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Ascorbic acid prevents ischemia-reperfusion injury in the rat small intestine

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Abstract Ischemia-reperfusion injury by free radicals and lipid peroxides is observed in various organs. Ascorbic acid (AsA) or glutathione (GSH) in various doses (AsA:2, 0.5, 0.1 mmol/kg, GSH:2 mmol/kg) was intraperitoneally administered to male Wistar rats. The entire small intestines were resected just before ischemia, after ischemia, and after 20 min of reperfusion (n = 7–10 at each time point). At each time point, the specimens were subjected to assays of lipid peroxides, GSH, and glutaminase activity of the tissues; they were also examined histologically. In the AsA group, the production of lipid peroxides after reperfusion was significantly suppressed in a dose-dependent manner, and the ratio of oxidized GSH to total GSH was also significantly low. Tissue glutaminase activity decreased to a lesser extent, and the degree of injury was apparently less marked in the AsA group. This study indicates that AsA acts as an antioxidant against peroxidative tissue injury, possibly by scavenging radicals, preserving reduced GSH, and reducing the peroxidative reaction.

Key words Small intestine, ascorbic acid, reperfusion · Ascorbic acid, small intestine, reperfusion · Reperfusion, ascorbic acid, small intestine · Ischemia, ascorbic acid, reperfusion

Introduction

It is well known that structural and functional injuries are caused by ischemia-reperfusion (I/R) in various organs [5, 22, 30]. The exact details of the underlying mechanism are still unclear, but some oxygen-derived free radicals generated during reperfusion are believed to play an important role [2, 3, 35]. McCord [16] first postulated that oxygen-derived free radicals may cause tissue injuries during reperfusion. According to McCord's theory, ATP undergoes degradation during ischemia and leads to the generation of superoxide anions which may eventually cause injury to tissues during reperfusion. Superoxide anions first change to hydrogen peroxide in the presence of superoxide dismutase and then to hydroxyl radicals with ferrous ions (Habour-Weiss reaction [10]). In recent years, however, superoxide anions have been shown not to be able to extract hydrogen atoms from unsaturated fatty acids [20], highlighting the importance of both hydroxyl radicals and iron-oxygen complexes. These are thought to be more reactive free radicals and to possibly react with cell membranes, producing lipid peroxides and peroxidative intermediate metabolites. Lipid peroxides, as well as alkoxy/peroxy radicals, may directly injure the cell membrane [14, 25, 32, 38]. In fact, many investigators have already reported that peroxidative products are observed in damaged tissues during the postischemic period [8, 25, 38]. Because such radicals have highly reactive properties, they immediately react with cell membranes consisting of polyunsaturated fatty acids (PUFA), and they may initiate lipid peroxidation of the membranes. Peroxidized lipids generate many potentially cytotoxic products [14], among which stoichiometrically the major products are lipid hydroperoxides (LPO). LPO are highly toxic in vivo [2]; they are also ca-

pable of inactivating enzymes in vitro [7] and further promoting free radical-mediated destruction of PUFA [32] and proteins [14].

Ascorbic acid (AsA), a water-soluble vitamin, has both reducing and chelating properties. In the very early stage of reperfusion, AsA radicals (monodehydro-AsA) have been observed in an e.p.r.-spin trapping study, followed by hydroxyl radicals [34]. Their appearance may be explained by a washing out of the radicals after oxidation by free radicals produced during ischemia, and by AsA scavenging of reactive oxygen intermediates produced immediately after reperfusion [34]. With its strong reducing property and its property of scavenging free radicals, AsA is well known as a strong antioxidant agent [37]. One of its major functions is to protect tissues from harmful oxidative products and to keep certain enzymes in their reduced states [27]. It has also been demonstrated that, in addition to reacting with and scavenging superoxide and hydroxyl radicals [27], AsA can also scavenge singlet oxygen [28]. AsA is known to promote peroxidation at low concentrations [1, 13] but to inhibit it at higher concentrations [15, 27, 28, 37].

