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PREVENTIVE EFFECTS OF ASCORBIC ACID ON THE ISCHEMIA/REOXYGENATION INJURY IN THE RAT SMALL INTESTINE

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Ischemia/reoxygenation (I/R) injury is observed in various organs, where some free radicals and lipid peroxides are believed to play an important role. We examined the preventive effects of ascorbic acid (AsA), one of antioxidant agents, on the I/R injury of the rat small intestine. AsA or glutathione was intraperitoneally administered to male Wistar rats weighing 250~300 g after one-day fasting. The entire small intestine was resected at three different points, just before ischemia, after ischemia, and after 20 min of reoxygenation. The specimens were used for the assays of lipid peroxides, glutathione, and glutaminase activity and for histological examination. In AsA-treated group, the production of lipid peroxides after reoxygenation was significantly suppressed and the ratio of reduced form of glutathione to the total glutathione was also significantly high. Tissue glutaminase activity decreased to less extent, and the degree of injury by reoxygenation was apparently less marked in this AsA-treated group. AsA works as an antioxidant against the peroxidative tissue injury possibly by scavenging free radicals and reducing the peroxidative reaction. The effects of AsA are expected to be very useful in small bowel transplantation and various surgical procedures.

Introduction

It is well known that structural and functional injuries are caused by ischemia reoxygenation in various organs[1)~3)]. The precise mechanism is still unclear, but some species of oxygen-derived free radicals generated by a reoxygenation are believed to play an important role[4)~6)]. McCord[7)] postulated that oxygen-derived free radicals may cause tissue injuries during reoxygenation period. According to his hypothesis. ATP undergoes degradation during ischemia and leads to the generation of superoxide anion which may finally cause injury to the tissues during reoxygenation period. Superoxide anion firstly changes to hydrogen peroxide in the presence of superoxide dismutase and then to hydroxyl radicals with ferrous ions (Harbour-Weiss reaction[8)]). In recent years, however, superoxide anion is not able to extract hydrogen from unsaturated fatty acid[9)]. It suggests the importance of hydroxyl radical and also iron-oxygen complexes, which are thought to be more reactive species of free radicals and may react with the cell membranes and produce lipid peroxides and peroxidative intermediate metabolites. Lipid peroxides may directly injure the cell membrane[10)~13)]. In fact, many investigators have already reported that the peroxidative products are observed in the damaged tissues during the postischemic period[12)~14)]. Because such radicals have highly reactive properties, they immediately react with cell membranes consisting of polyunsatur-

ated fatty acids (PUFA) and may initiate lipid peroxidation of the membranes. Peroxidised lipids generate many potentially cytotoxic products[10], among which stoichiometrically the major products are lipid hydroperoxides (LPO). LPO are highly toxic *in vivo*[4], and are capable of inactivating enzymes[15] *in vitro* and further promoting free radical-mediated destruction of FUFA[11] and proteins[10].

Ascorbic acid (AsA), a water-soluble vitamin, has both reducing and chelating properties at once. With its strong reducing property and its property of scavenging free radicals, AsA is well known as a strong antioxidant agent[16]. One of the major funcitons of AsA is to protect tissues from the harmful oxidative products and to keep certain enzymes in their reduced states[17]. It is also demonstrated that, in addition to reacting with and scavenging superoxide radical and hydroxyl radical[17], AsA can also scavenge single oxygen[18]. AsA is known to promote peroxidation at lower concentrations[19] but ot inhibit it at higher concentrations[16][17][20].

I/R injury of the heart and the liver has been well studied biochemically and histologically from the aspect of the production of free radicals an the adherence and accumulation of neutrophils[21][22]. However, few studies have been undertaken to investigate the I/R injury of small intestine[23]~[25].

Though small intestine tissue is commonly known to contain AsA in rats, the effects of AsA on the postischemic lipid peroxidative injury have not been throughly understood. At present, much remains unknown *in vivo* about the mechanism of preventive effects of AsA and the interaction between AsA and glutathione (GSH) on the I/R injury of the small intestine. The aim of the present study was to examine the effects of AsA on the I/R injury of the rat smal intestine from the biochemical and histological aspects. We discussed the possible mechanism of the preventive effects of AsA and GSH and the interaction between them.

