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Effects of Zinc Acexamate (NAS-501) on Superoxide Radicals and Lipid Peroxidation of Rat Gastric Mucosa

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Key Words

Zinc acexamate · Superoxide radical · Lipid peroxidation · Acute gastric mucosal injury · Inflammatory cell

Abstract

Zinc acexamate (NAS-501), an anti-ulcer agent, has been reported to prevent various acute experimental gastric mucosal lesions and duodenal ulcers in rats. In order to clarify the mechanisms by which NAS-501 exhibits the anti-ulcer effects, we investigated the anti-oxidative effects of NAS-501 in vitro and in vivo. NAS-501 significantly reduced the superoxide radical-dependent chemiluminescence, generated by hypoxanthine-xanthine oxidase, rat neutrophils and guinea-pig macrophages in vitro. These in vitro effects were also confirmed by electron spin resonance using a 5,5-dimethyl-1-pyrroline-N-oxide spin-trapping method. In addition, NAS-501 significantly inhibited lipid peroxidation induced by increasing concentrations of Fe^{2+}/ascorbate in rat gastric mucosal homogenate in vitro. Oral administration of NAS-501 (30 mg/kg) significantly inhibited production of thiobarbituric acid-reactive substance in rat gastric mucosa following per os instillation of 60% ethanol in 150 mmol/l HCl in vivo. These results suggest that NAS-501 exhibits the preventive effect from acute gastric mucosal lesions by the anti-oxidative activity.

Introduction

Recent studies have demonstrated that *Helicobacter pylori*- and nonsteroidal anti-inflammatory drug-induced gastropathies and active chronic gastritis are localized in areas of inflammation dominated by infiltration with inflammatory cells such as neutrophils, monocytes and macrophages [1–3]. These inflammatory cells activated in local areas generate reactive oxygen species and cytokines [4, 5]. In particular, superoxide radicals have been proposed to play important roles in tissue injury, because superoxide radical gener-

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ated in vivo is converted into the other more reactive oxygen species, and gives more tissue injury [6]. Thus a number of studies have suggested a possible contribution of free radicals in the development of digestive injury.

Zinc, a cofactor of more than 200 metalloenzymes, has been known to play an important role in the DNA and RNA synthesis [7], the stabilization of biological membranes [8, 9], wound healing [10, 11] and protection of lipid peroxidation [12]. Zinc acexamate (NAS-501), an anti-ulcer agent, has been reported to prevent various experimental acute gastric mucosal lesions and duodenal ulcers in rats [13]. Polaprezinc, a zinc complex of carnosine, has also been reported to have anti-oxidative activity [14], membrane-stabilizing activity [15], and wound-healing activity [16]. This drug is more effective than zinc sulfate, carnosine or a combination of zinc sulfate and carnosine on anti-oxidative effects in vivo [17].

In the present study, we investigated the anti-oxidative effects of NAS-501 in comparison with polaprezinc in vitro and in vivo to determine the mechanism by which NAS-501 protects the acute gastric mucosal lesions.

Materials and Methods

Animals

Male Jbc:Sprague-Dawley rats, weighing 200–300 g, and male Hartley guinea pigs, weighing 320–370 g, from Keari (Osaka, Japan) were used. The animals were kept at 23 ± 2°C under a 12-hour light-dark cycle. They were kept in individual cages with raised mesh bottoms to prevent coprophagy. They were deprived of food, but allowed free access to tap water for 24 h before the in vivo experiments.

Drugs

The following chemicals were obtained from commercial sources: 2-methyl-6-phenyl-3,7-dihydro-imidazo[1,2-a]pyrazin-3-one (CLA; Tokyo Kasei Co., Tokyo, Japan); N-formyl-methionyl-leucyl-phenylalanine (FMLP), phorbol 12-myristate 13-acetate (PMA), hypoxanthine (HX), allopurinol and bovine serum albumin (Sigma, St. Louis, Mo., USA); superoxide dismutase (SOD) and xanthine oxidase (XO; Boehringer Mannheim, Mannheim, Germany); 5,5-dimethyl-1-pyroline-N-oxide (DMPO; Labotec, Tokyo, Japan); diethylenetriamine pentaacetic acid (DETAPAC), α-tocopherol, NADPH and 3-t-butyl-4-hydroxyanisole (BHA; Nacalai Tesque, Kyoto, Japan); malonaldehyde bis(dimethylacetal) (MDA; Aldrich, Milwaukee, Wisc., USA); Diff-Quik® (International Reagents, Kobe, Japan); sucralfate (Chugai Pharmaceutical, Tokyo, Japan). Polaprezinc was synthesized in our laboratory. Zinc acexamate (NAS-501) was supplied from Vinas (Barcelona, Spain). Rabbit anti-rat neutrophil serum was prepared as described by Murakami et al. [6].