This peroxidative reaction at an early stage after reperfusion, i.e., primary I/R injury, may cause a variety of secondary events, such as the release of several types of interleukins and adhesion molecules of the cell membrane, and leukocyte infiltration to the tissues, secondary I/R injury [5]. I/R injury to the small intestine has been researched biochemically and histologically with regard to the production of free radicals and the adherence and accumulation of neutrophils [6, 16, 21, 31]. Almost the same mechanism may be responsible for I/R injury in the case of the small intestine.

Though the small intestinal tissue in rats is commonly known to contain AsA, the effects of AsA on postischemic lipid peroxidative injury are not yet thoroughly understood. At present, much remains unknown about the in vivo effects of AsA on I/R injury. In the present study, we examined the effects of AsA on I/R injury to the rat small intestine both biochemically and histologically. We then considered the possible mechanism underlying these effects on postischemic tissue injury caused by the lipid peroxidative reaction.

Materials and methods

Materials

The reduced form of glutathione (GSH), glyoxalase I, methylglyoxal, NADPH, glutathione reductase, and thiobarbituric acid (TBA) were obtained from Sigma (St. Louis, Mo., USA). The hemoglobin-methylene blue (HMB) test kit for the LPO assay was obtained from Kyowa Medex (Tokyo, Japan) and the ascorbic acid (AsA) from Wako Pure Chemical Industries (Osaka, Japan). All other chemicals were of analytical grade and were used without further purification.

Methods

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 1 day of fasting. The same volume of physiological saline was intraperitoneally administered to rats in a control group. Animals were divided into the following groups: (1) Controls ($n = 30$); (2) AsA: 2 mmol/kg ($n = 30$), 0.5 mmol/kg ($n = 21$), 0.1 mmol/kg ($n = 21$); and (3) GSH: 2 mmol/kg ($n = 30$). Parentheses indicate the number of rats examined in total.

Laparotomy was performed 1 h after administration of the drugs under anesthesia with Nembutal (pentobarbital sodium, 60 mg/100 body weight g), and the superior mesenteric artery was clamped to induce ischemia over the entire small intestine. The artery was unclamped after maintaining ischemia for 1 h. Each of the five groups of rats was divided into three subgroups based on the time points just before ischemia (B.I.), after ischemia (A.I.), and after 20 min of reperfusion (A.R.; K = 7–10 at each time point). At each time point, the entire small intestine was resected. The resected tissues were subjected to the following assays and histological examination.

Assay of thiobarbituric acid reactive substance (TBA-RS) and LPO of the small intestine tissue

Small intestinal tissues were homogenized with 10 volumes of 1.15% KCl solution and used to determine TBA-RS and LPO. TBA-RS was assayed following the method of Ohkawa et al. [23].

Assay of tissue LPO with the HMB test kit

The HMB test has a higher specificity for peroxides than the TBA method because it uses the peroxidase activity of hemoglobin. When hemoglobin reduces peroxides to their corresponding alcohols, N-methylcarbamoyl derivatives of methylene blue (leuco form) are oxidized and colored blue [11].

Assay of the reduced form of glutathione (GSH) and the oxidized form of glutathione (GSSG) of the small intestinal tissue

Small intestinal tissues were homogenized with 5 volumes of 6% perchlorate and subjected to the assay following the method of Bergmeyer et al . [12].

Assay of AsA and dehydro-AsA of the small intestinal tissue

Small intestinal tissues were also homogenized with 5 volumes of 5% TCA and centrifuged at 3000 rpm for 10 min. The supernatant was subjected to the assay of AsA and dehydro-AsA following the method of Okamura et al. [24].

Glutaminase activity of the intestinal tissue

Small intestinal tissues were homogenized in 125 mM potassium phosphate, 330 mM sucrose, and 2 mM dithiothreitol and subjected to the assay following the method of Pinkus and Windmueller [29].

