Aggravation by Paroxetine, a Selective Serotonin Reuptake Inhibitor, of Antral Lesions Generated by Nonsteroidal Anti-Inflammatory Drugs in Rats

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Received April 24, 2011; accepted June 21, 2011

ABSTRACT

Recent clinical studies have suggested a risk of adverse gastric reactions from the concomitant use of selective serotonin (5-HT) reuptake inhibitors (SSRIs) with nonsteroidal anti-inflammatory drugs (NSAIDs). We examined the adverse effects of SSRIs on antral lesions produced by indomethacin in rats. Rats fasted for 24 h were refed for 1 h, then administered indomethacin (30 mg/kg s.c.) 1 h after the refeeding and killed 6 h later. Paroxetine (1–10 mg/kg) was given orally 30 min before indomethacin. Indomethacin caused antral lesions in refed rats. Paroxetine dose-dependently aggravated these lesions, despite provoking no damage by itself. Similar results were obtained when other NSAIDs such as diclofenac, flurbiprofen, and loxoprofen were coadministered with paroxetine or when indomethacin was coadministered with other antidepressants such as fluvoxamine and milnacipran, but not imipramine or maprotiline. Exogenous 5-HT also worsened the indomethacin-induced antral damage, whereas the aggravating effect of paroxetine was attenuated by ondansetron, a selective 5-HT3 antagonist, but not antagonists for other 5-HT receptor subtypes. Indomethacin plus paroxetine had no effect on gastric secretion but significantly decreased mucosal superoxide dismutase (SOD) activity as well as GSH content. The antral damage induced by indomethacin plus paroxetine was significantly prevented by antiserotery (acid or pepsin) agents and mucosal protective agents as well as SOD and allopurinol. These results suggest that SSRIs aggravate NSAID-induced antral lesions, probably via the activation of 5HT3 receptors, and the mechanism of aggravation may involve the corrosive action of acid/pepsin as well as an impaired antioxidative system.

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs), such as indomethacin, cause gastrointestinal lesions, especially in the antrum (Lanza, 1984; Fries et al., 1989). The gastric lesions generated by NSAIDs in experimental animals, however, occur mainly in the corpus, not the antrum, of the stomach under fasting conditions (Takeuchi et al., 1986; Tanaka et al., 2001). These drugs, however, produce lesions in the small intestine but not the stomach under nonfasting conditions (Kunikata et al., 2001; Tanaka et al., 2002). It is noteworthy that indomethacin caused antral lesions in rats subjected to 24 h of fasting and subsequent 1 h of refeeding (Satoh et al., 1982, 1983). Likewise, high doses of NSAIDs produced antral lesions in hamsters under normal feeding conditions (Kolbasa et al., 1988).

Paroxetine, a selective serotonin (5-HT) reuptake inhibitor (SSRI), is often prescribed for the treatment of depression or mental disorders. Studies have suggested a risk of adverse gastric reactions to SSRIs, showing frequent gastrointestinal bleeding in patients taking SSRIs and a markedly increased risk of bleeding with the coadministration of NSAIDs (Dalton et al., 2003; De Jong et al., 2003; Mort et al., 2006; Lewis et al., 2008; Ahsberg et al., 2010; Dall et al., 2010), although there is controversy about the association (Tata et al., 2005; Itatsu et al., 2011). Consistent with the clinical findings, we found that SSRIs, given as a single injection, markedly aggravated the development of antral lesions in response to

ABBREVIATIONS: NSAID, nonsteroidal anti-inflammatory drug; 5-HT, serotonin; SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin/norepinephrine reuptake inhibitor; PGE, prostaglandin E; COX, cyclooxygenase; SOD, superoxide dismutase; DDC, diethyldithiocarbamate; MPO, myeloperoxidase; SC-560, 5-(4-chloro-phenyl)-1-(4-methoxyphenyl)-3-trifluoromethylpyrazole; SB204070, (1-butyl-4-piperidinyl)methyl-8-amino-7-chloro-1,4-benzodioxane-5-carboxylate.
indomethacin in refed rats (Kojo et al., 2010). Several models of antral lesions have been established with various agents, including NSAIDs, and the pathogenesis of these models is reportedly associated with the impairment of the mucosal antioxidative system, such as a decrease in superoxide dismutase (SOD) activity or an increase in oxyradical production (Oka et al., 1991; Chen et al., 1993; Ohashi et al., 2009). However, the mechanism underlying the aggravation by SSRIs of NSAID-induced antral damage remains unknown.

