Free Rad. Res. Comms., Vol. 7, No. 3–6, pp. 285–291
Reprints available directly from the publisher
Photocopying permitted by license only

© 1989 Harwood Academic Publishers GmbH
Printed in the United Kingdom

ROLE OF OXYGEN-DERIVED FREE RADICALS IN GASTRIC MUCOSAL INJURY INDUCED BY ISCHEMIA OR ISCHEMIA-REPERFUSION IN RATS

TOSHIKAZU YOSHIKAWA, SIGENOBU UEDA, YUJI NAITO, SHUJI TAKAHASHI, HIROKAZU OYAMADA, YUTAKA MORITA, TOMOYUKI YONETA and MOTOHARU KONDO

First Department of Medicine, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602, Japan

Free Radic Res Downloaded from informahealthcare.com by University of Illinois Chicago on 11/06/11
For personal use only.

Oxygen-derived free radicals have been implicated as possible mediators in the development of tissue injury induced by ischemia and reperfusion. Clamping of the celiac artery in rats reduced the gastric mucosal blood flow to 10% of that measured before the clamping. The area of gastric erosions and thiobarbituric acid (TBA) reactants in gastric mucosa were significantly increased 60 and 90 min after clamping. These changes were inhibited by treatment with SOD and catalase. Thirty and 60 min after reoxyganation, produced by removal of the clamps following 30 min of ischemia, gastric mucosal injury and the increase in TBA reactants were markedly aggravated compared with those induced by ischemia alone. SOD and catalase significantly inhibited these changes. The serum α-tocopherol/cholesterol ratio, an index of *in vivo* lipid peroxidation, was significantly decreased after long periods of ischemia (60 and 90 min), or after 30 and 60 min of reperfusion following 30 min of ischemia. These results indicated that active oxygen species and lipid peroxidation may play a role in the pathogenesis of gastric mucosal injury induced by both ischemia alone and ischemia-reperfusion. Although, allopurinol inhibited the formation of gastric mucosal injury and the increase in TBA reactants in gastric mucosa, the depletion of polymorphonuclear leukocytes (PMN) counts induced by an injection of anti-rat PMN antibody did not inhibit these changes. As compared with the hypoxanthine-xanthine oxidase system, PMN seem to play a relatively small part in the formation of gastric mucosal injury induced by ischemia-reperfusion.

KEY WORDS: Ischemia-reperfusion, oxygen free radicals, superoxide dismutase, catalase, gastric mucosal injury, lipid peroxidation.

INTRODUCTION

The ischemia itself causes tissue damage and eventual death, but further injuries can occur while oxygen in reintroduced to the tissue. Much evidence suggests the possible contribution of free radicals and active oxygen species derived from molecular oxygen in tissue injury which accompanies ischemia and reperfusion in several organs.[1-7] Ischemia in a stomach that contains acid may produce severe gastric mucosal damage. It has been suggested that superoxide radical or hydroxyl radical may be the major oxygen radicals contributing to ischemia or reperfusion injury in the stomach.[6,8] These reactive species can attack and damage important biological molecules. Within cellular membranes, hydroxyl radical can initiate a free radical chain reaction known as lipid peroxidation, in which polyunsaturated fatty acids are broken down into water-

Correspondence; Toshikazu Yoshikawa, MD, PhD First Department of Medicine, Kyoto Prefectural Univeristy of Medicine, Kamigyo-ku, Kyoto 602, Japan.

RIGHTSLINK

soluble products and toxic lipid peroxides are produced with the consequent distruption of membrane integrity. Peroxidation of lysosomal membranes may result in cell death through the release of lysosomal hydrolases into the cytoplasm. These pathological changes may attack the gastric mucosa with acid and pepsin to produce erosion or ulcer. The major source of active oxygen species produced after ischemia or ischemia-reperfusion seems to be the enzyme xanthine oxidase and activated polymorphonuclear leukocytes (PMN).[9] The purpose of this study was to determine the extent to which active oxygen species are a contributing factor in the pathogenesis of gastric mucosal injury from ischemia alone or ischemia and reperfusion. The roles of tissue xanthine oxidase and activated PMN as sources of oxygen radicals were investigated by the use of a xanthine oxidase inhibitor, allopurinol, and anti-rat PMN antibody.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 190–210 g from Keari Co., Ltd., Osaka were used. The animals were not fed 18 h prior to the experiments, but allowed free access to water. Ischemia was created under i.p. pentobarbital anesthesia (25 mg/kg) by applying small clamps to the celiac artery, and reoxygenation was produced by removal of the clamps. After ischemia or ischemia-reperfusion, the rats were killed by exsanguination via the abdominal aorta. The gastric mucosa was carefully examined macroscopically and microscopically, and the extent of the gastric mucosal lesion was expressed by the total area of the erosions.