Table 1 Criteria of histological grading 20 min after reperfusion

	Grading			
	0	1	2	3
1. Cell infiltration	None	Minor	Moderate	Severe
2. Separation of the villus epithelium from the lamina propria	None	Minor, in apical parts of the villi	Moderate, exceeding more than half the length of the villi (almost reaches the villus bases)	Denuded villi
3. Villus bases and crypts	Intact	Intact	Mainly intact	Destroyed
4. Edema	None or minor	Minor	Moderate	Severe

Table 2 Production of LPO during ischemia and reperfusion. All data represent mean ± SE for ten preparations in each group

	Before ischemia	After ischemia	After reperfusion
Control group	13.50 ± 1.25	16.38 ± 0.60	26.30 ± 1.98*
AsA group	12.00 ± 0.75	13.65 ± 0.61	16.75 ± 1.43**

(nmol/g tissue)
* $P < 0.05$ vs after ischemia; ** $P < 0.01$ vs control group

Histological grading

Small intestinal tissues were fixed in 10% buffered formalin and processed for standard light microscopy. The severity of the pathological lesions in small intestinal tissues resected 20 min after reperfusion ranged from 0 to 3, based on the following four parameters: (1) cell infiltration, (2) separation of the villus epithelium from the lamina propria, (3) villus bases and crypts, and (4) edema (Table 1) [19].

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.

Results

The following AsA group means AsA 2 mmol/kg treated group except for Fig. 2.

Lipid peroxidation of small intestinal tissue during ischemia and reperfusion. (Fig. 1, Table 2)

In the control group, the production of TBA-RS was significantly increased after 20 min of reperfusion of the small intestinal tissue (B.I. 0.020 ± 0.002, A.I. 0.081 ± 0.001, A.R. 0.155 ± 0.049 absorbance at 535 nm; $P < 0.01$ vs B.I.; $P < 0.05$ vs A.I.). The production was also significantly increased in the AsA 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 production of TBA-RS in small intestinal tissues obtained 20 min after reperfusion was significantly suppressed in the AsA groups compared with the control group ($P < 0.01$; Fig. 1), while in the GSH group the production of TBA-RS was significantly increased (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 intestinal tissue after reperfusion in the control group. However, the administration of AsA significantly suppressed the production of LPO (Table 2).

Dose-dependent effects of AsA on lipid peroxidation of the reperfused small intestinal tissue (Fig. 2)

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

Assay of the tissue level of AsA and dehydro-AsA in the rat small intestine during ischemia and reperfusion (Table 3)

In the control group, after 20 min of reperfusion, AsA content had decreased and dehydro-AsA increased; the ratio of dehydro-AsA to total AsA had also increased as a result of tissue reperfusion. In the AsA group, almost the same results were obtained. Before ischemia, the ratio of dehydro-AsA to total AsA was not significantly different between the control and AsA group (control group 7.8% ± 4.3%, AsA group 12.3% ± 2.7%, $P = 0.275$). However, in the AsA group, after reperfusion, the ratio was significantly lower than that of the control group.

Tissue glutathione levels in the small intestine during ischemia and reperfusion (Table 4)

In both groups, tissue levels of reduced glutathione (GSH) in the small intestine decreased due to ischemia, and they were further lowered after 20 min of reperfusion. Tissue levels of oxidized glutathione (GSSG) increased after reperfusion in both groups; however, the level of GSSG in the control group was higher than that in the AsA group. Though the difference in total glutathione levels after reperfusion was not significant between the control and AsA groups, the ratio of GSSG to total glutathione was significantly lower in the AsA group than in the control group.

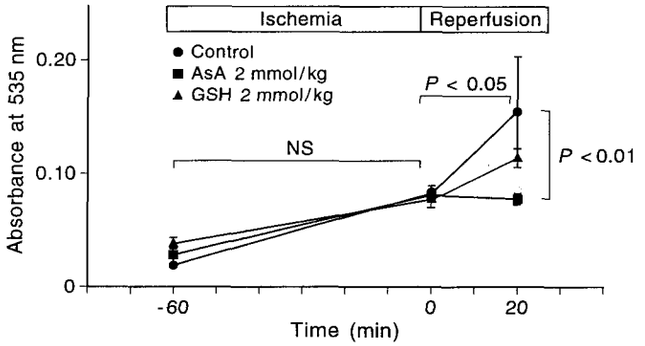

Fig. 1 Lipid peroxidation of the reperfused small intestinal tissue. AsA (2 mmol/kg body weight) was administered intraperitoneally 60 min before ischemia. The small intestine was then reperfused for 20 min. The production of TBA-RS in the control group (●) was significantly increased after the 20 min of reperfusion. Production in the AsA group (■) was significantly suppressed compared with the control group ($P < 0.01$). In the GSH group (▲) it was not suppressed. All data represent mean ± SE for ten preparations in each group