In the present study, we examined the effect of paroxetine, a SSRI, on the antral mucosa in rat stomachs when administered concomitantly with NSAIDs, in comparison with other antidepressants, such as tricyclic or tetracyclic antidepressants and a 5-HT₄-norepinephrine reuptake inhibitor (SNRI), and investigated the underlying mechanism of the aggravation by paroxetine of NSAID-induced antral lesions, in relation to endogenous 5-HT and its receptor subtypes. In addition, we examined the effects of various antiulcer drugs, such as antisecretory and mucosal protective drugs as well as antioxidative agents, on the antral lesions produced by indomethacin plus paroxetine.

**Materials and Methods**

**Animals.** Male Sprague-Dawley rats (220–260 g; Nippon Charles River, Shizuoka, Japan) were acclimatized to standard laboratory conditions (12-h light-dark cycle, temperature 22 ± 1°C). Experiments were carried out using approximately four to six rats per group under unanesthetized conditions, unless otherwise specified. All experimental procedures involving animals were approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University.

**Induction of Antral Lesions.** For the induction of antral lesions, the animals were first deprived of food for 24 h, repositioned for 1 h, given indomethacin (30 mg/kg) subcutaneously 1 h after the refeeding, and killed 6 h later. SSRIs or other antidepressants were given orally 30 min before the administration of indomethacin. The SSRIs used were paroxetine (1–10 mg/kg) and fluvoxamine (100 mg/kg), and the other antidepressants were imipramine (a tricyclic antidepressant; 50 mg/kg), maprotiline (a tetracyclic antidepressant; 30 mg/kg), and milnacipran (a SNRI; 30 mg/kg). In some cases, other conventional NSAIDs (diclofenac, 30 mg/kg; flurbiprofen, 30 mg/kg; loxoprofen, 60 mg/kg) or selective cyclooxygenase (COX)-2 inhibitors [5-(4-chloro-phenyl)-1-(4-methoxyphenyl)-1H-pyrazole-3-carboxylic acid (SC-560), 10 mg/kg; rofecoxib, 10 mg/kg] were given subcutaneously or orally, respectively, together with paroxetine, in place of indomethacin, whereas 5-HT₄ (10 mg/kg) was given subcutaneously together with indomethacin, in place of paroxetine. In addition, the effects of various 5-HT antagonists on the severity of the antral lesions generated by indomethacin (30 mg/kg) plus paroxetine (10 mg/kg) were examined. The 5-HT antagonists used were methysergide (a nonselective 5-HT₁/₂A,₂B,₂C antagonist; 1 mg/kg), sarpogrelate (a 5-HT₃ antagonist; 100 mg/kg), ondansetron (a 5-HT₃ antagonist; 10 mg/kg), and (1-butyl-4-piperidinyl) methyl-8-amino-7-chloro-1,4-benzodioxane-5-carboxylate (SB204070) (a 5-HT₃ antagonist; 1 mg/kg) (Sanders-Bush and Mayer, 2005), and they were given subcutaneously 30 min before the administration of paroxetine. Furthermore, the effects of various other agents on the antral lesions produced by indomethacin plus paroxetine were examined; antisecretory drugs such as pepstatin (isovaleryl-l-lvalyl-4-amino-3-hydroxy-5-methyl heptanoyl-l-ananyl-4-amino-3-hydroxy-6-methyl heptanoylic acid, a specific antipeptic agent; 1 mg/kg), atropine (an anticholinergic agent; 3 mg/kg), and omeprazole (a proton pump inhibitor; 30 mg/kg), and mucosal protective drugs such as rebamipide [2-(4-chlorobenzoylamino)-3-(2H-quinolin-4-yl) propionic acid; 10 mg/kg], teprenone (6,10,14,18-tetramethyl-5,9,13,17-nonaacetaetetraen 2-one; 300 mg/kg), and irsogladine [2-(4-dimino-6-(2,5-dichlorophenyl)-s-triazine malate; 10 mg/kg], as well as antioxidative drugs such as allopurinol (a xanthine oxidase inhibitor; 50 mg/kg) and SOD (30,000 units/kg). In addition, we examined the effect of diethyldithiocarbamate (DDC; 750 mg/kg), a SOD inhibitor, on the antral lesions produced by indomethacin. These agents, except SOD and DDC, were given orally 1 h before indomethacin, whereas SOD or DDC was administered intraperitoneally or subcutaneously, respectively, 1 h before indomethacin.