Preparation of Neutrophils and Macrophages

Rat peritoneal neutrophils were harvested 15–16 h after intraperitoneal injection of 100 ml/kg saline containing 0.3% oyster glycogen. Peritoneal macrophages of guinea pigs were obtained 4 days after intraperitoneal injection of 25 ml/animal paraffin liquid. After removing erythrocytes by hypotonic lysis, the peritoneal neutrophils or macrophages were washed twice and resuspended in Hanks' balanced salt solution, then kept on ice until assay. The purity and viability of these cells were both more than 95%, as determined by Diff Quik® staining and the trypan blue dye exclusion test, respectively. NAS-501 (0.001–10 mmol/l) did not significantly change the viability of these cells, as determined by the trypan blue dye exclusion test.

Measurement of Superoxide Radicals Generated by the HX-XO System, Neutrophils or Macrophages Using the CLA-Dependent Chemiluminescence Method

Superoxide radicals derived from the HX-XO system, neutrophils or macrophages were assayed using the CLA-chemiluminescence method, as described by Sugioka et al. [18]. In the HX-XO system, the reaction mixture containing 150 µl of 125 mmol/l Tris-HCl buffer (pH 6.7) with 50 µmol/l HX, 1 µmol/l CLA and various concentrations of test agents was preincubated for 2 min at 25°C, then the reaction was initiated by addition of 50 µl of 2×10^{-3} untis/ml XO. After incubating for 1 min at 25°C, total chemiluminescence intensity was measured for 0.5 min with a luminometer (model 20e, Turner, USA).

For measurement of superoxide radicals generated by neutrophils or macrophages, 150 µl of 50 mmol/l Tris-HCl buffer (pH 6.7) containing 10^6 cells/ml cell suspension, 1 µmol/l CLA and various concentrations

of test agents was preincubated at 37°C for 5 min, then 50 µl of stimulant (10^{-6} mol/l FMLP or 250 ng/ml PMA) was added to generate superoxide radicals. Maximum intensity of chemiluminescence was measured for 3 min at 37°C with a luminometer.

Measurement of Superoxide Radicals Generated by the HX-XO System, Neutrophils or Macrophages Using the Electron Spin Resonance (ESR) Spin-Trapping Method

Superoxide radicals were assayed by the ESR spin-trapping method using DMPO as a spin-trapping agent [19]. In the HX-XO system, 150 µl of 50 mmol/l Tris-HCl buffer (pH 6.7) containing 0.5 mmol/l HX, 0.1 mmol/l DETAPAC, 0.44 mol/l DMPO and various concentrations of test agents was mixed with 5×10^{-3} units/ml XO. The mixture was transferred into a standard quartz flat cell (Labotec, Tokyo, Japan) and the ESR spectra of DMPO-OOH, the spin-trapped adduct of superoxide radical, was measured by the ESR spectrometer (JEOL-JES-FE1X ESR spectrometer, JEOL, Tokyo, Japan). The conditions of the spectrometer were as follows: microwave power 10 mW; magnetic field 3,320 ± 100 G; modulation frequency 100 kHz, 1 G; amplitude 6.3 × 100; response 0.1 s; sweep time 2 min; temperature 25°C.

For the measurement of superoxide radicals generated by neutrophils and macrophages, 150 µl of reaction medium containing 10^6 cells/ml cell suspension and various concentrations of test agents was preincubated for 2 min, then 0.1 mmol/l DETAPAC, 36.8 mmol/l DMPO and stimulant (250 ng/ml PMA) were added to the mixture. The reaction mixture was transferred into a quartz cell and the signal of DMPO-OOH was measured using the ESR spectrometer 4 min after addition of PMA. The conditions of the spectrometer were as follows: microwave power 20 mW; magnetic field 3,326 ± 100 G; modulation frequency 100 kHz, 1 G; amplitude 2.0 × 100; response 0.1 s; sweep time 2 min; temperature 25°C.

Effects of NAS-501 and Allopurinol on the Activity of XO

In order to rule out a possible effect of NAS-501 on XO activity, uric acid generated during superoxide radical generation in the xanthine-XO system was measured [20]. Enzyme reaction was initiated by adding XO (16.4×10^{-3} units/ml) to the reaction mixture containing 0.1 mmol/l xanthine, 0.01% H_2O_2, 43.3 units/ml catalase and various concentrations of NAS-501 or allopurinol, then absorbance at 293 nm was measured for 1 min at 25°C.

