

Reduction of dehydroascorbic acid by homocysteine

Jae B. Park *

Phytonutrients Laboratory, Bldg. 307, Rm. 313, BHNRC, ARS, USDA, Beltsville, MD 20705, USA

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Abstract

To determine the reductive process of extracellular dehydroascorbic acid (DHA), molecules (homocysteine, homocysteine thiolactone, methionine, cysteine, and homoserine) were tested to identify those with the potential to reduce DHA to ascorbic acid (AA). Homocysteine (Hcy) was the most potent of the molecules tested. The efficacy of Hcy was compared with that of other molecules able to reduce DHA (reduced glutathione (GSH) and cysteine (Cy)). Although all three molecules were able to reduce DHA, GSH and Cy were not to reduce DHA to AA at concentrations lower than 100 $\mu\text{mol/l}$, and only less than 5% DHA was reduced to AA at concentrations of 200–300 $\mu\text{mol/l}$. In contrast, Hcy reduced DHA to AA stoichiometrically at concentrations as low as 10 $\mu\text{mol/l}$. In Jurkat and U937 cells, the increasing concentrations of extracellular Hcy suppressed intracellular dehydroascorbic acid uptake, indicating that extracellular reduction of DHA by Hcy leads to decreasing extracellular DHA available for its intracellular uptake. Simultaneous oxidation and reduction of Hcy and DHA were accelerated extracellularly in the presence of quercetin, an inhibitor of DHA uptake, suggesting that extracellular ascorbic acid concentration increased via blocking DHA uptake by quercetin and reducing extracellular DHA by Hcy. The effect of homocysteine on DHA reduction and uptake was confirmed with human umbilical vein endothelial cells. The oxidation of Hcy also prevented the decrease in DNA synthesis in human umbilical vein endothelial cells, which would occur following exposure to Hcy. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Ascorbic acid (vitamin C) is a potent antioxidant, the deficiency of which leads to scurvy in humans. Intracellular accumulation of ascorbic acid occurs via two different mechanisms: sodium-independent dehydroascorbic acid transport and sodium-dependent ascorbic acid transport. Under physiological conditions, a significant amount of ascorbic acid accumulates intracellularly [1]. Neutrophils, in particular, accumulate ascorbic acid up to 6–9 mmol/l when oxygen burst occurs in them. This large intracellular accumulation of ascorbic acid was shown to result from the prompt uptake and reduction of dehydroascorbic acid in neutrophils [2,3].

Because the intracellular reduction of dehydroascorbic acid caused the extensive accumulation of ascorbic acid in neutrophils, the reductive mechanism was investigated to identify the reductive compounds and mediator proteins involved. Reduced glutathione and glutaredoxin were re-

ported to be involved in reducing dehydroascorbic acid to ascorbic acid in neutrophils [3–5]. However, dehydroascorbic acid reduction involving only reduced glutathione and glutaredoxin has been challenged because the depletion of reduced glutathione by buthionine sulfoximine (BSO) failed to completely prevent the reduction of dehydroascorbic acid to ascorbic acid [6]. Also, NADPH-dependent dehydroascorbic acid reductase [7], a 31 kDa protein [8], α -lipoic acid dehydrogenase [9], and thioredoxin reductase [10] were reported to reduce dehydroascorbic acid to ascorbic acid, which suggest that intracellular reduction of dehydroascorbic acid may occur via multiple processes.

Vitamin C exists primarily as ascorbic acid (a reduced form) in human plasma [11]. Transiently produced dehydroascorbic acid seems to be transported into cells for the reduction or to be reduced immediately in plasma, because no measurable dehydroascorbic acid is detected in human blood [11,12]. However, the reported mechanisms for intracellular dehydroascorbic acid reduction cannot explain the reduction of dehydroascorbic acid in plasma, because all enzymes involved in the reduction are located in the cytoplasm and are not excreted extracellularly. Therefore, this study investigates the mechanism for the reduction of

* Fax: +1-301-504-9456;
E-mail: park@307.bhnrc.usda.gov

dehydroascorbic acid in human plasma by examining the potential reductive capability of molecules found in plasma.