**Macroscopic Evaluation of Gastric Lesions.** Animals with various treatments were killed for examination of the gastric mucosa by deep ether anesthesia 6 h after the NSAID treatment. The stomach was excised, treated with 2% formalin for fixation of the tissue walls, and then opened along the greater curvature or the antimesenteric attachment, and the mucosa was examined for damage under a dissecting microscope (10×). The area (mm²) of macroscopically visible lesions was measured separately for hemorrhagic and nonhemorrhagic damage, summed for each tissue, and used as a lesion score. The person measuring the lesions did not know the treatments given to the animals. In some cases, the gastric mucosa was examined with a light microscope after the administration of indomethacin (30 mg/kg) with or without paroxetine (10 mg/kg). The animals were killed 6 h after the indomethacin treatment, and the stomachs were excised. The tissue samples were then immersed in 10% neutralized formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin.

**Measurement of Myeloperoxidase Activity.** Myeloperoxidase (MPO) activity in the antral mucosa was measured as described by Krawisz et al. (1984) with some modifications. The animals were killed 6 h after the administration of indomethacin (30 mg/kg p.o.) with or without paroxetine (10 mg/kg s.c.) given 30 min before. In some cases, ondansetron (10 mg/kg s.c.) was given 1 h before the administration of indomethacin. All blood was withdrawn from the heart by perfusing it with saline, and the stomach was excised and opened along the greater curvature. After the tissue was rinsed with cold saline, the antral mucosa was scraped with glass slides, weighed, and homogenized in a 50-mmol phosphate buffer containing 0.5% hexadecyl-trimethyl-ammonium bromide (pH 6.0; Sigma-Aldrich, St. Louis, MO) and centrifuged at 2000 rpm for 10 min at 4°C. MPO activity in the supernatant was determined using O-dianisidine dihydrochloride (Sigma-Aldrich). The changes in absorbance at 450 nm were recorded on a microplate reader (VERSAMax; Molecular Devices, Sunnyvale, CA). Sample protein content was estimated by spectrophotometric assay (Pierce Protein Assay Kit; Thermo Fisher Scientific, Rockford, IL). The MPO activity was obtained from the slope of the reaction curve, based on the following equation: specific activity (μmol H₂O₂/min/mg protein) = (OD/min/OD/μmol H₂O₂ × mg protein).

**Determination of Mucosal PGE₂ Content.** Levels of PGE₂ in the gastric antral mucosa were measured after the oral administration of indomethacin (30 mg/kg) and paroxetine (10 mg/kg), either alone or in combination. The animals were killed under deep ether anesthesia 2 h after the administration of indomethacin or paroxetine, and the antral tissue was isolated, weighed, and placed in a tube containing 100% ethanol plus 0.1 M indomethacin (Putaki et al., 1994). Then, the tissues were homogenized by polytron homogenizer (IKA, Tokyo, Japan) and centrifuged at 10,000 rpm for 10 min at 4°C. After the supernatant of each sample had been evaporated with N₂ gas, the residue was resolved in assay buffer and used for the determination of PGE₂. The concentration of PGE₂ was measured using a PGE₂ enzyme immunoassay kit (GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK).

**Determination of SOD Activity in Gastric Mucosa.** SOD activity was measured in the gastric mucosa, according to the method reported by Ukeda et al. (1997). In brief, the rats were killed under deep ether anesthesia, and the stomachs were removed. After the tissue was rinsed with cold saline, the antral mucosa was scraped with glass slides and kept cold on ice. The mucosal scrapings were weighed, minced, and homogenized in a sucrose buffer solution (0.25
M sucrose, 10 mM Tris-HCl buffer solution, pH 7.4, 1 mM EDTA), the volume of which was approximately six to eight times the tissue weight. After centrifugation at 3500 rpm for 60 min, the supernatant was used for the determination of SOD activity. The absorbance was measured at 450 nm on a Hitachi spectrophotometer (U1100; Hitachi, Ibaraki, Japan), and the results were expressed as units per milliliter per gram of tissue weight. Paroxetine (10 mg/kg) was given orally 30 min before indomethacin (30 mg/kg), whereas ondansetron (10 mg/kg s.c.) or rebamipide (10 mg/kg p.o.) was given 1 h before paroxetine.