The microcirculatory blood flow in the gastric mucosa was measured by a laser doppler flowmeter (ALF 2100, Advance Co., Ltd., Tokyo). Thiobarbituric acid (TBA) reactive substances, an index of lipid peroxidation, were measured in serum by the method of Yagi,[10] and in tissue by that of Ohkawa *et al.*[11]. The level of TBA reactants was expressed as nmol of malondialdehyde. TBA (BDH Chemical, Poole, England) and 1,1,3,3-tetramethoxypropane (Takyo Kasei Co., Tokyo) were used for the TBA assay, and all other chemicals were of reagent grade. The serum α-tocopherol content was determined by the method of Abe *et al.*,[12] using a high-speed LC-6A liquid chromatograph (Shimazu Co., Kyoto). The serum cholesterol level was assayed according to the method of Richmond.[13]

To assess the effect of superoxide dismutase (SOD) and catalase, human Cu-ZnSOD (Nippon Kayaku Co., Ltd, Tokyo) at a dose of 50,000 U/kg and/or catalase from bovine liver (Sigma Chemical Co., St Louis, MO) at a dose of 90,000 U/kg were s.c. injected 1 h before ischemia, and 10,000 U/kg of SOD was i.v. injected just before the reoxygenation. The control rats were treated with physiological saline in the same manner. Allopurinol (Sigma Chemical Co., St Louis, MO), an inhibitor of xanthine oxidase, dissolved in distilled water (pH 10.8) was orally administered to rats at a dose of 100 mg/kg 48 and 24 h before the experiments. For the control, distilled water adjusted to pH 10.8 by 0.5 N NaOH was administered in the same manner. Anti-rat PMN antibody (10 ml/kg) obtained from immunized rabbits according to the modified method of Ward *et al.*,[14] was i.p. injected 18 h before the experiments. The control rats were i.p. injected with normal rabbit serum in the same manner.

Free Radic Res Downloaded from informahealthcare.com by University of Illinois Chicago on 11/06/11
For personal use only.

RIGHTSLINK

RESULTS

Gastric mucosal blood flow

Clamping of the celiac artery reduced the gastric mucosal blood flow to 10% of that measured before the clamping (Figure 1). By removing clamps, the gastric mucosal blood flow recovered promptly to the levels before clamping. However, when the clamping period was longer than 45 min, prompt recovery from ischemia was inhibited. The decrease in gastric mucosal blood flow after the clamping and prompt recovery of the blood flow following removal of the clamps were not influenced by the treatment with SOD and/or catalase.

Gastric mucosal injury

The total area of erosions, a morphological index of gastric injury, increased gradually after clamping of the celiac artery. However, the gastric mucosal lesions were significantly increased after the reperfusion following 30 min of gastric mucosal ischemia (Figure 2).

TBA reactive substances

TBA reactants in the gastric mucosa, an index of lipid peroxidation, did not increase 30 min after ischemia. However, TBA reactive substances in the gastric mucosa significantly increased 60 and 90 min after clamping, and 30 and 60 min after reoxygenation following 30 min of ischemia (Figure 3). Serum TBA reactants did not increase significantly after ischemia or ischemia-reperfusion.

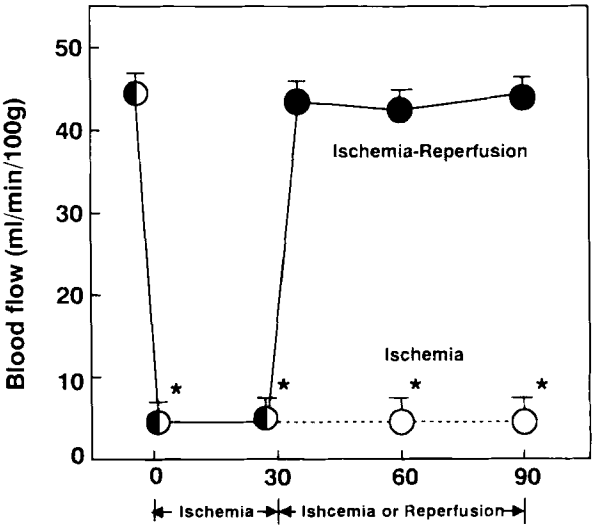

FIGURE 1 Changes in gastric mucosal blood flow. Each value indicates the mean ± SE of 6 rats. *$p < 0.001$ for difference to the values of rats before clamping of the celiac artery.