Homocysteine appeared to be the molecule with the most potential for involvement in the mechanism because it was able to reduce dehydroascorbic acid more efficiently than any other molecules tested (homocysteine thiolactone, methionine, cysteine, homoserine, and homocysteine). The efficiency of the reduction was compared with that of other potentially involved molecules (cysteine and reduced glutathione) in U937 and Jurkat cells, by determining the effect on intracellular reduction of dehydroascorbic acid. The effect of homocysteine on the reduction of dehydroascorbic acid was also reconfirmed using human umbilical vein endothelial cells. All these data indicate that homocysteine is capable to reduce dehydroascorbic acid to ascorbic acid, thereby being oxidized to homocystine. Because homocysteine has been considered to be an independent factor for heart disease, the reductive process between homocysteine and dehydroascorbic acid in plasma was discussed with respect to implication for improving heart disease.

2. Materials and methods

2.1. Materials

DL-Homocysteine, L-homocysteine thiolactone, DL-homocystine, L-homoserine, DL-methionine, L-cysteine, and glutathione were purchased from Sigma (St. Louis, MO). U937, Jurkat, and human umbilical vein endothelial cells were purchased from ATCC (Rockville, MD).

2.2. Cell culture conditions

U937 and Jurkat cells were cultured in RPMI medium supplemented with 10% fetal bovine serum. Human umbilical vein endothelial cells were cultured in Ham's F12K medium with heparin, 0.1 mg/ml; endothelial cell growth supplement, 0.04 mg/ml; and 10% fetal bovine serum. Cell viability was determined microscopically by trypan blue exclusion, and the number of cells was counted by hemacytometer [13]. The cells were grown to 1×10^6 /ml for the uptake experiments.

2.3. Dehydroascorbic acid uptake assay

$1-2 \times 10^6$ cells were resuspended in 1 ml HEPES/phosphate buffer. [^{14}C]Dehydroascorbic acid was added and the reaction was allowed to proceed for 5 min. To terminate the reaction, the cells were washed twice in cold phosphate-buffered saline (PBS; pH 7.4) [11]. [^{14}C]Dehydroascorbic acid was prepared from [^{14}C]ascorbic acid (NEN Life Science Products, 61 Bq/mmol) as described [14]. Uptake activity was measured in whole cells using

scintillation spectrometry or calculated as ascorbic acid reduced from dehydroascorbic acid using high-performance liquid chromatography with coulometric electrochemical detection as described previously [14,15]. To test the sodium dependence of dehydroascorbic acid uptake, the sodium-free buffer was prepared by replacing NaCl and Na_2HPO_4 in HEPES/phosphate buffer with choline chloride and K_2HPO_4 , respectively.

2.4. Reduction of dehydroascorbic acid by homocysteine

Dehydroascorbic acid at 100 $\mu\text{mol/l}$ and various concentrations of homocysteine were simultaneously added to 1 ml HEPES/phosphate buffer. The reduction of dehydroascorbic acid by homocysteine was determined by measuring ascorbic acid as described above. The reduction of dehydroascorbic acid by reduced glutathione, cysteine, homocysteine thiolactone, and homoserine were determined by the method used for homocysteine. To confirm that reduction of dehydroascorbic acid leads to oxidation of homocysteine, the amount of homocysteine was also measured directly, as described previously [16].