Measurement of Sulfhydryl Content in Gastric Mucosa. The amount of nonprotein sulfhydryl (GSH) was measured in the antral mucosa of the stomach, according to a modified version of the method originally described by Kaplowitz et al. (1980). After the tissue was rinsed with cold saline, the antral mucosa was scraped with glass slides and kept cold on ice. The mucosal scrapings were weighed, homogenized in 2 ml of phosphate buffer (0.1 M NaH2PO4 plus 0.25 M sucrose, pH 7.4), and centrifuged at 4000 rpm for 15 min at 4°C. A 0.5-ml aliquot of 25% trichloroacetic acid was added to 1 ml of the supernatant of each sample, and the sample was kept for 30 min at 4°C. After centrifugation at 3000 rpm for 15 min, the supernatant was used to determine GSH content by using 5,5-dithiobis(2-nitrobenzoic acid). Absorbance was measured at 412 nm on the Hitachi spectrophotometer, and the results were expressed as micromoles per gram of wet tissue. Paroxetine (10 mg/kg) was given orally 30 min before the subcutaneous administration of indomethacin (30 mg/kg), whereas ondansetron (10 mg/kg s.c.) or rebamipide (10 mg/kg p.o.) was given 1 h before paroxetine.

Determination of Gastric Secretion. Effects of indomethacin and paroxetine, either alone or in combination, on the secretion of gastric acid and pepsin were examined in pylorus-ligated rats. With the rats under light ether anesthesia, a small incision (5–7 mm) was made along the midline of the abdomen from the xyphoid process, the pyloric sphincter (Kolm et al., 1945). The animals were then allowed to recover from the anesthesia. Four hours after the ligation, the animals were killed with an overdose of ether, the stomachs were refed for 1 h, administered indomethacin at 30 mg/kg s.c. 1 h after the refeeding, and killed 6 h later. Paroxetine (1–10 mg/kg p.o.) was administered 30 min before indomethacin. Data are presented as the mean ± S.E. for four to six rats per group. Statistical analyses were performed using a two-tailed unpaired t test and Dunnett’s multiple comparison test, and values of P < 0.05 were regarded as significant.

Results

Effect of Paroxetine on Antral Lesions Caused by NSAIDs

Indomethacin (30 mg/kg s.c.) produced nonhemorrhagic antral lesions within 6 h in the refed rats. Pretreatment with paroxetine (1–10 mg/kg p.o.) dose-dependently aggravated the antral lesions generated by indomethacin, the lesion score at 10 mg/kg being significantly greater than the control value (Fig. 1A). In addition, indomethacin alone produced nonhemorrhagic lesions, but additional treatment with paroxetine generated hemorrhagic lesions deep in the mucosa. Paroxetine alone did not cause any injury in the stomach (data not shown). Likewise, 5-HT given subcutaneously at 10 mg/kg also significantly worsened the indomethacin-induced antral damage, the degree of aggravation being equivalent to that caused by paroxetine at 10 mg/kg. Histological examination revealed that indomethacin alone damaged only the

![Fig. 1. A, effects of paroxetine and 5-HT on the antral lesions produced by indomethacin in rats. Animals fasted for 24 h were refed for 1 h, administered indomethacin at 30 mg/kg s.c. 1 h after the refeeding, and killed 6 h later. Paroxetine (1–10 mg/kg p.o.) or 5-HT (10 mg/kg s.c.) was administered 30 min before indomethacin. Data are presented as the mean ± S.E. for four to six rats. * indicates significant difference from saline, P < 0.05. B, macroscopic (I and II) and microscopic (III and IV) appearances of the antral lesions generated by indomethacin in rats, in the absence or presence of paroxetine (10 mg/kg). I and III, indomethacin alone; II and IV, indomethacin plus paroxetine. Inserts in I and II show enlargements of the antral lesions. Note that the antral lesions produced by indomethacin were markedly aggravated by and became hemorrhagic with the combined administration of paroxetine. Arrows indicate the location of damage.](https://jpet.aspetjournals.org/doi/fig/1.)

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epithelial cell layer, whereas the combination of indomethacin and paroxetine produced deep damage reaching to the muscularis mucosa, with severe edema in the submucosa (Fig. 1B).