Free Radic Res Downloaded from informahealthcare.com by University of Illinois Chicago on 11/06/11
For personal use only.

RIGHTSLINK

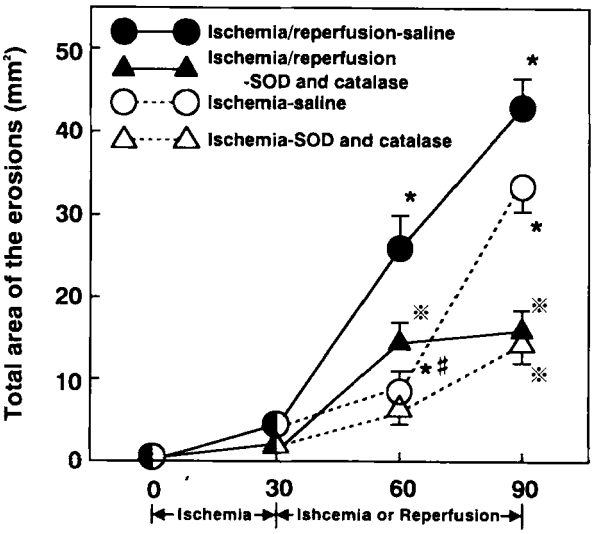

FIGURE 2 Changes in total area of gastric erosions after ischemia or ischemia-reperfusion, and the effects of SOD and catalase on these changes. Each value indicates the mean ± SE of 5–12 rats. # $p < 0.05$ for difference to the values of rats 30 min after reperfusion following 30 min of ischemia. ※ $p < 0.01$ for difference to the values of rats treated with physiological saline. *$p < 0.001$ for difference to the values of rats before clamping of the celiac artery.

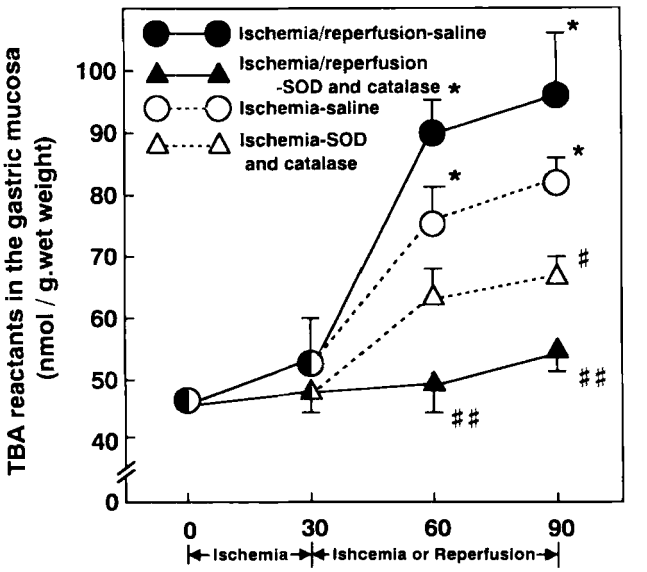

FIGURE 3 Changes in TBA reactive substances in gastric mucosa after ischemia or ischemia-reperfusion, and the effects of SOD and catalase on these changes. Each value indicates the mean ± SE of 5–14 rats. #$p < 0.05$ and ##$p < 0.01$ for difference to the values of rats treated with physiological saline. *$p < 0.001$ for difference to the values of rats before clamping of the celiac artery.

Free Radic Res Downloaded from informahealthcare.com by University of Illinois Chicago on 11/06/11
For personal use only.