Effect of NAS-501 on NADPH Oxidation in Rat Neutrophils

In order to confirm suppressive effect of NAS-501 on the generation of superoxide radicals, the rate of NADPH oxidation was measured [21, 22]. Rat neutrophils were isolated as described above. The neutrophils (a final cell concentration of 2×10^7 cells/ml in RPMI 1640) were prewarmed for 5 min at 37°C and activated by the addition of PMA at a final concentration of 1 µg/ml. After 5 min, the activation was terminated by addition of 5 vol of ice-cold 0.34 mol/l sucrose. The cells were centrifuged at 200 *g* for 10 min at 4°C, resuspended in 1 ml of distilled water and immediately disrupted by sonication four times for 4 s on melting ice. Then, 1 ml of ice-cold 0.68 mol/l sucrose was added to sonicate, and unbroken cells and cell debris were removed by centrifugation at 500 *g* for 10 min at 4°C. The supernatant was centrifuged at 27,000 *g* for 30 min at 4°C. The final pellet was resuspended at a concentration equivalent to 4×10^6 cells/ml in 0.34 mol/l sucrose. Reaction mixtures were prepared in a total volume of 0.5 ml, using 50 mmol/l Tris HCl buffer (pH 7.0) with 1 mmol/l sodium azide. The tubes containing enzyme fraction were prewarmed for 3 min at 25°C and after addition of 50 µl of NAS-501 (0.01–1 mmol/l) or above buffer (as a control) incubated for a further 5 min. Enzyme reaction was initiated by adding 50 µl of NADPH (0.2 mmol/l) and absorbance at 340 nm was measured for 5 min at 25°C.

Assay of Lipid Peroxidation in Homogenate of Rat Gastric Mucosa in vitro

Homogenate of rat gastric mucosa was prepared as follows: the gastric mucosa of 24-hour fasted normal rat was removed by a glass slide and homogenized in a Potter homogenizer at a concentration of 100 mg wet tissue in 1.8 ml of 10 mmol/l Tris-HCl buffer (pH 6.7). Five hundred microliters of the homogenate was added to a reaction mixture containing 0.5 ml of 10 mmol/l Tris-HCl buffer (pH 6.7), 0.1 mmol/l *L*-ascorbic acid, 0.1 mmol/l $FeCl_3$, 1 mmol/l H_2O_2 and various concentrations of test agents, then the mixture was incubated for 3 h at 37°C. The reaction was terminated by adding 50 µl of 100 mmol/l BHA. Thiobarbituric acid-reactive substances (TBARS) in the supernatant (1,100 *g*, 10 min) of the reaction mixture were measured by the method of Ohkawa et al. [23] using MDA as an external standard.

Assay of TBARS in Rat Gastric Mucosa following HCl/Ethanol Instillation in vivo

Acute gastric injury was produced by HCl/ethanol instillation according to the method of Mizui and

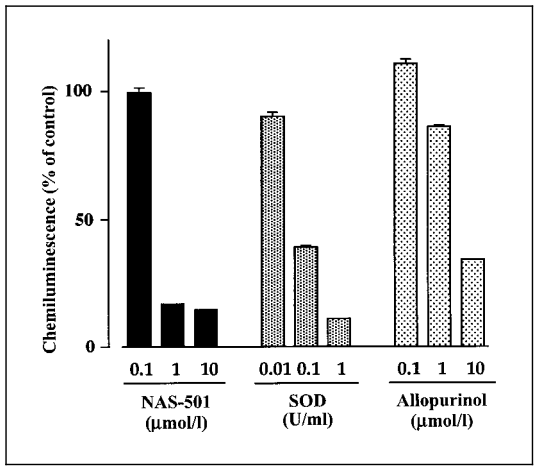

Fig. 1. Effects of NAS-501, SOD and allopurinol on HX-XO-derived superoxide radicals in the CLA-chemiluminescence method. Data are expressed as the mean ± SEM for 3 assays.

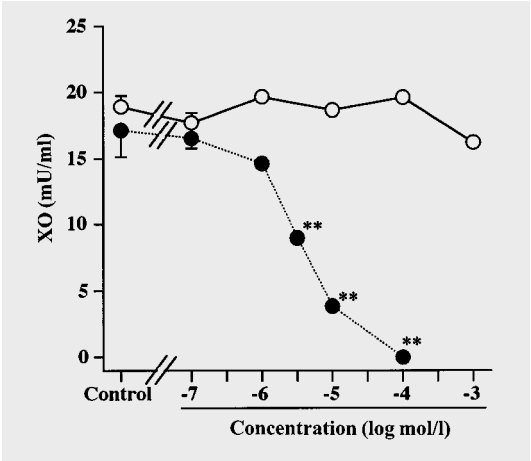

Fig. 2. Effects of NAS-501 and allopurinol on XO activity. The generation of uric acid in the presence of various concentrations of NAS-501 (○) or allopurinol (●) was measured to assess XO activity. Each point represents the mean ± SEM for 3 assays. Significant difference from the control: ** $p < 0.01$.