2.5. Glucose uptake assay

For inhibition experiments, $1-2 \times 10^6$ cells were resuspended in 1 ml HEPES/phosphate buffer (pH 7.4) containing NaCl, 147 mmol/l; KCl, 5 mmol/l; KH_2PO_4 , 1.9 mmol/l; Na_2HPO_4 , 1.1 mmol/l; glucose, 5.5 mmol/l; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.3 mmol/l; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 1 mmol/l; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.3 mmol/l; and HEPES, 10 mmol/l. The reaction was initiated by adding 37 Bq 2-[1,2- ^3H (N)]deoxy-D-glucose (specific activity 969.4 Bq/mmol, NEN Life Science Products). After 5 min, the cells were washed twice in cold PBS (pH 7.4) [10], and uptake activity was measured in whole cells using scintillation spectrometry.

2.6. Measurement of DNA synthesis

Human umbilical vein endothelial cells in 12-well dishes were incubated for 72 h in Ham's F12K medium with heparin, 0.1 mg/ml, 0.04 mg/ml; and 0.4% fetal bovine serum. For 12 h before treatments, the endothelial cells in 12-well dishes were incubated in the same medium with 20% fetal bovine serum and without endothelial cell growth supplement. The cells were treated with 200 $\mu\text{mol/l}$ homocysteine (Hcy), 400 $\mu\text{mol/l}$ Hcy, 200 $\mu\text{mol/l}$ Hcy and 400 $\mu\text{mol/l}$ dehydroascorbic acid (DHA), or 400 $\mu\text{mol/l}$ Hcy and 800 $\mu\text{mol/l}$ DHA. The cells were labeled with 37 KBq [*methyl*- ^3H]thymidine (specific activity 969.4 Bq/mmol, NEN Life Science Products) for 3 h. After labeling, the cells were washed with cold PBS (pH 7.4), fixed in cold 10% trichloroacetic acid, and washed with 95% ethanol. Incorporated [^3H]thymidine was extracted in 0.2 M NaOH and measured in a liquid scintillation counter.

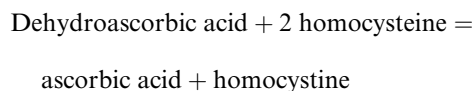
2.7. Analyses

All data points in the figure and tables represent the mean of three or more samples \pm S.D.

3. Results

3.1. Reduction of dehydroascorbic acid by homocysteine

Because it is well known that reduced glutathione reduces dehydroascorbic acid, numerous biological molecules (homocysteine, homocysteine thiolactone, methionine, cysteine, homoserine, and homocystine) were tested to determine their capacity for reducing dehydroascorbic acid to ascorbic acid compared with that of reduced glutathione (Table 1). Even though glutathione, cysteine, and homocysteine were able to reduce dehydroascorbic acid, glutathione and cysteine could not reduce dehydroascorbic acid to ascorbic acid at concentrations lower than 100 μ mol/l, and approx. 5% of the dehydroascorbic acid was reduced to ascorbic acid at concentrations between 200 and 300 μ mol/l. Homocysteine reduced dehydroascorbic acid to ascorbic acid at concentrations as low as 10 μ mol/l (Table 1). The reduction of dehydroascorbic acid by homocysteine seems to proceed stoichiometrically (Table 1).



The reaction between homocysteine and dehydroascorbic acid was so fast that the reduction of dehydroascorbic acid was complete within less than 3 min.

Previously, five different mechanisms were reported for the reduction of dehydroascorbic acid, and five different proteins (glutaredoxin, a 31 kDa reducing protein, 3 α -hydroxysteroid dehydrogenase, α -lipoic acid dehydrogenase, and thioredoxin reductase) were shown to contain the reductive activity. Of them, glutaredoxin exhibited the highest dehydroascorbic acid reducing activity (15 nmol/min/mg protein) [3]. Glutaredoxin is able to reduce dehydroascorbic acid to ascorbic acid via reduced glutathione. Whether glutaredoxin could use homocysteine or cysteine