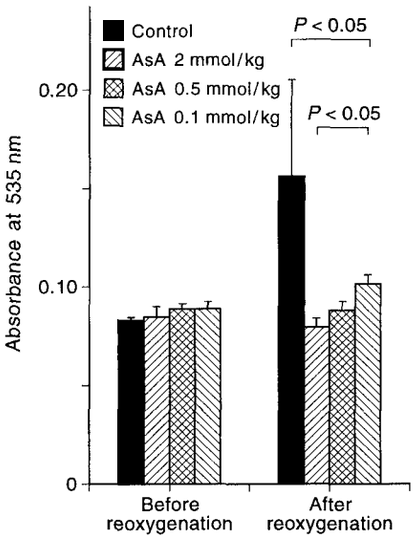

Fig. 2 Dose-dependent effects of AsA on lipid peroxidation of the reperfused small intestinal tissue. AsA [2 mmol/kg body weight (BW), 0.5 mmol/kg BW, and 0.1 mmol/kg BW] was administered intraperitoneally 60 min before ischemia. The small intestine was then reperfused for 20 min. AsA suppressed the production of TBA-RS in a dose-dependent manner, with significant differences between the 0.1 and 2.0 mmol/kg groups ($P < 0.05$). All data represent as mean ± SE for seven preparations in each group

Glutaminase activity in small intestinal tissues (Table 5)

In the control group, tissue glutaminase activity in the small intestine after reperfusion following ischemia decreased by 69 % compared with the activity before reperfusion. In the AsA group, however, tissue glutaminase activity decreased by only 34 %, and there were significant differences between the AsA and control groups.

Table 3 Tissue levels of ascorbic acid (AsA) and dehydro-AsA during ischemia and reperfusion. All data represent mean ± SE for ten preparations in each group

	Total AsA (μg/g tissue)		
	Before ischemia	After ischemia	After reperfusion
Control group	440.95 ± 14.25	477.50 ± 8.30	308.35 ± 12.13*
AsA group	558.66 ± 39.33	619.45 ± 26.58	372.10 ± 16.38**

* $P < 0.05$ vs after ischemia; ** $P < 0.05$ vs control group

	Dehydro-AsA (μg/g tissue)		
	Before ischemia	After ischemia	After reperfusion
Control group	36.76 ± 21.05	83.53 ± 7.45	103.93 ± 7.03*
AsA group	75.03 ± 18.93	90.75 ± 7.45	97.98 ± 10.00*

* $P < 0.05$ vs after ischemia

	Dehydro-AsA/Total AsA (%)		
	Before ischemia	After ischemia	After reperfusion
Control group	7.8 ± 4.3	16.9 ± 0.8	33.0 ± 0.9*
AsA group	12.3 ± 2.7	14.3 ± 1.2	25.8 ± 2.8*, **

* $P < 0.05$ vs after ischemia; ** $P < 0.05$ vs control group

Histology (Figs. 3, 4)

Histological examination of the reperfused small intestine showed marked mucosal congestion, edema, and epithelial exfoliation. The degree of injury ranged from 3 to 6 in the control group, from 1 to 4 in the AsA group, and from 1 to 5 in the GSH group, according to the modified grading system of Muller et al. (Fig. 4). The reperfusion injury following ischemia was apparently less marked in the AsA groups.

Discussion

Since it was first reported by Granger et al. in 1981 [5], I/R injury has attracted much attention as a major cause of functional disorders of various organs. At present, it is regarded as an important event in the pathophysiological processes involved in organ transplantation, some types of surgical treatments, cardiac [33] and cerebrovascular disorders, and hepatic [34] and gastrointestinal bleeding.