Materials and Methods

1. Materials

Reduced form of glutathione (GSH), glyoxalase 1, methylglyoxal, nicotinamide adenine dinucleotide phosphate (NADPH), glutathione reductase, thiobarbituric acid (TBA), trichloroacetic acid (TCA), sodium dodecyl sulfate (SDS), $\alpha\alpha'$-dipyridyl, dithiothreitol (DTT), and N-ethylmaleimide (NEM) were obtained from Sigma Co., Ltd. (St. Louis, MO). Hemoglobin-methylene blue (HMB) test kit for LPO assay was obtained by Kyowa Medex Co., Ltd. (Tokyo, Japan) and ascorbic acid from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals were of analytical grade and were used without further purification.

This study was performed according to the principles of laboratory animal care, and was approved by the animal studies committee of Tokyo Women's Medical College.

2. Methods (Fig. 1)

AsA or GSH dissolved at various concentrations in physiological saline was intraperitoneally administered (2 ml in total) to male Wistar rats weighing 250~300 g after one-day fasting. The same volume of physiological saline was intraperitoneally administered to the rats of the control group. Animals were divided into the following groups, ① Control (n=10), ② AsA: 2 mmol/kg (n=10), 0.5 mmol/kg (n=7), 0.1 mmol/kg (n=7), ③ GSH: 2 mmol/kg (n=

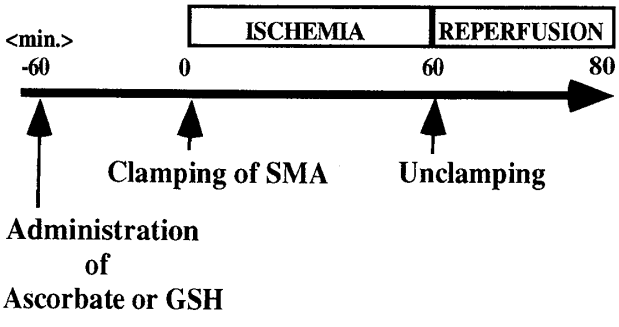

Fig. 1 Experimental protocol
Intestinal tissue was excised at time 0, 60 and 80 min, and was applied to the following examination: TBA-RS, LOOH, GSH, GSSG, glutaminase, histology.

10). Parenthesis indicates the number of rats examined. Laparotomy was performed 1 hour after the administration of the drugs under the anesthesia with Nembutal (pentobarbital sodium: 60 mg/100 g of B.W.), and the superior mesenteric artery (SMA) was clamped to induce ischemia over the entire small intestine. The artery was unclamped after maintaining ischemia for 1 hour. The entire small intestine was excised just before ischemia (B.I.), after ischemia (A.I.), and after 20 min of reoxygenation (A.R.). The excised tissues were used for the following assays and histological examination.

Assay of thiobarbituric acid reactive substance (TBA-RS) and LPO of the small intestine tissue: small intestine tissues were homogenized with 10 vol. of 1.15% KCl solution and 0.2 ml of this 10% tissue homogenate were added 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid solution adjusted to pH 3.5 with NaOH, and 1.5 ml of 0.8% aqueous solution of TBA. The mixture was made up to 4.0 ml with distilled water, and then heated in an oil bath at 95°C for 60 min. After cooling with tap water, 1.0 ml of distilled water and 5.0 ml of the mixture of n-butanol and pyridine (15 : 1, v/v) were added and shaken vigorously. After centrifugation at 4,000 rpm for 10 min, the organic layer was taken and its absorbance at 535 nm was measured[26].

Assay of tissue LPO was performed using the HMB test kit (Kyowa Medex Co., Tokyo): The hemoglobin-methylene blue test has a higher specificity for peroxides than the TBA method, because it uses the peroxidase activity of hemoglobin. When hemoglobin reduces peroxides to the corresponding alcohols, N-methylcarbamoyl derivatives of methylene blue (leuco form) are oxidized and colored blue[27].

Assay of reduced form of GSH and oxidized form of glutathione (GSSG) of the small intestine tissue: small intestine tissues were homogenized with 5 vol. of 6% perchlorate and applied to the assay, using the method of Klotsh et al[28].