as reducing molecules to reduce dehydroascorbic acid was therefore investigated. Various concentrations of homocysteine or cysteine were tested with glutaredoxin. Glutaredoxin was found to be superfluous in the reaction, and no substantial increase of ascorbic acid was observed (data not shown). This finding indicates that homocysteine or cysteine could not be substituted for reduced glutathione in the reduction of dehydroascorbic acid by glutaredoxin. All these data showed clearly that homocysteine was able to reduce dehydroascorbic acid faster than cysteine or reduced glutathione, thus being a powerful molecule for reducing dehydroascorbic acid, but it was not to reduce dehydroascorbic acid via glutaredoxin. Because the reduction of dehydroascorbic acid by homocysteine can occur extracellularly, the effect of the extracellular dehydroascorbic acid reduction on the intracellular accumulation of ascorbic acid is to be investigated next, to demonstrate that homocysteine can reduce dehydroascorbic acid, thereby decreasing the concentrations of dehydroascorbic acid available for the intracellular reduction.

3.2. Inhibition of intracellular accumulation of ascorbic acid by extracellular reduction of dehydroascorbic acid by homocysteine

Dehydroascorbic acid is transported into cells via sodium-independent glucose transporters (Glut 1 and Glut 3) [2,12]. In Jurkat and U937 cells, dehydroascorbic acid uptake was demonstrated and characterized in detail [13]. In these cells, dehydroascorbic acid uptake is fast, and significant dehydroascorbic acid uptake can be measured for less than 20 min [12,13]. In contrast, ascorbic acid uptake is much slower than dehydroascorbic acid uptake, being negligible for 20 min. These characteristic uptakes make it possible to determine dominant dehydroascorbic acid uptake in less than 20 min. If homocysteine reduces extracellular dehydroascorbic acid, intracellular dehydroascorbic acid uptake will be inhibited because of the decreased concentration of dehydroascorbic acid. To determine the effect of homocysteine on intracellular dehydroascorbic acid uptake, cells were suspended in buffer containing various concentrations of homocysteine, and dehydroascorbic acid was added to initiate the reaction. As shown in Table 2, the increasing concentrations of

Table 1
Reduction of dehydroascorbic acid by biological molecules

	Concentration (μ M)				
	10	25	100	200	300
GSH	ND ^a	ND	ND	3.7 \pm 0.9	7.9 \pm 1.5
Hcy	4 \pm 0.8	12 \pm 2.2	45 \pm 6.5	89 \pm 7.3	95 \pm 7.1
Cys	ND	ND	ND	3.4 \pm 1.1	6 \pm 1.7

The reactions proceeded in the presence of 100 μ M dehydroascorbic acid. Dehydroascorbic acid reduced by the molecules (GSH, reduced glutathione; Hcy, homocysteine; Cys, cysteine) was represented as ascorbic acid (μ M). Homocysteine thiolactone, homocystine, homoserine, and methionine could not reduce dehydroascorbic acid at concentrations higher than 300 μ M. The values represent the mean of three or more samples \pm S.D.

^aND, not detected.

Table 2
Inhibition of intracellular accumulation of ascorbic acid by homocysteine

Hcy (μM)	U937 (nmol/ 10^6 cells)	Jurkat (nmol/ 10^6 cells)
0	8 ± 1.2	5.7 ± 1.1
25	7 ± 1.5	4.2 ± 1.5
50	6.5 ± 1.3	3.8 ± 0.9
100	5.2 ± 1.8	2.6 ± 1.1
200	1.8 ± 0.7	1.08 ± 0.6

$1\text{--}2 \times 10^6$ U937 and Jurkat cells were resuspended in 1 ml HEPES/phosphate buffer. [^{14}C]Dehydroascorbic acid was added and the reaction was allowed to proceed for 5 min. Intracellular ascorbic acid was calculated as ascorbic acid reduced from dehydroascorbic acid using high-performance liquid chromatography with coulometric electrochemical detection as described previously [14,15]. The values represent the mean of three or more samples \pm S.D.

homocysteine suppressed dehydroascorbic acid uptake in Jurkat and U937 cells, indicating that the reduction by homocysteine decreased the amount of dehydroascorbic acid available for intracellular uptake. However, dehydroascorbic acid uptake still occurred competitively, even for homocysteine at 200 μM /l. It suggested that dehydroascorbic acid uptake was so competitive that transiently produced dehydroascorbic acid was available for both the reduction and the uptake even though homocysteine reduced dehydroascorbic acid quickly.