RIGHTSLINK

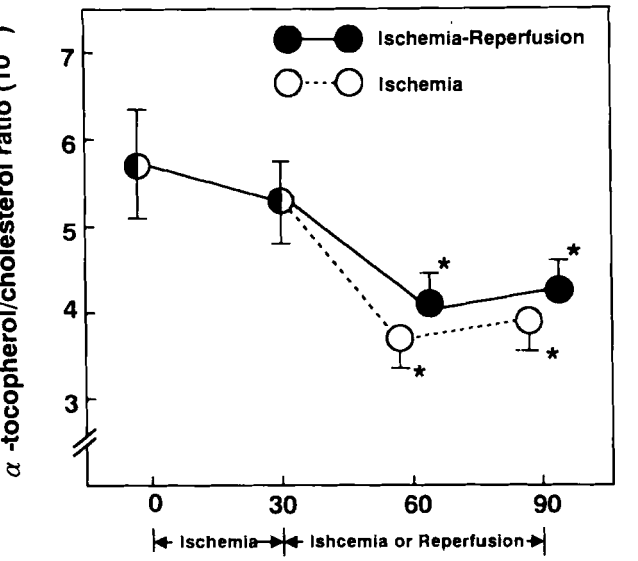

FIGURE 4 Changes in serum α-tocopherol/cholesterol ratio after ischemia or ischemia-reperfusion. Each value indicates the mean ± SE of 5–14 rats. *p < 0.05 for difference to the values of rats before clamping of the celiac artery.

Changes in serum α-tocopherol/cholesterol ratio

After ischemia or ischemia-reperfusion, the serum α-tocopherol levels were significantly decreased. To eliminate the influence of lipids, the α-tocopherol/cholesterol ratio was examined. The ratio was significantly decreased 60 and 90 min after ischemia, and 30 and 60 min after reperfusion following 30 min of ischemia (Figure 4).

Effects of SOD and catalase

The total area of gastric erosions induced by ischemia or ischemia-reperfusion significantly decreased by the treatment with SOD and catalase (Figure 2). The increase in TBA reactive substances in the gastric mucosa induced by ischemia of ischemia-reperfusion was significantly inhibited by the treatment with SOD and catalase (Figure 3).

Effects of allopurinol

Treatment with allopurinol attenuated the gastric mucosal injury induced by 60 min after reperfusion following 30 min of ischemia, and significantly inhibited the increase in TBA reactants in the gastric mucosa (Table 1).

Effects of anti-rat PMN antibody

The circulating PMN counts showed a 90% decrease 18 h after the administration of anti-rat PMN antibody. Platelet counts and red blood cell counts were not influenced by the antibody treatment. The depletion of PMN counts induced by the treatment

Free Radic Res Downloaded from informahealthcare.com by University of Illinois Chicago on 11/06/11
For personal use only.

RIGHTSLINK

TABLE 1
Effects of allopurinol and PMN-depletion on the total area of gastric erosions and on the increase in TBA reactive substances in the gastric mucosa 60 min after reperfusion following 30 min of ischemia.

Treatment	Total area of erosions (mm^2)	N#	TBA reactants in gastric mucosa (n mol/g wet weight)	N#
Distilled water (pH 10.8)	23.6 ± 4.0	10	62.1 ± 4.3	7
Allopurinol (100 mg/kg × 2)	11.3 ± 3.4*	9	48.5 ± 3.3*	7
Normal rabbit serum	28.0 ± 6.4	8	61.2 ± 5.4	8
Anti-rat PMN antibody	19.6 ± 4.6	8	58.0 ± 5.0	8

Each value indicates the mean ± SE *p < 0.05 for difference to the values of rats treated with distilled water (pH 10.8). # Number of rats.

with anti-rat PMN antibody did not show significant inhibition against the aggravation of gastric mucosal lesions or the increase in TBA reactive substances in gastric mucosa induced by ischemia or ischemia-reperfusion (Table 1).

DISCUSSION

The roles of free radical-mediated injuries have become increasingly accepted in many disease states.[1,2] Increasingly, interest has focused on their role in the setting of ischemia and reperfusion, where injury has been convincingly linked to free radical production in several organs. This study shows that gastric mucosal injury can be produced and TBA reactive substances in the gastric mucosa significantly increase after gastric mucosal ischemia induced by the clamping of the celiac artery, and also after reoxygenation following 30 min of ischemia. These results suggest that lipid peroxidation may play an important role in the formation of gastric mucosal lesions induced by ischemia or ischemia-reperfusion. These changes induced by reperfusion were more marked than those induced by ischemia. Mucosal injury and the increase in TBA reactants were clearly and significantly inhibited during ischemia or reperfusion in the SOD and catalase-treated rat stomachs without altering the reduced mucosal blood flow. These results indicate that the effectiveness of these enzymes may possibly occur not through the effect on gastric mucosal microcirculation, but through the catalysis of oxygen radicals. We reported that the serum α-tocopherol/cholesterol ratio is a good index for monitoring lipid peroxidation *in vivo*.[7] In the present investigation, the ratio was significantly decreased during both ischemia alone and ischemia-reperfusion. It has become clear that lipid peroxidation may occur not only during reperfusion but also during ischemia.