I/R injury is believed to result from lipid peroxidation, which may occur in various pathological and phys-

Table 4 Tissue glutathione levels in the small intestine during ischemia and reperfusion

	GSH (μmol/g tissue)		
	Before ischemia	After ischemia	After reperfusion
Control group	1.90 ± 0.12	1.75 ± 0.16	0.22 ± 0.02*
AsA group	1.94 ± 0.16	1.65 ± 0.15	1.34 ± 0.12**

* $P < 0.01$ vs after ischemia; ** $P < 0.01$ vs control group

	GSSG (μmol/g tissue)		
	Before ischemia	After ischemia	After reperfusion
Control group	0.42 ± 0.04	0.63 ± 0.02	1.56 ± 0.19
AsA group	0.35 ± 0.02	0.51 ± 0.02	0.84 ± 0.07**

** $P < 0.01$ vs control group

	Total glutathione (μmol/g tissue)		
	Before ischemia	After ischemia	After reperfusion
Control group	2.31 ± 0.13	2.38 ± 0.16	1.77 ± 0.18
AsA group	2.28 ± 0.15	2.17 ± 0.15	2.18 ± 0.15

	GSSG/Total glutathione (%)		
	Before ischemia	After ischemia	After reperfusion
Control group	18.1 ± 1.40	26.9 ± 2.10	87.2 ± 1.90
AsA group	15.7 ± 2.00	24.7 ± 1.40	38.6 ± 2.80**

** $P < 0.01$ vs control group

Table 5 Assay of glutaminase activity in tissues of small intestine. All data represent mean ± SE for ten preparations in each group

	Before reperfusion (BR)	After reperfusion (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*	0.66 ± 0.08*

(μmol glutamate/h per mg tissue)
* $P < 0.01$ vs control group

iological conditions at the cell membrane, which is composed of PUFA. LPO, the intermediate metabolite of lipid peroxidation, is believed to impair the structure and the function of the cell membrane [14, 25, 32, 38].

Evidence of hydroxyl radical generation in vivo in the early stage of reperfusion has already been found in the heart and the liver [33, 34]. The iron-catalyzed free radicals may extract hydrogen from PUFA in vitro and become effective initiators of lipid peroxidative reactions in vivo. Iron breaks LPO down into alkoxyl/peroxy radicals; these radicals extract hydrogen from PUFA and propagate the peroxidative chain reaction [9]. 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 reperfusion injury as a possible initiator of lipid peroxidation [9, 18]. When properly chelated or released, low-molecular weight iron may contribute to free radical-mediated tissue injury. Some in vitro experiments using iron and microsomes [20, 21] have shown that AsA acts as a pro-oxidant [22], while many in vivo studies using liver tissues have shown that it also serves as an antioxidant at high concentrations [17, 36, 37].

From the present study, we conclude that AsA acts mainly as an antioxidant, even if it does induce low-molecular weight iron and enhance reactivity of Fe ions to some extent, as in the reperfused rat liver [26].

We used an I/R 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 for only 60 min, so that the effects of ischemia itself would not be so severe (data not shown). The effects of reperfusion on small intestinal tissue were evaluated 20 min after reperfusion, at which time the infiltration of neutrophils into tissues was not yet observed [4, 26].

Dehydro-AsA has been reported to be less effective than AsA in the suppression of lipid peroxidation of reperfused liver tissue [26]. Ozaki et al. suggested that dehydro-AsA must be converted to AsA in order to possess an antioxidant property [26].

Glutathione, an endogenous antioxidant, is produced in the liver and distributed throughout the entire body, including the small intestine, via blood and bile juice. AsA, when oxidized in the early stage of reperfusion, changes to the dehydro-form, and then again to the original form when reduced. There may be some relationship between the redox states of both AsA and glutathione in vivo. That is, AsA acts as an antioxidant by being oxidized to dehydro-AsA and then again being reduced to AsA, coupled with the conversion of GSH to GSSG. In our AsA group, the ratio of GSSG to total glutathione was significantly low compared with that in the control group, suggesting that the level of GSH is maintained high and that its activity as an antioxidant is preserved. This observation may be explained by the following mechanism. AsA works more strongly as an antioxidant than GSH by being oxidized to dehydro-AsA, and AsA prevents GSH from being oxidized to GSSG in the very early stage of reperfusion.