If homocysteine inhibited dehydroascorbic acid uptake by directly affecting the transporters in Jurkat and U937 cells, homocysteine should also inhibit glucose uptake in the cells, because dehydroascorbic acid uptake occurs via sodium-independent glucose transporters. To verify that dehydroascorbic acid uptake occurred in a sodium-independent manner and to verify that homocysteine did not inhibit these sodium-independent transporters, dehydroascorbic acid and glucose uptakes were measured at various concentrations of homocysteine in buffer with and without sodium. Dehydroascorbic acid uptake and the reduction of dehydroascorbic acid were independent of sodium in the buffer (data not shown). Also, glucose uptake was not affected by the various concentrations of homocysteine (data not shown), suggesting that the inhibition of dehy-

droascorbic acid uptake by homocysteine was a result of the reduction of dehydroascorbic acid to ascorbic acid.

3.3. Increasing extracellular reduction of dehydroascorbic acid by blocking of its uptake

As described above, the competition between extracellular reduction of dehydroascorbic acid by homocysteine and cellular dehydroascorbic acid uptake seems to exist in vitro. If it does, the blocking of intracellular dehydroascorbic acid uptake results in the reduction of more dehydroascorbic acid and increased extracellular ascorbic acid. This assumption was investigated using flavonoids that block sodium-independent glucose transporters [13,17]. Previously, it was demonstrated in Jurkat and U937 cells that approx. 80% of dehydroascorbic acid uptake was inhibited by quercetin (a potent flavonoid) at 50 μM /l [13]. As anticipated, in the presence of quercetin, extracellular ascorbic acid concentration increased via blocking dehydroascorbic acid uptake by quercetin (Table 3).

Because quercetin was reported to have antioxidant properties, it is possible that it reduced dehydroascorbic acid to ascorbic acid, thus inhibiting dehydroascorbic acid uptake in the Jurkat and U937 cells. However, the previously reported data [17] and the data in this study could not validate this possibility. Flavonoids were not able to reduce dehydroascorbic acid even at concentrations higher than 200 μM /l (data not shown). This is a clear demonstration that the blocking of dehydroascorbic acid uptake increased extracellular reduction of dehydroascorbic acid. These observations were also confirmed using human umbilical vein endothelial cells (Table 4).

All these data indicate clearly that in Jurkat, U937, and human umbilical vein endothelial cells, the increasing concentrations of extracellular homocysteine reduces extracellular dehydroascorbic acid into ascorbic acid, thereby decreasing extracellular dehydroascorbic acid available for its intracellular uptake. Respective oxidation and reduction of homocysteine and dehydroascorbic acid to homocysteine and ascorbic acid seem to occur simultaneously. This simultaneous oxidation and reduction of homocysteine and dehydroascorbic acid can be accelerated extra-

Table 3
Increased extracellular and decreased intracellular reduction of dehydroascorbic acid by blocking of dehydroascorbic acid uptake in U937 cells

Hcy (μM)	Control		Quercetin (50 μM)	
	Extra AA (μM)	Intra AA (nmol/ 10^6 cells)	Extra AA (μM)	Intra AA (nmol/ 10^6 cells)
0	ND ^a	8 ± 1	ND	0.9 ± 0.1
25	14 ± 0.9	7 ± 0.5	10 ± 0.7	0.8 ± 0.1
50	26 ± 1.5	6.5 ± 0.6	20 ± 1.2	0.8 ± 0.09
100	56 ± 4.5	5.2 ± 0.4	52 ± 3.8	0.6 ± 0.05
200	89 ± 10	1.8 ± 0.1	83 ± 9	ND

Dehydroascorbic acid uptake occurred with and without 50 μM quercetin, and the uptake was measured in the presence of 100 μM dehydroascorbic acid. Extra AA and Intra AA represent extracellular ascorbic acid and intracellular ascorbic acid, respectively. The values represent the mean of three or more samples \pm SD.