Assay of AsA and dehydro-AsA of the small intestine tissue: small intestine tissues were homogenized with 5 vol. 3 mM Na_2 EDTA and centrifuged with 3,000 rpm for 10 min. The supernatant was applied to the following assay of AsA and dehydro-AsA by the modified method of Okamura[29]. The supernatant (0.2 ml) was neutralized with 0.8 ml of H_3PO_4 solution. The reactive mixture contains 1.0 ml of 10% TCA, 0.8 ml of 4% $\alpha\alpha'$-dipyridyl, 0.4 ml of 3% $FeCl_3$, and was incubated at 37°C for 60 min. The absorbance of the organic layer was measured at 525 nm. Dehydro-AsA was reduced to AsA with DTT at room temperature. After removing the excess DTT with NEM, total ascorbic acids, i.e. reduced and original, are determined by the same method mentioned above.

Glutaminase activity of the intestine tissue: small intestine tissues were homogenized in 125 mM potassium phosphate, 330 mM sucrose and 2 mM dithiothreitol, and applied to the assay using the method of Pinks and Windmueller[30].

Histological examination: small intestine tissues were fixed in 10% buffered formalin and processed for standard light microscopy. The degree of pathological lesions in small intestine tissues resected 20 min after reoxygenation was examined.

3. Statistical analysis

All values of data were presented as means ± SE. Statistical significance was determined by analysis of variance with unpaired Student's t test. A p value less than 0.05 was considered significant.

Results

1. Lipid peroxidation of the small intestine tissue during ischemia and reoxygenation (Fig. 2, Table 1)

In Control group, the production of TBA-RS was significantly increased by 20 min reoxygenation of the small intestine tissue (B.I.; 0.020 ± 0.002, A.I.; 0.081 ± 0.001, A.R.; 0.155 ± 0.049 OD at 535 nm $p<0.01$, vs B.I., $p<0.05$, vs A.I.). The production was also sig-

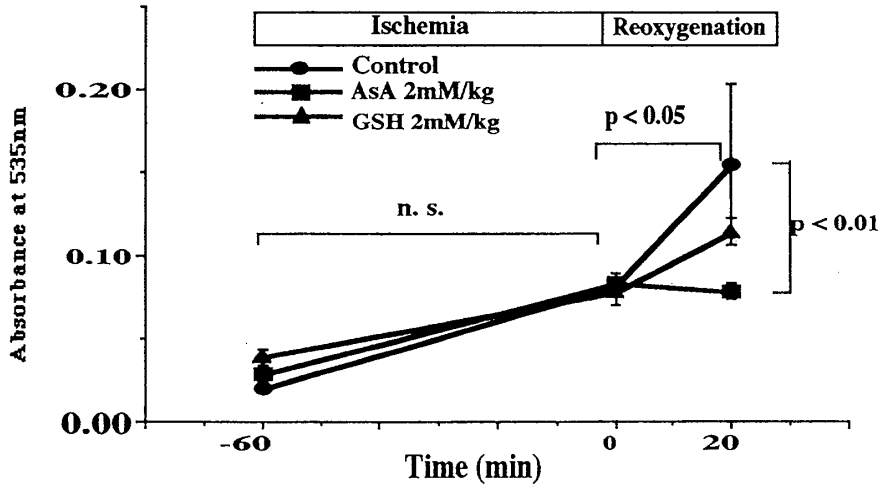

Fig. 2 Lipid peroxidation of the reoxygenated small intestine tissue
The concentration of TBA-RS in the small intestine during ischemia and reoxygenation was shown. ●: Control group, ■: AsA-treated group, ▲: GSH-treated group. AsA (2 mmol/kg B.W.) was administered intraperitoneally 60 min before ischemia. After the 60 min of ischemia, the small intestine was reoxygenated for 20 min. The entire small intestine was resected just before ischemia, after ischemia, and after 20 min of reoxygenation. All values of data are shown as mean ± SE for ten preparations in each group.