Similar to indomethacin, other NSAIDs such as diclofenac (30 mg/kg), flurbiprofen (30 mg/kg), and loxoprofen (60 mg/kg) given subcutaneously also produced antral lesions in the refed rats. Pretreatment of the animals with paroxetine (10 mg/kg p.o.) significantly aggravated the damage, resulting in deep, hemorrhagic lesions (Fig. 2A). The occurrence of antral lesions in refed rats was reproduced by the combined oral administration of SC-560 (10 mg/kg) and rofecoxib (10 mg/kg), although neither of these agents alone damaged the antrum (Fig. 2B). In addition, paroxetine did not damage the antral mucosa when given with rofecoxib, but it did provoke a few lesions when given with SC-560, although the damage was mostly nonhemorrhagic. However, the concurrent administration of paroxetine with SC-560 plus rofecoxib produced severe hemorrhagic lesions in the antrum, the lesion score being equivalent to that produced by paroxetine plus indomethacin.

**Effect of Various Antidepressants on Indomethacin-Induced Antral Lesions**

To clarify whether the NSAID-induced antral ulceration is aggravated by other antidepressants, such as tricyclic or tetracyclic drugs, we examined the effects of various antidepressants given orally on the indomethacin-induced antral lesions.

Fulvoxamine (100 mg/kg p.o.), another SSRI, and milnacipran (30 mg/kg), a SNRI, significantly aggravated the antral damage produced by indomethacin (30 mg/kg s.c.) and rendered nonhemorrhagic lesions hemorrhagic, similar to paroxetine (Fig. 3). However, the tricyclic antidepressant imipramine (50 mg/kg) and the tetracyclic antidepressant maprotiline (30 mg/kg) had no effect, with most of the lesions remaining nonhemorrhagic. None of the antidepressants used in this study by itself provoked any injury in the stomach (data not shown).

**Effect of 5-HT Receptor Antagonists on the Aggravation of Antral Lesions Produced by Indomethacin Plus Paroxetine**

Because the aggravating effect of paroxetine on NSAID-induced antral lesions was mimicked by 5-HT given exogenously, it may be accounted for by endogenous 5-HT accumulated in the tissue caused by the inhibition of neuronal reuptake. Then, to determine which type of 5-HT receptor is associated with the actions of paroxetine, we examined the effects of various 5-HT antagonists on the aggravation by paroxetine of the indomethacin-induced antral lesions.

The severity of the antral lesions generated by indomethacin (30 mg/kg s.c.) plus paroxetine (10 mg/kg p.o.) was not significantly affected by prior subcutaneous administration of either the 5-HT$_1$,2,5,7 receptor antagonist methysergide (1 mg/kg), the 5-HT$_2$ receptor antagonist sarpogrelate (100 mg/kg), or the 5-HT$_4$ antagonist SB204070 (1 mg/kg), and the

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**Fig. 2.** Effect of paroxetine on the antral lesions produced by conventional NSAIDs (diclofenac, flurbiprofen, and loxoprofen) (A) or selective COX-1 (SC-560) and COX-2 inhibitors (rofecoxib) (B) in rats. A, animals fasted for 24 h were refed for 1 h, then administered diclofenac (30 mg/kg), flurbiprofen (30 mg/kg), or loxoprofen (60 mg/kg) subcutaneously 1 h after the refeeding, and killed 6 h later. B, animals fasted for 24 h were refed for 1 h, then administered SC-560 (30 mg/kg) and rofecoxib (30 mg/kg) orally, either alone or in combination, 1 h after the refeeding, and killed 6 h later. In both A and B, paroxetine (10 mg/kg p.o.) was administered 30 min before the administration of other materials. Data are presented as the mean ± S.E. for four to six rats. * indicates significant difference from the corresponding saline group, $P < 0.05$.

**Fig. 3.** Effects of various antidepressants on the antral lesions produced by the combined administration of indomethacin in rats. Animals fasted for 24 h were refed for 1 h, then administered indomethacin (30 mg/kg) subcutaneously 1 h after the refeeding and killed 6 h later. Imipramine (50 mg/kg), maprotiline (30 mg/kg), fluvoxamine (100 mg/kg), or milnacipran (30 mg/kg) was given orally 30 min before indomethacin. Data are presented as the mean ± S.E. for four to six rats. * indicates significant difference from saline, $P < 0.05$. 

