of rats of each group is shown in parentheses. Different letters indicate significant differences in each column among groups.

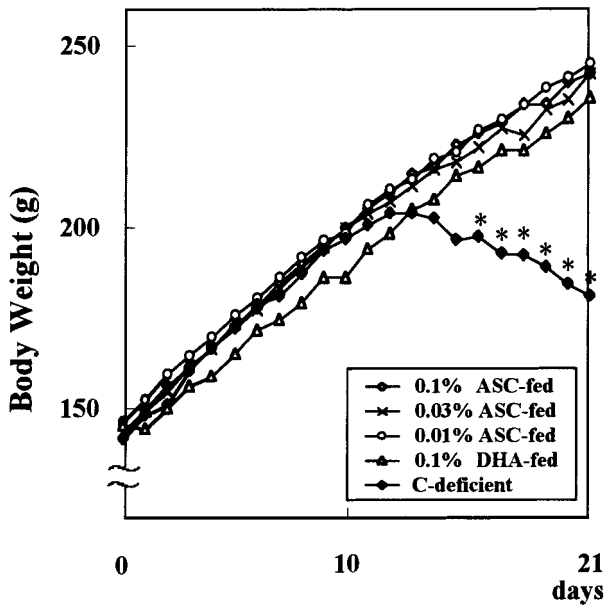

Figure 1. Change in body weight of five ODS rat groups (0.1% ASC-fed, 0.03% ASC-fed, 0.01% ASC-fed, 0.1% DHA-fed, and C-deficient). The body weight of rats was measured every day. Each point represents a mean value, and asterisks indicate a significant difference from the corresponding control group (ANOVA Bonferroni/Dunn procedure; *, $P < 0.05$).

requirement of the vitamin on the basis of both the ASC and DHA contents of foods.

ABBREVIATIONS USED

ASC, L-ascorbic acid; DHA, L-dehydroascorbic acid; ODS, osteogenic disorder Shionogi.

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