Doteuchi [24]. The 24-hour fasted rats were given 150 mmol/l HCl/60% ethanol orally at a volume of 1 ml/200 g body weight 30 min after the administration of NAS-501, polaprezinc and sucralfate. SOD (45,000 units/kg, i.v.) and allopurinol (30 mg/kg, i.p.) were given 5 or 60 min before HCl/ethanol instillation, respectively. Anti-neutrophil serum was given intravenously at a dose of 0.5 ml/kg 2 h before HCl/ethanol instillation. Each control animal received the vehicle (0.5% carboxymethylcellulose in saline for NAS-501, polaprezinc and sucralfate; saline for SOD and allopurinol; preimmune rabbit serum for anti-neutrophil serum) alone. Sixty minutes after the instillation of HCl/ethanol, the rats were killed by exsanguination via the abdominal aorta, and the stomach was removed. The gastric mucosa was scrapped with a glass slide and homogenized at a concentration of 100 mg wet tissue in 0.9 ml of 1.15% KCl. TBARS in gastric mucosa was measured, as described above. The level of TBARS is expressed as picomoles of MDA per milligram of protein. Protein was determined according to the method of Lowry et al. [25].

Statistical Analyses

The data are presented as the means ± SEM. Statistical significance was estimated by Dunnett's multiple comparison test, or Student's t test, and values of $p < 0.05$ were regarded as significant.

Results

Effects of NAS-501 on HX-XO-Derived Superoxide Radicals in the CLA-Chemiluminescence Method

Figure 1 shows the effects of NAS-501, SOD and allopurinol on HX-XO-derived superoxide radicals in the CLA-chemiluminescence method. CLA-dependent chemiluminescence in the HX-XO system was suppressed by NAS-501 at concentrations of 1–10 μmol/l. SOD and allopurinol also inhibited CLA-dependent chemiluminescence. To rule out a direct effect of NAS-501 on XO activity, we determined the effect of NAS-501 on uric acid generation by a xanthine-XO system in comparison with allopurinol (fig. 2). Allopurinol significantly inhibited XO activity at concentrations of 3×10^{-6} to 10^{-4} mol/l, while NAS-501 had no effect within the concentration range 10^{-7} to 10^{-3} mol/l. These results

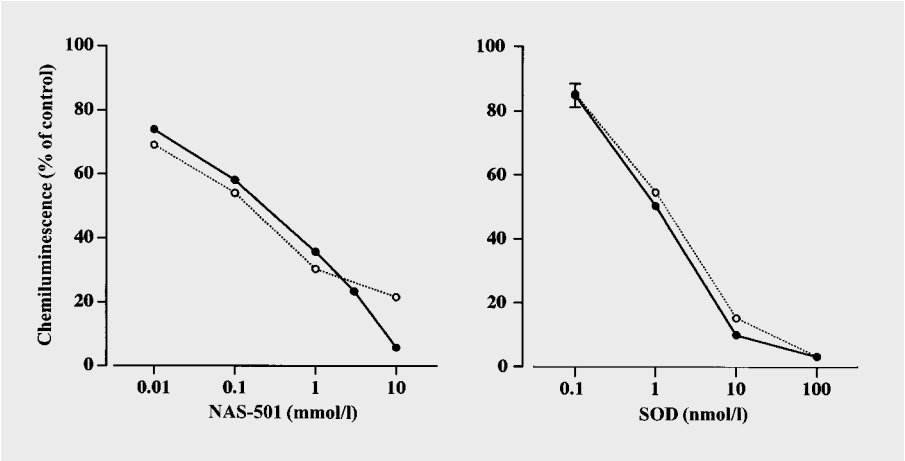

Fig. 3. Effects of NAS-501 and SOD on the generation of superoxide radicals in FMLP (●)- and PMA (○)-stimulated neutrophils of rats. Superoxide radicals were measured by the CLA-chemiluminescence method. Data are expressed as the mean ± SEM for 3 assays.

indicate that NAS-501 scavenges the superoxide radical without inhibiting the activity of XO.

Effects of NAS-501 on Neutrophils or Macrophage-Generated Superoxide Radicals in the CLA-Chemiluminescence Method

The effects of NAS-501 and SOD on FMLP- or PMA-induced production of superoxide radicals by rat neutrophils were studied by the CLA-chemiluminescence method. As shown in figure 3, NAS-501, as well as SOD, inhibited chemiluminescence induced by either FMLP or PMA in a concentration-dependent manner.

The effects of NAS-501 on FMLP- or PMA-induced production of superoxide radicals by guinea-pig macrophages were also examined by the CLA-chemiluminescence method. As shown in figure 4, NAS-501 inhibited FMLP- or PMA-induced chemiluminescence in a concentration-dependent manner.