Table 1 LOOH production during ischemia and reoxygenation

	Before ischemia	After ischemia	After reoxygenation
Control group	13.50±1.25	16.38±0.60	26.30±1.98[a)]
AsA-treated group	12.00±0.75	13.65±0.61	16.75±1.43[b)]

(nmol/g tissue)

a) $p<0.05$, vs after ischemia, b) $p<0.01$, vs Control group. All data are expressed by mean±SE for ten preparations in each group.

nificantly increased in the reoxygenated small intestine tissue in AsA-treated group (B.I.; 0.029 ± 0.005, A.I.; 0.084 ± 0.004, A.R.; 0.078 ± 0.005, $p<0.05$, vs B.I.). The TBA-RS production in small intestine tissues obtained 20 min after reoxygenation was significantly supressed in AsA-treated groups, compared with that in Control group ($p<0.01$) (Fig. 2). While in GSH-treated group, the TBA-RS production was significantly increased in the reoxygenated small intestine tissue (B.I.; 0.039 ± 0.005, A.I.; 0.078 ± 0.008, A.R.; 0.114 ± 0.008, $p<0.05$, vs B.I. and A.I.).

LPO production was also significantly increased in the small intestine tissue after reoxygenation in Control group (B.I.; 13.50 ± 1.25, A.I.; 16.38 ± 0.60, A.R.; 26.30 ± 1.98 nmol/g tissue, $p<0.01$, vs B.I., $p<0.05$, vs A.I.). However, the administration of AsA significantly suppressed the production of LPO in the reoxygenated small intestine tissue (16.75 ± 1.43 nmol/g tissue, $p<0.01$, vs Control group) (Table 1).

2. Dose dependent effects of AsA on the lipid peroxidation of the reoxygenated small intestine tissue (Fig. 3)

As shown in Fig. 2, AsA suppressed the production of TBA-RS in a dose dependent manner.

3. Assay of the tissue levels of AsA and dehydro-AsA of the rat small intestine during ischemia and reoxygenation (Table 2)

In Control group, at 20 min-reoxygenation, AsA content was decreased whereas that of dehydro-AsA was increased. And the ratio of dehydro-AsA to total AsA was also increased by the tissue reoxygenation (B.I.: 7.8 ± 4.3%,

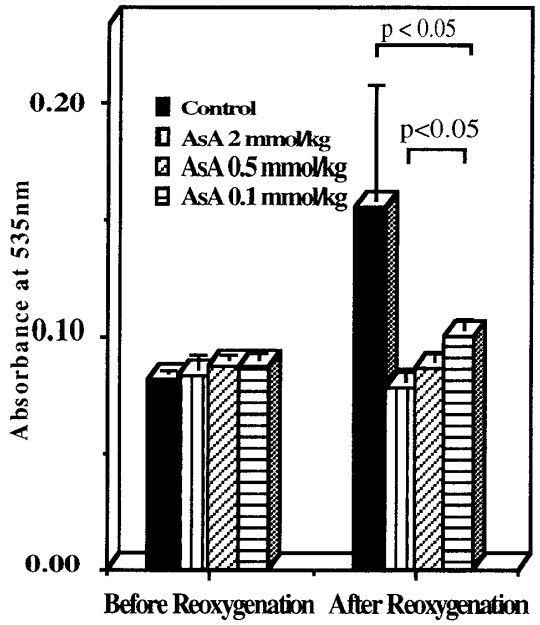

Fig. 3 Dose dependent effects of AsA on the lipid peroxidation of the reoxygenated small intestine tissue

The production of TBA-RS during ischemia and reoxygenation was shown. AsA was administered intraperitoneally 60 min before ischemia (2, 0.5, 0.1 mmol/kg B.W.). After 60 min of ischemia, the small intestine was reoxygenated for 20 min. The small intestine was resected before reoxygenation and after 20 min of reoxygenation. All values of data are shown as mean ± SE for seven preparations in each group.