Phosphate-dependent glutaminase (L-glutamine amidohydrolase) is one of the major glutamine-degrading enzymes in the intestine, and high activity of phosphate-dependent glutaminase has been found in intestinal mucosal epithelium, villus, and crypt cells of rats [29]. Glutaminase is observed rather uniformly along the entire length of the small intestine, and specific glutaminase activity is measured similarly in the duodenum, jejunum, and ileum [29]. In the present study,

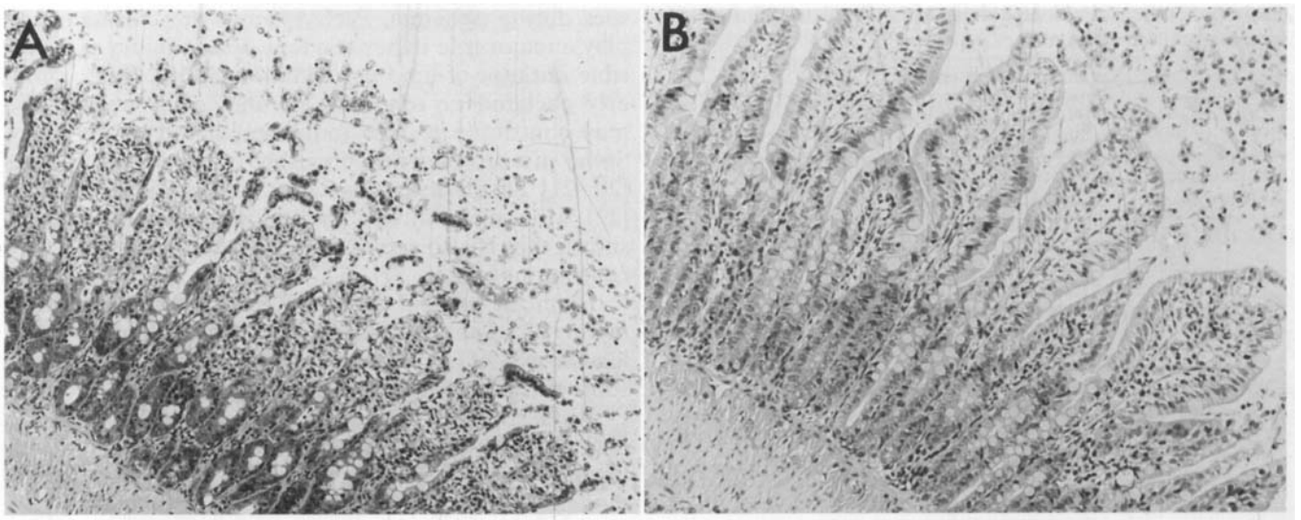

Fig. 3 A, B Histological appearance of the small intestine 20 min after reperfusion. After 60 min of ischemia followed by 20 min of reperfusion, full-thickness small intestinal tissues were fixed in 10% buffered formalin and processed for standard H&E stain and light microscopic examination (× 20): **A** control group: there is 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 group: there is mild cell infiltration, minor separation of the epithelium from the lamina propria, almost intact villus bases, and minor edema

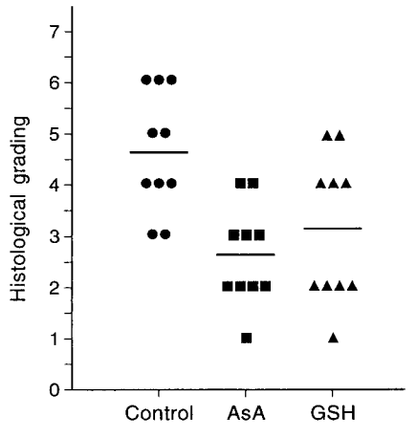

Fig. 4 Histological grading of the reperfused small intestinal tissue. The severity of pathological lesions in small intestinal tissues assessed 20 min after reperfusion was graded 0–3, based on the following four parameters: (1) cell infiltration, (2) separation of the villus epithelium from the lamina propria, (3) villus bases and crypts, and (4) edema. The degree of injury was graded from 3 to 6 in the control group (●), from 1 to 4 in the AsA group (■), and from 1 to 5 in the GSH group (▲)

glutaminase activity in the reperfused rat small intestinal tissues was well preserved by the administration of AsA.