^aND, not detected.

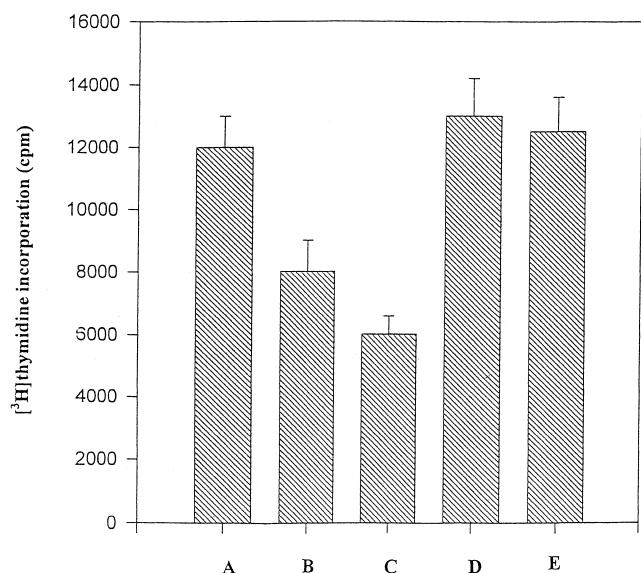


Fig. 1. Effect of homocysteine on DNA synthesis in human umbilical vein endothelial cells. A, control; B and C, [^3H]thymidine incorporation at 200 and 400 μM homocysteine; D and E, [^3H]thymidine incorporation at 200 μM homocysteine and 400 μM dehydroascorbic acid, and 400 μM homocysteine and 800 μM dehydroascorbic acid. Treatment and measurement were performed as described in Section 2. The error bars represent the mean of three or more samples \pm S.D.

cellularly in the presence of quercetin, an inhibitor of DHA uptake, suggesting that extracellular ascorbic acid concentration increased via blocking dehydroascorbic acid uptake by quercetin and reducing extracellular dehydroascorbic acid by homocysteine.

3.4. Prevention of Hcy-induced decrease in DNA synthesis by the oxidation of Hcy by dehydroascorbic acid

As suggested in several studies [18–20], the imbalance in cellular redox state may have pleiotropic effects on the cells: modifying activities of cellular enzymes, inducing genes, and manifesting functional abnormalities. In relatively high concentrations of homocysteine, DNA synthesis in human umbilical vein endothelial cells was reported to decrease in a dose-dependent manner, and this decrease in DNA synthesis was proposed as a mechanism to ex-

plain homocysteine-induced atherosclerosis [19]. Because the reduction of dehydroascorbic acid was to oxidize homocysteine to homocystine, the antiproliferative effect of homocysteine on the endothelial cells was investigated in the presence of various concentrations of dehydroascorbic acid. The oxidation of homocysteine by dehydroascorbic acid prevented homocysteine-induced decrease in DNA synthesis in the endothelial cells (Fig. 1). Because dehydroascorbic acid and ascorbic acid were likely to exist in the oxidation-reduction process, they were investigated to contain any antiproliferative effect on human umbilical vein endothelial cells. Dehydroascorbic acid and ascorbic acid exhibited no antiproliferative effect on the endothelial cells (data not shown). The data indicate that the antiproliferative effect of homocysteine was null by the oxidation of homocysteine by dehydroascorbic acid, in human umbilical vein endothelial cells.