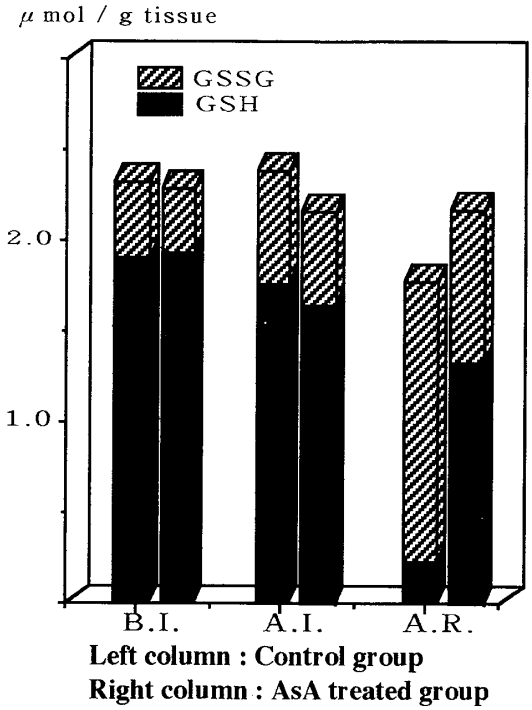

Fig. 4 Reduced and oxidized glutathione levels of small intestine during ischemia and reoxygenation period

Reduced form (GSH) and oxidized form (GSSG) of glutathione levels are shown as ■ and ▒, respectively. The entire small intestine was resected just before ischemia (B.I.), after ischemia (A.I.), and after 20 min of reoxygenation (A.R.). The total amount of glutathione after reoxygenation in small intestine tissue decreased in Control group, but not significantly changed in AsA group. However, the ratio of GSSG to the total glutathione in AsA group was significantly low compared with that in Control group (87.2 ± 1.9% vs 38.6 ± 2.8%, $p<0.01$).

A.I.; 16.9 ± 0.8%, A.R.; 33.0 ± 0.9%, $p<0.05$ vs B.I.; and A.I.). On the contrary, in AsA-treated group, both AsA and dehydro-AsA contents were increased before ischemia, but the ratio of dehydro-AsA to total AsA was not significantly different between Control and AsA-treated group. (Control group: 7.8 ± 4.3%, AsA-treated group: 12.3 ± 2.7%, $p=0.275$). In both groups immediately after reox-

Table 2 Tissue levels of AsA and dehydro-AsA during ischemia and reoxygenation

		Before ischemia	After ischemia	After reoxygenation
Total AsA (μg/tissue)	Control group	440.95±14.25	477.50±8.30	308.35±12.13[a)]
	AsA-treated group	558.66±39.33	619.45±26.58	372.10±16.38[b)]
Dehydro-AsA (μg/tissue)	Control group	36.76±21.05	83.53±7.45	103.93±7.03[c)]
	AsA-treated group	75.03±18.93	90.75±7.45	97.98±10.00[d)]
Dehydro-AsA/Total AsA (%)	Control group	7.8±4.3	16.9±0.8	33.0±0.9[e)]
	AsA-treated group	12.3±2.7	14.3±1.2	25.8±2.8[f)g)]

a) $p<0.05$, vs after ischemia, b) $p<0.05$, vs Control group, c) $p<0.05$, vs after ischemia, d) $p<0.05$, vs after ischemia, e) $p<0.05$, vs after ischemia, f) $p<0.05$, vs after ischemia, g) $p<0.05$, vs Control group. All data are expressed by mean±SE for ten preparations in each group.

ygenation, dehydro-AsA content was increased and the ratio of the dehydro-AsA content was also greatly decreased 20 min after reoxygenation with the increase of dehydro-AsA content. The ratio of dehydro-AsA to total AsA after the tissue reoxygenation was significantly low compared with that of Control group (Control group: 33.0 ± 0.9%, AsA-treated group: 25.8 ± 2.8%, $p<0.05$).

4. Tissue glutathione levels of the small intestine during ischemia and reoxygenation (Fig. 4, Table 3)

Tissue level of reduced glutathione (GSH) in small intestine tissues decreased due to ischemia, and the glutathione level was further lowered after 20 min-reoxygenation (B.I.: 1.90 ± 0.12, A.I.: 1.7 ± 0.16, A.R.: 0.22 ± 0.021 μmol/g tissue, $p<0.01$ vs B.I. and A.I.). Tissue level of GSSG increased after the reoxygenation and GSSG level in Control group was higher than that of AsA-treated group (Control group: 1.56 ± 0.19, AsA-treated group: 0.84 ± 0.07 μmol/g tissue, $p<0.01$). The difference in total glutathione levels after reoxygenation was not significant between Control and AsA-treated groups. In AsA-treated group, total glutathione level was decreased after ischemia, and after reoxygenation it was slightly recovered with low level of GSSG. The ratio of GSSG to total glutathione was significantly low in the AsS-treated group compared with those in Control group (87.2 ± 1.9% vs 38.6 ± 2.8%, $p<0.01$).