Histopathologically, in the AsA group, the structure of the intestinal mucosa was generally preserved. The reperfused small intestine of rats in the control group showed severe cellular infiltration, separation of the epithelia from the lamina propria down to the villus base, mostly disarrayed villus bases, and moderate edema. Severe reperfusion injury following ischemia mainly occurrs in the mucosal layer of the small intestine in rats [19]. The mucosa contains over 90% of the total glutaminase activity [29]. This is why glutaminase activity remained so high in the AsA group. These results indicate that by administering AsA, the reperfused small intestinal tissues of rats were effectively protected from reperfusion injury, both biochemically and histologically.

In this in vivo study, AsA worked exclusively as an antioxidant and protected the postischemic intestinal tissue from peroxidative tissue injury. This protective mechanism may be explained, in part, as follows: AsA protects postischemic small intestinal tissue by scavenging radicals and/or reducing the peroxidative reactions. It also preserves the high glutathione level of postischemic small intestinal tissue. As the result, the cellular integrity of postischemic small intestinal tissue is well preserved.

In conclusion, our findings show that AsA acts as an antioxidant in the small intestine and reduces injury due to reperfusion. These effects should be very useful in preserving mucosal function or accelerating its recovery after certain types of surgical treatments, such as enterectomy, and especially small bowel transplantation.