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lesion score of hemorrhagic damage as well as total area in these groups were all but equivalent to those in the vehicle-treated group (Fig. 4). However, pretreatment of the animals with ondansetron (10 mg/kg s.c.), the 5-HT3 receptor antagonist, almost totally abrogated the aggravating effect of proxetine on the indomethacin-induced antral lesions, and the severity of the lesions was reduced to the level observed in control animals given indomethacin alone. Likewise, the aggravation by milnacipran of the antral lesions was also significantly abrogated by the prior administration of ondansetron (data not shown).

Effect of Various Agents on Antral Lesions Produced by Indomethacin Plus Paroxetine

Antisecretory Drugs. A proton pump inhibitor, omeprazole (30 mg/kg p.o.), and an anticholinergic agent, atropine (3 mg/kg p.o.), had no effect on the severity of antral damage induced by indomethacin (30 mg/kg s.c.) alone (data not shown), but totally inhibited the aggravation by paroxetine (10 mg/kg p.o.) of these lesions; both hemorrhagic and total lesions were significantly decreased in area compared with those produced by indomethacin plus paroxetine (Fig. 5A). Likewise, the aggravation of indomethacin-induced antral damage was significantly attenuated by pepstatin, a specific pepsin inhibitor (1 mg/kg p.o.). Macroscopically, indomethacin-induced nonhemorrhagic lesions became hemorrhagic on the coadministration of paroxetine, but these changes were apparently inhibited by the prior administration of pepstatin (Fig. 5B). Furthermore, this agent slightly decreased the severity of antral lesions caused by indomethacin alone (data not shown).

Mucosal Protective Drugs. Similar to antisecretory drugs, the mucosal protective drugs, such as rebamipide (10 mg/kg p.o.) and irsogladine (10 mg/kg p.o.), significantly inhibited the aggravation by proxetine of indomethacin-induced antral lesions; the areas of hemorrhagic and total lesions were markedly decreased compared with the vehicle-treated group (Fig. 6). Teprenone (300 mg/kg p.o.) also protected against these lesions, although its effect was less than...
that of rebamipide or irsogladine and significant only for the area of hemorrhagic lesions.

**Antioxidative Drugs.** The severity of the antral lesions produced by indomethacin plus paroxetine was significantly reduced when the animals were pretreated with allopurinol (50 mg/kg p.o.), a xanthine-oxidase inhibitor, the inhibition of hemorrhagic and total lesions being 81.0 and 64.3%, respectively (Fig. 7A). Likewise, the area of hemorrhagic lesions was significantly suppressed by pretreatment with SOD (30,000 units/kg i.p.), although the effect on total lesions was not statistically significant. On the other hand, the development of antral lesions in response to indomethacin was significantly exacerbated by cotreatment with DDC (100 mg/kg s.c.), a SOD inhibitor that by itself provoked no damage, and the degree of aggravation was all but equivalent to that by paroxetine (Fig. 7B).

**Changes in SOD Activity and GSH Content Induced in Antral Mucosa by Indomethacin Plus Paroxetine**

Mucosal SOD activity in the normal rat antrum was 330 ± 26 units/g tissue. In the animals given indomethacin (30 mg/kg s.c.), the SOD activity was low, 246 ± 25 units/g tissue, but not significantly different from that in normal rats (Fig. 8A). However, additional treatment with paroxetine (10 mg/kg p.o.) further decreased the activity to 223 ± 19 units/g tissue, which was significantly lower than the control group. The decreased SOD activity in response to indomethacin plus paroxetine was significantly reverted by pretreatment with ondansetron, a 5-HT₃ antagonist (10 mg/kg s.c.) as well as rebamipide (10 mg/kg p.o.). On the other hand, the amount of GSH in the normal rat antrum was 2.4 ± 0.10 μmol/g tissue (Fig. 8B). The mucosal GSH content was slightly but significantly decreased by indomethacin alone. This decrease was further enhanced by additional treatment with paroxetine, the value being 1.6 ± 0.08 μmol/g tissue, which is approximately 50% of that in normal rats. The decrease in GSH caused by indomethacin plus paroxetine was almost totally restored by pretreatment with ondansetron and rebamipide, the value being 2.3 ± 0.26 and 1.9 ± 0.17 μmol/g tissue, respectively.