5. Glutaminase activity in small intestine tissues (Table 4)

Tissue glutaminase activity in small intestine after reoxygenation following ischemia decreased by 69% compared with the activity before reoxygenation in Control group. In AsA-treated group, however, tissue glutaminase activity decreased only by 34%, and there were significant differences between Control and AsA-treated groups (Control group: 0.68 ± 0.07, AsA treated-group: 1.70 ± 0.08 μmol glutamate/hr/mg tissue, $p<0.01$ vs Control group).

6. Histology (Fig. 5)

In Control group, histological examination of the reoxygenated small intestine showed marked mucosal congestion, edema, and epithelial exfoliation. The injury by reoxygenation following ischemia was apparently less marked in AsA-treated group.

Table 3 Tissue glutathione levels of small intestine during ischemia and reoxygenation period

		Before ischemia	After ischemia	After reoxygenation
GSH (μmol/g tissue)	Control group	1.90±0.12	1.75±0.16	0.22±0.02[a)]
	AsA-treated group	1.94±0.16	1.65±0.15	1.34±0.12[b)]
GSSG (μmol/g tissue)	Control group	0.42±0.04	0.63±0.02	1.56±0.19
	AsA-treated group	0.35±0.02	0.51±0.02	0.84±0.07[c)]

a) $p<0.01$, vs after ischemia, b) $p<0.01$, vs Control group, c) $p<0.01$, vs Control group. all data are expressed by mean±SE for ten preparations each group.

Table 4 Assay of glutaminase activity in small intestine tissues

	Before reoxygenation (BR)	After reoxygenation (AR)	AR/BR ratio
Control group	2.25±0.08	0.68±0.07	0.31±0.03
AsA group	2.37±0.28	1.70±0.08[a)]	0.66±0.08[b)]

(μmol glutamate/hr/mg tissue)

a) $p<0.01$, vs Control group, b) $p<0.01$, vs Control group. All data are expressed by mean±SE for ten preparations in each group.