Effect of NAS-501 on NADPH Oxidation in Rat Neutrophils

The particulate fractions from activated neutrophils are known to generated superoxide radicals in the presence of NADPH [26, 27]. In this study, we measured a decrease in absorbance at 340 nm by NADPH oxidation. As shown in figure 5, NAS-501 inhibited oxidation of NADPH in a concentration-dependent manner.

Effects of NAS-501 on HX-XO- or Cell-Derived Superoxide Radicals in the ESR Spin-Trapping Method

The in vitro effect of NAS-501 on superoxide radicals was also studied by the ESR spin-trapping method. As shown in figure 6, the DMPO-OOH signal (the hyperfine splittings; A_N = 14.28 G, A_H^β = 11.28 G, and A_H^γ = 1.27 G) appeared, when XO was added to the reaction mixture of the HX-XO system or PMA to that of neutrophils or macrophages. The intensity of the DMPO-OOH signal in the HX-XO system was suppressed in the

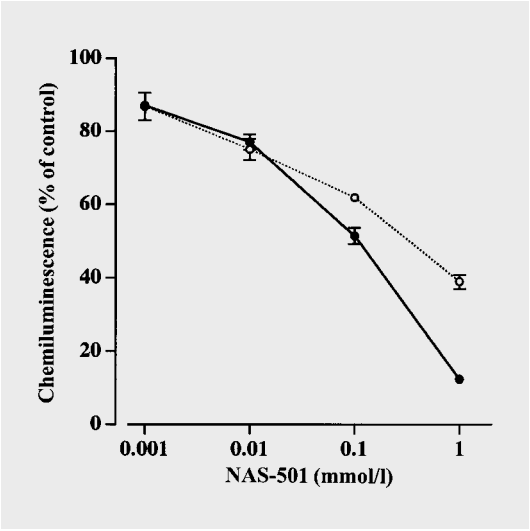

Fig. 4. Effects of NAS-501 on the generation of superoxide radical in FMLP (●)- or PMA (○)-stimulated macrophages of guinea pigs. Superoxide radicals were measured by the CLA-chemiluminescence method. Data are expressed as the mean ± SEM for 3 assays.

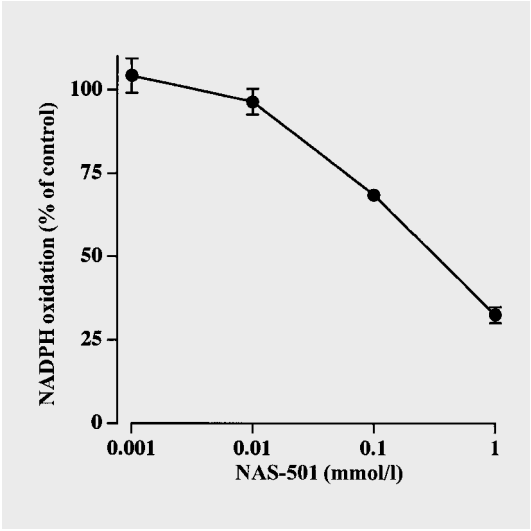

Fig. 5. Effect of NAS-501 on NADPH oxidation in rat neutrophils. The rate of NADPH oxidation in the presence of various concentrations of NAS-501 was recorded at 340 nm at 25 °C. Data are expressed as the mean ± SEM for 3 assays.

presence of NAS-501 (1 mmol/l) and SOD. NAS-501 (1 mmol/l) or SOD also reduced the DMPO-OOH signal intensity that was induced by neutrophils or macrophages. These results indicate that NAS-501 has a suppressive activity on the production of superoxide radicals by neutrophils, macrophages or an ability to scavenge these radicals.

Effects of NAS-501 on the Lipid Peroxidation of Rat Gastric Mucosal Homogenate in vitro

Effects of NAS-501 on lipid peroxidation were examined in vitro using rat gastric mucosal homogenate by measuring the levels of TBARS in comparison to those of polaprezinc and α-tocopherol. As shown in figure 7, NAS-501 significantly inhibited lipid peroxidation of mucosal homogenate at concentrations of 0.3–10 mmol/l. Polaprezinc revealed an effect only at a high concentration (10 mmol/l), while α-tocopherol exhibited a potent effect at concentrations of 0.03–10 mmol/l.