4. Discussion

Actual reduction of dehydroascorbic acid by homocysteine results in the oxidation of homocysteine to homocystine. What implication does the simultaneous oxidation and reduction of homocysteine and dehydroascorbic acid have in vivo? Abnormal metabolism of homocysteine has been considered an independent risk factor for heart diseases [21–23]. For example, a moderate elevation of circulating homocysteine was reported to be common in patients with cardiovascular disease, and a moderate-to-high elevation of homocysteine (31–160 $\mu\text{mol/l}$) was considered a risk factor leading to the progress of cardiovascular disease [24–27]. Hyperhomocysteinemia (high concentrations of homocysteine in plasma) is due to rare genetic defects leading to deficiencies in cystathionine β -synthase, methylenetetrahydrofolate reductase, or enzymes involved in methyl-B12 synthesis and homocysteine methylation [27]. Several studies indicated that high levels of homocysteine in plasma had a variety of deleterious effects on endothelial or smooth muscle cells in culture [23,28,29]. The high level of homocysteine is also considered to affect multifactorially both the vascular wall structure and the

Table 4

Increased extracellular and decreased intracellular reduction of dehydroascorbic acid by blocking of dehydroascorbic acid uptake in human umbilical vein endothelial cells

Hcy (μM)	Control		Quercetin (50 μM)	
	Extra AA (μM)	Intra AA (nmol/ 10^6 cells)	Extra AA (μM)	Intra AA (nmol/ 10^6 cells)
0	ND ^a	2.6 \pm 0.2	ND	0.8 \pm 0.1
25	14 \pm 1.1	1.8 \pm 0.1	10 \pm 1.2	0.6 \pm 0.1
50	26 \pm 1.9	1.4 \pm 0.1	20 \pm 1.5	ND
100	56 \pm 6.4	0.8 \pm 0.01	52 \pm 4.3	ND
200	100 \pm 12	ND	83 \pm 7.9	ND

Dehydroascorbic acid uptake occurred with and without 50 μM quercetin, and the uptake was measured in the presence of 100 μM dehydroascorbic acid. Extra AA and Intra AA represent extracellular ascorbic acid and intracellular ascorbic acid, respectively. The values represent the mean of three or more samples \pm SD.

^aND, not detected.

blood coagulation system [30–32]. Several intervention studies performed with vitamin C to determine its long-term effect on cardiovascular disease have had positive results [33–36]. However, the mechanism underlining the effect of vitamin C has not been clearly elucidated yet.

The data presented here demonstrate that homocysteine reduces dehydroascorbic acid to ascorbic acid and that homocysteine is oxidized to homocystine in the process of reducing dehydroascorbic acid. As mentioned above, these observations were confirmed using human umbilical vein endothelial cells. On basis of these data, a mechanism was proposed to explain the beneficial effect of vitamin C on endothelial cells via reducing the total amount of extracellular homocysteine. The mechanism has one assumption: that homocysteine, not total homocyst(e)ine, is an independent risk factor for heart diseases [37]. In the mechanism, endothelial cells were directly influenced by elevated concentrations of homocysteine, which might be due to genetic defects of the metabolizing enzymes or a postmethionine load. Under physiological conditions, a steady-state equilibrium between homocysteine and homocystine may exist. Because ascorbic acid exists as such or as dehydroascorbic acid, dehydroascorbic acid can shift this equilibrium toward producing homocystine as a result of the reduction of dehydroascorbic acid to ascorbic acid. The reduction of dehydroascorbic acid leads to decreasing the concentrations of homocysteine, thus attenuating the deleterious effects of hyperhomocysteinemia on neighboring cells including endothelial cells. Because dehydroascorbic acid is competitively driven toward two pathways, intracellular uptake of dehydroascorbic acid and extracellular reduction of dehydroascorbic acid by homocysteine, dehydroascorbic acid may shift the equilibrium further toward homocystine if inhibitors block intracellular uptake of dehydroascorbic acid.