Many physiological processes are known to result in the production of oxygen-derived free radicals:enzymatic reactions, electron transport processes within the mitochondria and endoplasmic reticulum, intracellular autooxidation of various compounds, arachidonic acid metabolism, and the activation of phagocytic cells. The source of oxygen free radicals in tissues subjected to ischemia and reperfusion is not yet clear. Xanthine oxidase was the first documented biological source of superoxide radical.[15]

In the presence of oxygen, xanthine oxidase catalyzes the conversion of hypoxanthine to xanthine with the simultaneous generation of superoxide.[9] While one substrate (hypoxanthine) accumulates during ischemia as a result of adenosine 5′-triphosphate

Free Radic Res Downloaded from informahealthcare.com by University of Illinois Chicago on 11/06/11
For personal use only.

RIGHTSLINK

degradation, the other (molecular oxygen) is provided by reperfusion, which could theoretically result in a burst of superoxide production. In a gastric injury model induced by hemorrhagic shock, Itoh and Guth[8] reported that gastric lesions were reduced by treatment with SOD or allopurinol, a competitive inhibitor of xanthine oxidase. In our model of ischemia-reperfusion, pretreatment with allupurinol largely prevented the formation of gastric mucosal injury, indicating that xanthine oxidase in a major source of reactive oxygen metabolites in gastric injury induced by ischemia-reperfusion.

Another postulated source of oxygen free radicals is activated PMN.[9] When PMN are stimulated by particles or specific soluble inflammatory mediators, they produce superoxide radicals via the activation of NADPH oxidase in the cell membrane. Most of the hydrogen peroxide released during the stimulation of phagocytic cells appears to be directly derived from the dismutation of superoxide radicals. To clarify the role of PMN on ischemia-reperfusion injury in the stomach, we produced PMN depleted rats by the administration of anti-PMN antibody obtained from immunized rabbits. However, by the treatment with anti-PMN antibody, the gastric mucosal injury induced by ischemia-reperfusion was not inhibited. As compared with the hypoxanthine-xanthine oxidase system catalyzed by xanthine oxidase, PMN seem to play a relatively small part in the formation of gastric mucosal injury induced by ischemia-reperfusion. Broader knowledge concerning the source of free radicals, the mechanism of injury initiation, and the biochemical regulation of antioxidant defence mechanisms should provide insight into new therapeutic strategies for the modulation of many disorders induced by ischemia and ischemia-reperfusion.

Free Radic Res Downloaded from informahealthcare.com by University of Illinois Chicago on 11/06/11
For personal use only.

References

1. Bulkley, G.B. *Surgery,* **94,** 407–411, (1983).
2. Weisiger, R.A. *Gastroenterol.,* **90,** 494–496, (1986).
3. Granger, D.N., Rutili, G. and McCord, J.M. *Gastroenterol.,* **81,** 22–29, (1981).
4. Gardner, T.J., Stewart, J.R., Casale, A.S., Dawney, J.M. and Chambers, D.E. *Surgery,* **94,** 423–427, (1983).
5. Arthur, M.J.P., Bentley, I.S., Tanner, A.R., Kowalski Saunders, P., Millward-Sadler, G.H. and Wright, R. *Gastroenterol.,* **89,** 1114–1122, (1985).
6. Perry, M.A., Wadwa, S., Parks, D.A., Pickard, W. and Granger, D.N. *Gastroenterol.,* **90,** 362–367, (1986).
7. Yoshikawa, T., Takemura, T., Tanigawa, T., Miyagawa, H., Yoshida, N., Sugino, S. and Kondo, M. *Bioelectrochem. Bioenerg.* **18,** 295–300, (1987).
8. Itoh, M and Guth P.H. *Gastroenterol.* **88,** 1162–1167, (1985).
9. McCord, J.M. *N. Engl. J. Med.,* **312,** 159–163, (1985).
10. Yagi, K. *Biochem. Med.,* **15,** 212–216, (1976).
11. Ohkawa, H., Ohishi, N. and Yagi, K. *Anal. Biochem.,* **95,** 351–358, (1979).
12. Abe, K., Yoguchi, Y. and Katsui, G. *J. Nutr. Sci. Vitaminol.,* **21,** 183–188, (1975).
13. Richmond, W. *Clin Chem.,* **19,** 1350–, (1973).
14. Ward, P.A. and Cochrance, S.G. *J. Exp. Med.,* **121,** 215–237, (1965).
15. McCord, J.M. and Fridovich, I. *J. Biol. Chem.,* **243,** 5753–5760, (1968).

Accepted by Prof. T.F. Slater

RIGHTSLINK