Effects of NAS-501 on the Lipid Peroxidation of Rat Gastric Mucosa by Acute Gastric Injury in vivo

Effects of NAS-501 on the lipid peroxidation of gastric mucosa were studied in rats with acute gastric injury. Acute gastric injury was induced by an instillation of HCl/ethanol after administration of NAS-501 or other drugs. TBARS levels in gastric mucosa of the non-treated group significantly elevated 5–60 min after instillation of HCl/ethanol in a time-dependent manner. Elevation of mucosal TBARS 60 min after instillation was completely inhibited by NAS-501 at a dose of 30 mg/kg (fig. 8, 9). A similar effect was obtained by sucralfate (300 mg/kg, p.o.) or SOD

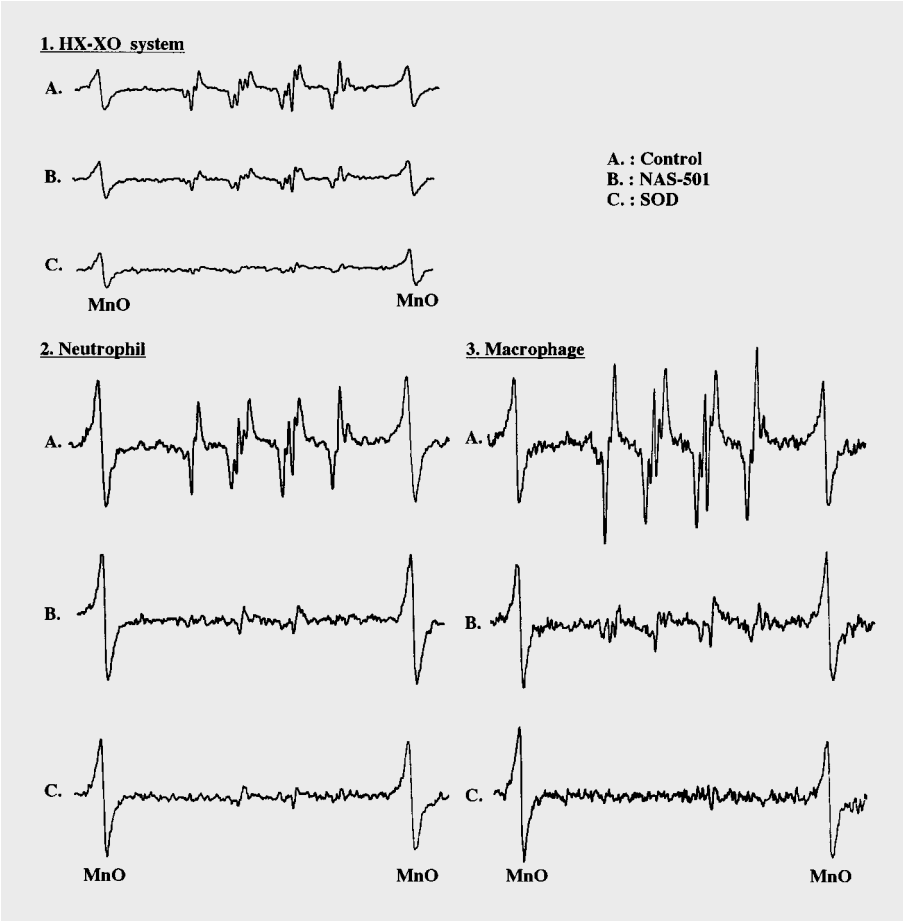

Fig. 6. Scavenging effects of NAS-501 and SOD on the formation of DMPO spin-trapped adduct by ESR spectrometer. Superoxide radicals were generated by the HX-XO system, PMA-stimulated neutrophils or PMA-stimulated macrophages, then the ESR spectra of DMPO-OOH, the spin-trapped adduct of superoxide radical, was measured by the ESR spectrometer. A = Control; B = NAS-501 (1 mmol/l), and C = SOD (100 mmol/l in the HX-XO system and macrophages, and 10 nmol/l in neutrophils).

(45,000 units/kg, i.v.), but not by polaprezinc (10–100 mg/kg, p.o.) or allopurinol (30 mg/kg, i.p.). Interestingly, an intravenous injection of rabbit anti-rat neutrophil serum exhibited an inhibitory effect on mucosal lipid peroxidation by a HCl/ethanol instillation, suggesting an involvement of neutrophils in this model.

Discussion

NAS-501 has been reported to prevent various acute experimental gastric mucosal lesions and duodenal ulcers in the rat [13]. However, the mechanisms of anti-ulcer action of NAS-501 has not yet been clarified. Since reactive oxygen species generated by ethanol,