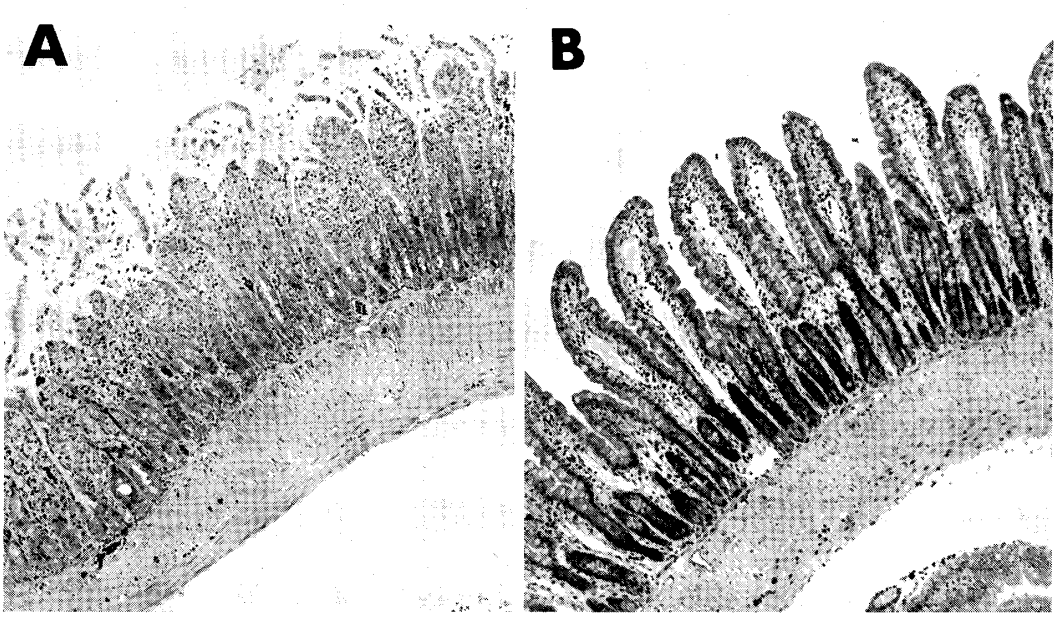

Fig. 5 Histological appearance of the small intestine 20 min after reoxygenation
After 60 min of ischemia, the small intestine was reoxygenated for 20 min. And full-thickness small intestine tissues were fixed in 10% buffered formalin and processed for standard H-E stain and light microscopic examination. (magnification ×10)
(A) Control group; The specimen appears severe cell infiltration, separation of the epithelium from the lamina propria down to the villus base, mostly disarrayed villus bases, and moderate edema.
(B) AsA-treated group; The specimen appears mild cell infiltration, minor separation of the epithelium from the lamina propria, almost intact villus bases, and minor edema.

Discussion

Since it was first reported by Granger et al in 1981[1], I/R injury has attracted much attention as a major cause of functional disorders of various organs due to ischemic microcirculatory impairment. At present, it is regarded as an important event involved in the pathophysiological processes in organ transplantation, some types of surgical treatments including the ischemic procedures, cardiac[21] and cerebrovascular disorders, and hepatic[23] and gastrointestinal bleeding. I/R injury is believed to be mainly attributed to lipid peroxidation, which possibly occurs in various pathological and physiological conditions at the cell membrane that is composed of PUFA. LPO, the intermediate metabolite of lipid peroxidation, is believed to be impaired the cell membrane structure and function[10)~13)].

The evidence of the generation of hydroxyl radical *in vivo* in the early stage of reoxygenation has already been shown in heart and liver[21)22)]. In fact, the iron-catalyzed free radicals potentially extract hydrogen from PUFA *in vitro* and may become effective initiators of lipid peroxidative reaction *in vivo*. Iron breaks down LPO into alkoxyl/peroxy radicals, and these radicals further extract hydrogen from PUFA and also propagate the peroxidative chain reaction[31]. AsA is also reported to have chelating effects and to release low-molecular-weight iron from hepatic tissues during ischemia. Perferryl ions are considered to play a major role in hepatic reoxygenation injury as a possible initiator of lipid peroxidation[3]. Low-molecular-weight iron may cause the free radical-mediated tissues injury when released from chelated form. Some *in vitro* experiments using iron and microsomes[18)19)] showed that AsA acted as a pro-oxidant[20], while many *in vivo* studies using the liver tissues showed that

it also served as an antioxidant at high concentrations[16)32)33)].

In the present study, we achieved the following conclusion, AsA may mainly acted as an antioxidant, even if it might induce low-molecular-weight iron and enhance reactivity of Fe ions to some extent, which was the same as the reoxygenated rat liver[34)].

We used an ischemia-reoxygenation model in the small intestine of rats in order to study peroxidation and tissue injury and the effect of AsA on this injury. To prepare the appropriate model of I/R injury, ischemia was maintained only for 60 min, during which the effects of ischemia itself would not be so severe (data not shown). This peroxidative reaction at an early stage after reoxygenation, i.e., primary I/R injury, possibly causes a variety of secondary events, such as the release of several types of interleukins, the expression of adhesion molecules of the cell membrane, and leukocyte infiltration into the tissues, i.e., secondary I/R injury[6)]. Effects of reoxygenation on small intestine tissue were evaluated 20 min after reoxygenation, at which the infiltration of neutrophils into tissues has not been observed yet[34)35)].

Dehydro-AsA has been reported to be less effective than AsA on the suppression of the lipid peroxidation in the reoxygenated liver tissue[34)]. Ozaki et al suggested that dehydro-AsA must have been converted to AsA in order to have the anti-oxidant property[34)].