Recently, flavonoids were demonstrated to be a potential blocker for dehydroascorbic acid uptake in myelocytic and lymphocytic cells [17]. Therefore, endothelial cells were used to investigate whether there was more oxidation of homocysteine to homocystine in the presence of flavonoids. As shown in Table 4, dehydroascorbic acid uptake was inhibited by flavonoids. Therefore, dehydroascorbic acid may further oxidize homocysteine into homocystine in the presence of flavonoids. Because flavonoids inhibit dehydroascorbic acid uptake via blocking sodium-independent glucose transporters and because flavonoids cannot reduce dehydroascorbic acid, the shift of the equilibrium toward homocystine is due to increasing extracellular dehydroascorbic acid susceptible to reduction by homocysteine. Adding dehydroascorbic acid instead of ascorbic acid can further accelerate the decrease of homocysteine, because the directly added dehydroascorbic acid does not require incidental oxidation of ascorbic acid under physiological conditions. Therefore, an entire amount of added dehydroascorbic acid could be used directly to oxidize homocysteine, thereby shifting the equilibrium of homo-

cysteine and homocystine toward a dramatic decrease of extracellular homocystine. Blocking dehydroascorbic acid uptake can further accelerate this process by flavonoids. As mentioned previously, only homocysteine, not total homocyst(e)ine, was considered to be a potential risk factor for heart disease in the proposed mechanism. If this assumption stands, co-existence of dehydroascorbic acid and inhibitors of dehydroascorbic acid uptake (flavonoids) in plasma is the most effective way to lower the concentration of extracellular homocysteine, thus attenuating its deleterious effect on endothelial cells.

In physiological conditions, extracellular reduction of dehydroascorbic acid by homocysteine may be further influenced by other systems to reduce dehydroascorbic acid extracellularly. It was reported in erythrocytes that dehydroascorbic acid could be reduced extracellularly without entering cells [37]. Also, transmembrane ascorbic acid free radical reductase has been described in the plasma membrane of liver cells [38], which may reduce ascorbic acid free radicals into ascorbic acid extracellularly. If these two systems can reduce extracellular dehydroascorbic acid, they may have potential to shift the equilibrium between homocysteine and dehydroascorbic acid. Therefore, these systems are worthwhile to be investigated in the future in order to determine their potential effects on the oxidation of homocysteine.

Increased homocysteine in human plasma seems to be correlated with some types of heart disease, such as occlusive arterial diseases and atherosclerosis. If this is true, ascorbic acid (especially an oxidized form of ascorbic acid, dehydroascorbic acid) may attenuate the deleterious effect at least by reducing the concentrations of homocysteine in plasma. Historically, epidemiologic studies have suggested that humans who consume larger quantities of antioxidants in food and vitamin C and E supplements have lower chronic heart disease morbidity and mortality [39]. Ascorbic acid (vitamin C) is particularly effective in blocking the effect of hyperhomocysteinemia; orally administered vitamin C (2 g) prevented methionine-induced endothelial dysfunction in both conduit and resistance vessels [40]. It was also reported in humans that an acute impairment of vascular endothelial function resulting from elevated homocysteine concentrations could be prevented by pretreatment with vitamins [33]. Data even suggested that homocysteine might accumulate in scurvy-like conditions [41]. Although numerous studies indicate that ascorbic acid from supplements exerts a beneficial effect on cardiovascular disease, the only mechanism suggested thus far for this effect is that ascorbic acid may attenuate the increased oxidant stress that occurs under hyperhomocysteinemia. In this paper, the data showed that homocysteine is a potent molecule for reducing dehydroascorbic acid extracellularly, and the reduction of dehydroascorbic acid by homocysteine may attribute to attenuating the deleterious effects (including the decrease in DNA synthesis) of homocysteine on human umbilical vein endothelial

cells. Based on these, the reduction of dehydroascorbic acid by homocysteine was cautiously proposed as a possible mechanism for the beneficial effect of ascorbic acid on cardiovascular disease. However, the mechanism proposed should be evaluated concertedly with previously reported mechanisms for the effect of ascorbic acid on heart diseases, because heart diseases are considered to arise as the consequence of numerous risk factors.

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