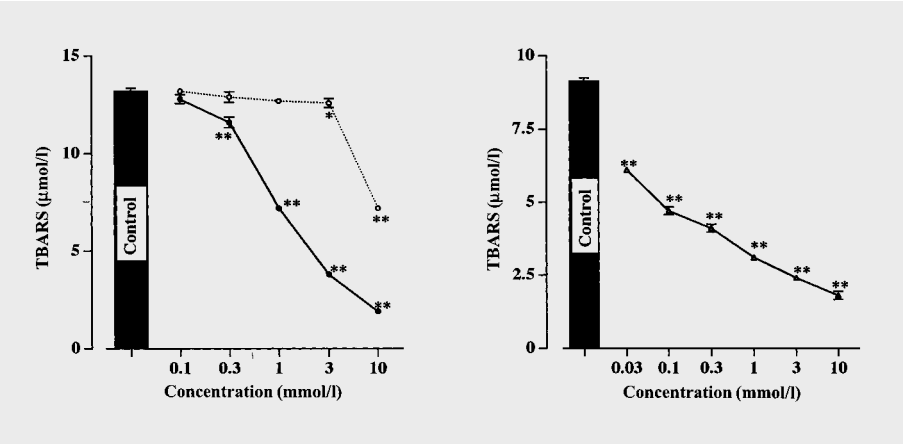

Fig. 7. Effects of NAS-501, polaprezinc and α-tocopherol on lipid peroxidation of rat gastric mucosal homogenate in vitro. Gastric mucosal homogenate was incubated for 3 h at 37 °C with Fe^{2+}/ascorbate and various concentrations of NAS-501 (●), polaprezinc (○) or α-tocopherol (△). TBARS were assayed as described in the text. Each point represents the mean ± SEM for 3 assays. Significant difference from the control: * $p < 0.05$; ** $p < 0.01$.

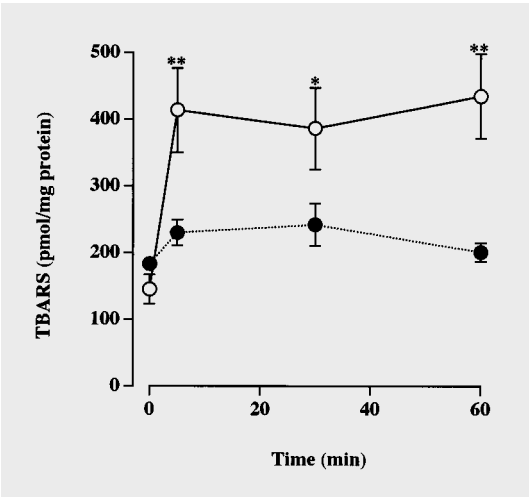

Fig. 8. Changes in the lipid peroxidation of rat gastric mucosa after HCl/ethanol instillation by treatment with NAS-501 (●, 30 mg/kg) or vehicle (○, 0.5% carboxymethylcellulose in saline). Each point represents the mean ± SEM for 6 rats. Significant difference from the values of before HCl/ethanol instillation: * $p < 0.05$; ** $p < 0.01$.

nonsteroidal anti-inflammatory drugs and *Helicobacter pylori* have been recognized as the partial cause of gastric ulcer or gastritis [5, 28], we have studied the anti-oxidative activity of NAS-501 in vitro and in vivo.

The ability of NAS-501 to scavenge superoxide radicals was tested in the HX-XO system in vitro using the CLA-chemiluminescence method. NAS-501 revealed an inhibitory effect on the HX-XO-induced chemiluminescence without inhibiting XO activity, indicating that NAS-501 has a scavenging effect on superoxide radicals. This scavenging effect of NAS-501 was also confirmed by the ESR spin-trapping method. Since there is much evidence suggesting that the inflammatory cells, such as neutrophils, monocytes and macrophages, are sources of superoxide radicals in gastritis [1–3], we examined the effects of NAS-501 on superoxide radicals generated by activated neutrophils or macrophages using two different methods: the CLA-chemiluminescence method, and the ESR spin-trap-

