Inhibition by Aminosalicylates of Lipid Peroxidation in Large Intestinal Mucosa after Mesenteric Ischemia/Reperfusion in the Rat

Takumi Kumamoto, Akihiko Matsuda, Mikiko Kataoka and Yukifumi Kokuba

Development Laboratories, Research and Development Division, Nippon Hoechst Marion Roussel Ltd., Shiga 520–23, Japan

Received May 19, 1997
Accepted July 25, 1997

ABSTRACT—To clarify the mode of action of aminosalicylates, which are generally used as therapeutic agents for ulcerative colitis, we investigated the effect of some of the aminosalicylates on lipid peroxidation in the large intestinal mucosa after mesenteric ischemia/reperfusion in the rat. Lipid peroxidation was assessed by measuring the level of thiobarbituric-acid-reactive substances. It was found that aminosalicylates dose-dependently inhibited the elevation of the level of thiobarbituric-acid-reactive substances in the large intestinal mucosa after ischemia/reperfusion. This effect may partly contribute to the therapeutic actions of aminosalicylates in ulcerative colitis.

Keywords: Lipid peroxidation, Ischemia/reperfusion, Large intestinal mucosa

Although the cause of ulcerative colitis is unclear, Wilson (1) and Granger et al. (2) reported that it is partly due to oxygen-derived free radicals and the inflammation and tissue injury in this disease and that salicylazosulphapyridine (SASP) and its metabolite [5-aminosalicylic acid (5-ASA)] might scavenge the oxygen-derived free radicals in gastrointestinal and small intestinal ischemia/reperfusion models. SASP is effective in the treatment and prophylaxis of ulcerative colitis. Although ulcerative colitis is generated in the large intestine, no study using large intestinal ischemia/reperfusion models has been reported. Therefore, we investigated the effect of aminosalicylates on mucosal lipid peroxidation after ischemia/reperfusion in the rat large intestine.

We used 77 male Sprague-Dawley rats weighing 205–410 g (7- to 9-weeks-old; Charles River, Yokohama) in this study. After an overnight fast, the rats were anesthetized with urethane (50 mg/kg, i.p.). The body temperature was maintained at 37°C by a heating pad. The rats underwent laparotomy, and the large intestinal lumen was flushed with 25–30 ml of warm sodium-phosphate-buffered saline (pH 7.4, bubbled with argon gas). The superior mesenteric artery and inferior mesenteric artery were ligated according to the method of Megison et al. (3). Collateral arcades from the right coeliac artery and the jejunal arteries proximal to the site of occlusion were ligated to avoid the variable contribution of collateral circulation to the distal ileum. To produce ischemia, atraumatic vascular clamps were then placed across the superior mesenteric artery and inferior mesenteric artery, and the bowel was then placed across the superior mesenteric artery and inferior mesenteric artery and returned to the abdominal cavity (10 min). After 10-min ischemia, for reperfusion, the clamps were removed from the superior mesenteric artery and inferior mesenteric artery (60 min). The rats in the sham-operated group underwent laparotomy, and the superior mesenteric artery, inferior mesenteric artery and collateral vessels were isolated but not occluded. In 6 rats in each group 3 ml of 5-[4-(2-carboxyethylcarbamoyl)-phenylazo]salicylic acid disodium salt dianhydride (BX661A), SASP, 5-ASA (0.01, 0.1, 1 mM), N-(4-aminobenzoyl)-γ-alanine (4-ABA, 1 mM) or sulphapyridine (SP, 1 mM) solution in phosphate-buffered saline was injected into the large intestinal lumen 1 min before reperfusion. Three milliliters of a mixture of superoxide dismutase (SOD, at 990 units/rat) and catalase (CAT, at 12,060 units/rat) was injected into the large intestinal lumen 1 min before reperfusion (4). Five rats in the control group were given 3 ml of phosphate-buffered saline into the large intestinal lumen 1 min before reperfusion. Three milliliters of a mixture of superoxide dismutase (SOD, at 990 units/rat) and catalase (CAT, at 12,060 units/rat) was injected into the large intestinal lumen 1 min before reperfusion (4).

After 60 min reperfusion, the large intestine was excised, and then we removed BX661A and SASP solutions from the large intestinal lumen by flushing it with 25–30 ml of ice cold sodium phosphate-buffered saline (pH 7.4, bubbled with argon gas). The mucosa was removed by the method of Kasai et al. (5). The level of thiobarbituric-acid-reactive substances (TBARS), an index of lipid peroxidation in rat large intestinal mucosa, was measured by the method of...
Buege and Aust (6). We combined 500 µl of homogenised large intestinal mucosa with 5 ml of TCA-TBA-HCl (15% w/v trichloroacetic acid, 0.375% w/v thiobarbituric acid, 0.25 N hydrochloric acid) solution and mixed them thoroughly. The mixed solution was heated for 15 min in a boiling water bath. After cooling, the precipitate was removed by centrifugation at 1,200 x g for 15 min. The absorbance of the sample was determined at 535 nm against a blank that contained all the reagents except for the large intestinal mucosa. The TBA concentration of the sample was determined using an extinction coefficient of 1.56 x 10^5 M^-1 cm^-1. All values are expressed as the mean ± S.E. The TBARS levels were evaluated using analysis of variance followed by a multiple comparison procedure (Bonferroni's test).