Glutathione, an endogenous antioxidant, is produced in the liver and distributed over the whole body including the small intestine via blood and bile. In the very early stage of reoxygenation, AsA radical (monodehydro-AsA) was obsrved by electro paramagnetic resonance-spin trapping study, followed by hydroxyl radical[23)]. In the AsA-treated group the ratio of dehydro-AsA to total AsA was significantly higher than that of Control group after reoxygenation. AsA, when oxidized at the early stage of reoxygenation, changes to dehydro form, and again to the origial form when reduced. There may be some relationship between the redox state of both AsA and glutathione *in vivo*. That is, AsA acts as an anti-oxidant by being oxidized finally to dehydro-AsA, and again is reduced to AsA, coupled with the conversion of GSH to GSSG. In the AsA-treated group, total glutathione level after reoxygenation is not significantly different from that of Control group. However, the ratio of GSSG to the total glutathione is significantly lowered compared with that of Control group, which suggests that GSH is maintained at high levels, and the activity of GSH is maintained at high level and the activity of GSH as an antioxidant is preserved. These observations will be explained by the following mechanism; AsA works strongly as an antioxidant more than GSH by being oxidized to dehydro-AsA, and AsA prevents GSH from being oxidized to GSSG in the very early stage of the reoxygenation.

Phosphate-dependent glutaminase (L-glutamine amidohydrolase) is one of the major glutamine-degrading enzymes in intestine, and high activity of phosphate-dependent glutaminase was found in the intestinal mucosal epithelium, villus, and crypt cells of rats[30)]. Glutaminase was observed fairly uniformly in whole small intestine, and the specific glutaminase activity was measured similarly in duodenum, jejunum, and ileum[30)]. In the present study the glutaminase activity in the reoxygenated rat small intestine tissues was well preserved, by the administration of AsA before ischemia.

Histopathologically, in AsA-treated group, the intestine mucosa was relatively preserved in its structure. The reoxygenated small intestine of rats in Control group showed the cellular infiltration, separation of the epithelia from the lamina propria down to the villus base, mostly disarrayed villus bases, and moderate edema. The severe injury by reoxygenation following ischemia mainly occurred in the mucosal layer of the small intestine of rats[36)]. the mucosa contains more than 90% of the total

glutaminase activity[30]. This is the reason only glutaminase activity was preserved significantly higher in AsA-treated group. These results indicate that the administration of AsA can effectively protect small intestine tissue from reoxygenation injury both biochemically and histologically.

A part of the protective mechanism of AsA which was shown in this *in vivo* study can be explained as follows: 1) AsA protects the postischemic small intestine tissue by scavenging radicals and/or reducing the peroxidative reactions by itself, 2) AsA preserves the high glutathione level of the postischemic small intestine tissue. As a result, the cellular integrity of the postischemic small intestine tissue was also preserved well.

In conclusion, our findings showed that AsA acts as an antioxidant in the small intestine and reduces injury due to reoxygenation. These effects are expected to be very useful in preserving mucosal function or accelerating its recovery after some types of surgical treatments such as strangulated ileus, SMA thrombosis, enterectomy, and especially small bowel transplantation.

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ラット小腸の虚血再酸素化傷害に対するアスコルビン酸の抑制効果について

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様々の臓器における虚血後再酸素化（ischemia/reoxygenation; I/R）時の細胞や機能の傷害は，発生する種々のフリーラジカルや，過酸化脂質によると考えられている．一方，アスコルビン酸（AsA）は，低濃度では過酸化を促進し，高濃度では逆に抑制することが知られている．ラット小腸においてI/R時の傷害の程度とAsAによる傷害抑制効果について生化学的，病理学的に検討した．AsAとグルタチオン（GSH）を，雄性Wistar系ラットに腹腔内投与し，60分後開腹，上腸間膜動脈を遮断し，全小腸を60分間虚血状態とした．再酸素化後20分で，全小腸を摘出して検索し，以下の結果をえた．AsA投与群では，過酸化脂質の産生は，有意に抑制され（LOOH Control: 26.30±1.98, AsA: 16.75±1.43 nmol/g tissue, $p<0.01$），また，glutaminase活性は，再酸素化後も有意に高く保たれ（Control: 0.41±0.04, AsA: 1.02±0.05μmol glutamate/h/mg tissue, $p<0.05$），組織傷害も軽微であった．また，還元型GSHレベルは，再酸素化後も高値に保たれた（Control:0.22±0.02, AsA:1.34±0.12 μmol/g tissue, $p<0.01$）．AsAは，ラット小腸において抗酸化剤として作用し，I/R傷害を軽減させることが明らかとなった．