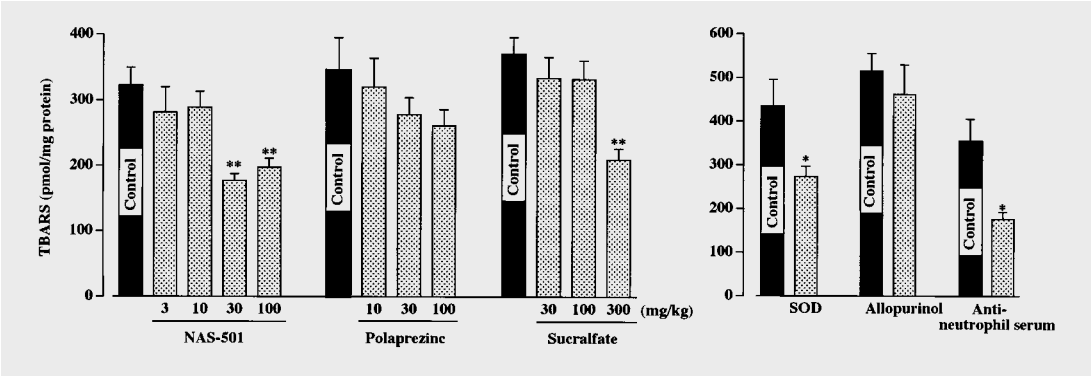

Fig. 9. Effects of NAS-501, polaprezinc, sucralfate, SOD, ALP and anti-neutrophil serum on the lipid peroxidation of rat gastric mucosa following HCl/ethanol instillation. One hour after instillation of 60% ethanol in 150 mmol/l HCl, animals were sacrificed, and the TBARS levels in gastric mucosa were measured as described in the text. NAS-501, polaprezinc, sucralfate and control (0.5% carboxymethylcellulose in saline) were given orally 30 min before HCl/ethanol instillation. SOD (45,000 units/kg, i.v.) and allopurinol (30 mg/kg, i.p.) were given 5 or 60 min before HCl/ethanol instillation, respectively. Anti-neutrophil serum or preimmune rabbit serum was given intravenously at a dose of 0.5 ml/kg 2 h before HCl/ethanol instillation. Each column represents the mean ± SEM for 5 rats. Significant difference from the control: * $p < 0.05$; ** $p < 0.01$.

ping method. Either method demonstrated that NAS-501 was effective to reduce production of superoxide radicals from these inflammatory cells. Furthermore, NAS-501 also exhibited an inhibitory effect on the NADPH oxidation by the superoxide radical generating enzyme, NADPH oxidase in rat neutrophils. These results suggest that an anti-oxidative activity of NAS-501 depends upon its ability not only to scavenge superoxide radicals but also to inhibit the generation of superoxide radicals.

It is well documented that the lipid peroxidation by superoxide radicals or hydrogen peroxide in vivo depends upon a conversion of these peroxides into more reactive species, such as hydroxyl radicals [29, 30]. In fact, Dudeja and Brastitus [31] demonstrated that hydroxyl radicals play an important role in lipid peroxidation induced by the Fe^{2+}/ascorbate system in tissue homogenate. Thus it was suggested that NAS-501 prevented the mucosal membrane from lipid peroxidation by inhibiting a conversion of peroxide into more reactive hydroxyl radicals or scavenging injurious radicals.

In order to clarify whether NAS-501 exhibits an anti-ulcer action by preventing lipid peroxidation of gastric mucosa from injurious radicals, we studied the anti-oxidative effect of NAS-501 in experimentally induced acute gastric mucosal injury. We used HCl/ethanol as an acute necrotizing agent in this model, since NAS-501 has been demonstrated to be effective in this model [13], and the gastric mucosal injury induced by ethanol is associated with active oxygen species [32, 33]. We observed that an instillation of HCl/ethanol resulted in elevation of TBARS levels in gastric mucosa, and this elevation was inhibited by an intravenous injection of anti-neutrophil serum at a dose of 0.5 ml/kg; the numbers of

neutrophils in the peripheral blood were suppressed to about 10% of those in preimmune serum-treated animals for up to 12 h (data not shown). Pretreatment with NAS-501 at a dose of 30 mg/kg resulted in complete inhibition of HCl/ethanol-induced lipid peroxidation in gastric mucosa. NAS-501 at a dose of 30 mg/kg, but not 10 mg/kg, exhibited a potent anti-oxidative action as well as a protective action on the HCl/ethanol-induced lesions, suggesting that the in vivo effect of NAS-501 on gastric lesion may depend on its anti-oxidative effect. A concentration of NAS-501 in gastric contents after an oral administration of 30 mg/kg was estimated as approximately 30 mmol/l that was sufficient for exhibiting an anti-oxidative activity in vitro, supporting that the protective action of NAS-501 on gastric lesions is responsible for its anti-oxidative effect. However, a possible effect of NAS-501 on neutrophil infiltration remains to be clarified. Further studies will be necessary to resolve the effect of NAS-501 on leukocyte chemotaxis.

Polaprezinc, an organic zinc compound similar to NAS-501, has been reported to be a more effective anti-oxidative agent than inorganic zinc substance [17]. However, this drug failed to prevent lipid peroxidation of gastric mucosa by HCl/ethanol instillation even at a dose of 100 mg/kg, while NAS-501 was effective at 30 mg/kg. This difference in the in vivo effects of these drugs seems to be due to a difference in their potencies of anti-oxidative activity, as suggested by the following evidence: first, NAS-501 reduced the amount of superoxide radicals generated by PMA-stimulated neutrophils, while polaprezinc does not [14], and second, NAS-501 inhibited lipid peroxidation of gastric mucosal homogenate in vitro at lower concentrations than those of polaprezinc. In contrast to polaprezinc, sucralfate inhibited elevation of TBARS levels in gastric mucosa at 300 mg/kg. This observation might be supported by a previous reported that $AlCl_3$ protected cultured rat gastric mucosal cells from 15% ethanol-induced damage in which oxygen free radicals participate [34].

In conclusion, we demonstrated that NAS-501 has an anti-oxidative activity by reducing superoxide radical production in vitro. NAS-501 effectively inhibited lipid peroxidation injury by HCl/ethanol instillation, probably by inhibiting production of oxygen radicals from activated neutrophils in injured tissues.

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