Reference

1. Bodannes RS, Chan PC (1979) Ascorbic acid as a scavenger of singlet oxygen. FEBS Lett 105: 195–196
2. Caraceni P, Rosenblum ER, Thiel DHV, Borle AB (1994) Reoxygenation injury in isolated rat hepatocytes: relation to oxygen free radicals and lipid peroxidation. Am J Physiol 266: G799–G806
3. Flecha BG, Cutrin JC, Boveris A (1993) Time course and mechanism of oxidative stress and tissue damage in rat liver subjected to in vivo ischemia-reperfusion. J Clin Invest 91: 456–464
4. Grace PA (1994) Ischemia-reperfusion injury. Br J Surg 81: 637–647
5. Granger DN, Rutili G, McCord JM (1981) Superoxide radicals in feline intestinal ischemia. Gastroenterology 81: 22–29
6. Granger DN, Hollwarth ME, Parks DA (1986) Ischemic-reperfusion injury: role of oxygen-derived free radicals. Acta Physiol Scand 126: 47–63
7. Green RC, Little C, O'Brien PJ (1971) The inactivation of isocitrate dehydrogenase by a lipid peroxide. Arch Biochem Biophys 142: 598–605
8. Grune T, Siems WG, Schonheit K, Blasig IE (1993) Release of 4-hydroxynonenal, an aldehydic mediator of inflammation, during postischemic reperfusion of the myocardium. Int J Tissue React 15: 145–150
9. Halliwell B, Gutteridge JMC (1984) Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J 219: 1–14
10. Harbour JR, Chow V, Bolton JR (1974) An electron spin resonance study of the spin adducts of OH and HO2 radicals with nitrons in the ultraviolet photolysis of aqueous hydrogen peroxide solutions. Can J Chem 52: 3549–3553
11. Kanazawa K, Inoue N, Ashida H, Mizuno M, Natake M (1989) What do thiobarbituric acid and hemoglobin-methylene blue tests evaluate in the endogenous lipid peroxidation of rat liver ? J Clin Biochem Nutr 7: 69–79
12. Klotsh H, Bergmeyer HU (1974) Methods in enzymatic analysis, vol.4. Academic Press, New York
13. Laudicina DC, Marnett LJ (1990) Enhancement of hydroperoxide-dependent lipid peroxidation in rat liver microsomes by ascorbic acid. Arch Biochem Biophys 278: 73–80
14. Logani MK, Davies RE (1980) Lipid oxidation: biologic effects and antioxidants, a review. Lipids 15: 485–495
15. Mak IT, Weglicki WB (1985) Characterization of iron-mediated peroxidative injury in isolated hepatic lysosomes. J Clin Invest 75: 58–63
16. McCord JM (1985) Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med 312: 159–163
17. Miller DM, Aust SD (1989) Studies of ascorbate-dependent, iron-catalyzed lipid peroxidation. Arch Biochem Biophys 271: 113–119
18. Miller DM, Spear NH, Aust SD (1992) Effects of deferrioxamine on iron-catalyzed lipid peroxidation. Arch Biochem Biophys 295: 240–246
19. Muller AR, Nalesnik M, Platz KP, Langrehr JM, Hoffman RA, Schraut WH (1994) Evaluation of preservation conditions and various solutions for small bowel preservation. Transplantation 57: 649–655
20. Niki H (1987) Kasseisanso. Ishiyaku Press, Tokyo
21. Nilsson UA, Schoenberg MH, Aneman A, Poch B, Magadum S, Beger HG, Lundgren O (1994) Free radicals and pathogenesis during ischemia and reperfusion of the cat small intestine. Gastroenterology 106: 629–636
22. Nordstrom G, Seeman T, Hasselgren PO (1985) Beneficial effect of allopurinol in liver ischemia. Surgery 97: 679–683
23. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95: 351–358
24. Okamura M (1980) An improved method for determination of L-ascorbic acid and L-dehydroascorbic acid in blood plasma. Clin Chim Acta 103: 259–268
25. Ozaki M, Fuchinoue S, Teraoka S, Ota K (1994) Mobilization of low-molecular-weight iron and peroxidative damage during ischemia and reoxygenation of the rat liver. Transplant Proc 26: 918–921
26. Ozaki M, Fuchinoue S, Teraoka S, Ota K (1995) The in vivo cytoprotection of ascorbic acid against ischemia/reoxygenation injury of rat liver. Arch Biochem Biophys 318: 439–445
27. Padh H (1990) Cellular functions of ascorbic acid. Biochem Cell Biol 68: 1166–1173
28. Palamanda JR, Kehrer JP (1993) Involvement of vitamin E and protein thiols in the inhibition of microsomal lipid peroxidation by glutathione. Lipids 28: 427–431
29. Pinkus LM, Windmueller HG (1977) Phosphate-dependent glutaminase of small intestine: localization and role in intestinal glutamine metabolism. Arch Biochem Biophys 182: 506–517
30. Rao PS, Mueller HS (1983) Lipid peroxidation and acute myocardial ischemia. Adv Exp Med Biol 161: 347–363
31. Schoenberg MH, Poch B, Younes M, Schwarz A, Baczako K, Lundberg C, Haglund U, Beger HG (1991) Involvement of neutrophils in postischaemic damage to the small intestine. Gut 32: 905–912
32. Shimasaki H, Privett OS (1975) Studies on the role of vitamin E in the oxidation of blood components by fatty hydroperoxides. Arch Biochem Biophys 169: 506–512
33. Sun JZ, Kaur H, Halliwell B, Li XY, Bolli R (1993) Use of aromatic hydroxylation of phenylalanine to measure production of hydroxyl radicals after myocardial ischemia in vivo. Circ Res 73: 534–549
34. Togashi H, Shinzawa H, Yong H, Takahashi T, Noda H, Oikawa K, Kamada H (1994) Ascorbic acid radical, superoxide, and hydroxyl radical are detected in reperfusion injury of rat liver using electron spin resonance spectroscopy. Arch Biochem Biophys 308: 1–7
35. Weinberg JM, Davis JA, Abarzua M, Rajan T (1987) Cytoprotective effects of glycine and glutathione against hypoxic injury to renal tubules. J Clin Invest 80: 1446–1454
36. Younes M, Kayser E, Strubelt O (1992) Effect of antioxidants on hypoxia/reoxygenation induced injury in isolated perfused rat liver. Pharmacol Toxicol 71: 278–283
37. Zeng LH, WU J, Carey D, Wu TW (1991) Trolox and ascorbate: are they synergistic protecting liver cells in vitro and in vivo? Biochem Cell Biol 69: 198–201
38. Zimmerman BJ, Granger DN (1994) Mechanism of reperfusion injury. Am J Med Sci 307: 284–292