Fig. 1. Effects of SOD/CAT on elevation of TBARS levels in rat large intestinal mucosa following ischemia/reperfusion. []: Sham-operated (n = 6), ■: control (n = 6), □: SOD (990 U/rat)/CAT (12906 U/rat) (n = 6). Each point represents the mean ± S.E. Three milliliters of SOD and CAT mixture was injected into the large intestinal lumen 1 min before reperfusion. In the control group, 3 ml of phosphate-buffered saline was injected into the large intestinal lumen 1 min before reperfusion. In the sham-operated group, the rats underwent laparotomy, and the superior mesenteric artery, inferior mesenteric artery, and collateral vessels were isolated but not occluded. *P<0.05 vs control (Bonferroni’s test). TBARS: thiobarbituric-acid-reactive substances, SOD: superoxide dismutase, CAT: catalase.

After ischemia/reperfusion, the TBARS level was increased to 41 ± 2 pmol/mg of mucosa compared with 25 ± 2 pmol/mg in the sham-operated rats (P < 0.05). The SOD/CAT mixture completely inhibited the elevation of TBARS level in the large intestinal mucosa in the rats after ischemia/reperfusion (Fig. 1). Similar results have been reported by Buyukgebiz et al. (7). SOD and CAT are enzymes that catalyze the dismutation of O2•− and H2O2, respectively. Several studies indicated that SASP and 5-ASA scavenge reactive oxygen species (8). After oral administration of SASP and BX661A, SASP is cleaved into 5-ASA and its moieties (SP), and BX661A is cleaved into 5-ASA and its moieties (4-ABA) by large intestinal flora.

Fig. 2. Effects of BX661A and other test drugs on elevation of TBARS levels in rat large intestinal mucosa following ischemia/reperfusion. []: Sham-operated (n = 6), ■: control (n = 5), □: Test drug (0.01 mM, n = 5), ○: Test drug (0.1 mM, n = 5), △: Test drug (1 mM, n = 5). Each point represents the mean ± S.E. Three milliliters of BX661A, SASP, 5-ASA (0.01, 0.1, 1 mM), 4-ABA or SP (1 mM) in phosphate-buffered saline was injected into the large intestinal lumen 1 min before reperfusion (in each group). In the control group, 3 ml of phosphate-buffered saline was injected into the large intestinal lumen 1 min before reperfusion. In the sham-operated group, rats underwent laparotomy, and the superior mesenteric artery, inferior mesenteric artery, and collateral vessels were isolated but not occluded. *P<0.05, **P<0.01 vs control (Bonferroni's test). TBARS: thiobarbituric-acid-reactive substances.
SASP, BX661A and 5-ASA dose-dependently inhibited the elevation of the TBARS level in large intestinal mucosa induced by ischemia/reperfusion, but 1 mM 4-ABA (moiety of BX661A) and 1 mM SP (moiety of SASP) did not inhibit it (Fig. 2). Concerning BX661A and SASP, however, there is a possibility that the inhibition was caused by 5-ASA, the moiety of BX661A, and SASP, cleaved by the remaining flora. To clarify this, we collected the BX661A and SASP solutions from the large intestinal lumen to analyze 5-ASA in BX661A or SASP solution with HPLC after the ischemia/reperfusion experiments. However, 5-ASA was hardly detected. From this result, it was suggested that the inhibition was caused by BX661A or SASP itself.

In this study, we found that SASP, BX661A and 5-ASA inhibited the ischemia/reperfusion-induced lipid peroxidation in the rat large intestinal mucosa. This strongly suggests that these scavenging effects on the oxygen-derived free radicals partly contribute to the activity of aminosalicylates in ulcerative colitis.

Acknowledgments
We are grateful to G. Uchida, M. Kozaki, Y. Ishizuka and M. Sato for their help.

REFERENCES
1 Wilson SK: Role of oxygen-derived free radicals in acute angiotensin II-induced hypertensive vascular disease in the rat. Circ Res 66, 722–734 (1990)
2 Granger DN, Holwarth DO and Parks DA: Ischemia/reperfusion injury: role of oxygen free radicals. Acta Physiol Scand S48, 47–63 (1986)
3 Megison SM, Horton JW, Chao H and Walker PB: A new model for intestinal ischemia in the rat. J Surg Res 49, 168–173 (1990)
4 Yoshikawa T, Ueda S, Naito Y, Takahashi S, Oyamada H, Morita Y, Yoneta T and Kondo M: Role of oxygen-derived free radicals in gastric mucosal injury induced by ischemia-reperfusion in rats. Free Rad Res Commun 7, 285–291 (1989)
5 Kasai T, Tanaka T, Kiriya S and Sonoyama K: Facile preparation of rat intestinal mucosa for assay of mucosal enzyme activity. J Nutr Sci Vitaminol 39, 399–403 (1993)
6 Buege JA and Aust SD: Microsomal lipid peroxidation. Methods Enzymol 52, 302–310 (1978)
7 Buyukgebiz O, Aktan AO, Yegen C, Yalcin AS, Haklar G, Yalin R and A Ercam ZS: Captopril increases endothelin serum concentrations and preserves intestinal mucosa after mesenteric ischemia-reperfusion injury. Res Exp Med 194, 339–348 (1994)
8 Allgayer H, Hofer P, Schmidt M, Bohne P, Kruis W and Gugler R: Superoxide, hydroxyl and fatty acid radical scavenging by aminosalicylates. Biochem Pharmacol 43, 259–262 (1992)