**Effects of Various Agents on Changes in Antral MPO Activity Induced by Indomethacin Plus Paroxetine**

The MPO activity in the normal antral mucosa was 0.096 ± 0.004 μmol H₂O₂/min/mg protein and increased slightly in response to indomethacin (30 mg/kg s.c.), reaching 0.105 ± 0.035 μmol H₂O₂/min/mg protein (Fig. 9). The activity was further significantly potentiated by additional treatment with paroxetine (10 mg/kg p.o.), the value being 0.593 ± 0.062 μmol H₂O₂/min/mg protein, which is approximately five times greater than the control level. The increased MPO activity induced by indomethacin plus paroxetine was significantly suppressed by pretreatment with ondansetron (10 mg/kg) further decreased the activity to 223 ± 19 units/g tissue, which was significantly lower than the control group. The decreased SOD activity in response to indomethacin plus paroxetine was significantly reverted by pretreatment with ondansetron, a 5-HT₃ antagonist (10 mg/kg s.c.) as well as rebamipide (10 mg/kg p.o.). On the other hand, the amount of GSH in the normal rat antrum was 2.4 ± 0.10 μmol/g tissue (Fig. 8B). The mucosal GSH content was slightly but significantly decreased by indomethacin alone. This decrease was further enhanced by additional treatment with paroxetine, the value being 1.6 ± 0.08 μmol/g tissue, which is approximately 50% of that in normal rats. The decrease in GSH caused by indomethacin plus paroxetine was almost totally restored by pretreatment with ondansetron and rebamipide, the value being 2.3 ± 0.26 and 1.9 ± 0.17 μmol/g tissue, respectively.

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mg/kg s.c.), rebamipide (10 mg/kg p.o.), and allopurinol (50 mg/kg p.o.). Likewise, the antisecretory agents pepstatin (1 mg/kg p.o.), omeprazole (30 mg/kg p.o.) and atropine (3 mg/kg p.o.) significantly attenuated the increase in MPO activity in response to indomethacin plus paroxetine, and the values in all cases were equivalent to that in the control (data not shown).

**Effect of Paroxetine on Gastric Secretion and Mucosal PGE2 Content**

Normal rats secreted 5 to 7 ml of gastric juice for 4 h after ligation of the pylorus, the acid and pepsin output being 235 ± 30 μEq/h and 376 ± 120 μg/h, respectively. Neither output was significantly affected by the combined administration of indomethacin (30 mg/kg s.c.) and paroxetine (10 mg/kg intraduodenally) (Fig. 10A). On the other hand, indomethacin significantly decreased PGE2 content in the antral mucosa in refed animals (Fig. 10B). Paroxetine by itself had no effect on the mucosal PGE2 content and did not affect the inhibitory effect of indomethacin on PGE2 production.

**Discussion**

The present study showed that paroxetine, a SSRI, markedly aggravated indomethacin-induced antral damage in refed rats, changing superficial/nonhemorrhagic lesions into deep/hemorrhagic lesions. Other NSAIDs similarly produced antral lesions in the refed rats, and this property of NSAIDs was reproduced by the combined administration of selective COX-1 and COX-2 inhibitors. It is noteworthy that the effect of paroxetine was also reproduced by exogenous 5-HT and abrogated by ondansetron, a 5HT3 antagonist, suggesting the involvement of endogenous 5-HT/5-HT3 receptors in the pathogenic mechanism. Furthermore, we found that the generation of antral lesions by indomethacin plus paroxetine was suppressed by antisecretory and mucosal protective drugs as well as antioxidative agents. It is assumed that SSRIs aggravate NSAID-induced antral damage, probably via the activation of 5-HT3 receptors, and the mechanism of...
Aggravation by SSRIIs of NSAID-Induced Antral Damage

The present study confirmed in experimental animals the clinical findings that the risk of gastric bleeding in patients taking SSRIIs was markedly increased by the coadministration of NSAIDs (Dalton et al., 2003; De Jong et al., 2003; Mort et al., 2006; Lewis et al., 2008; Ahsberg et al., 2010; Dall et al., 2010). In this study, we used antral lesions caused by indomethacin in refed rats (Satoh and Guth, 1981, Satoh et al., 1983), because in patients the incidence of NSAID-induced injury in the antrum is reportedly high (Fries et al., 1989). Satoh and Guth (1981) first showed that indomethacin selectively produced antral lesions in refed rats without damaging the corpus mucosa. We further showed that this action of indomethacin was mimicked by other conventional NSAIDs such as diclofenac, flurbiprofen, or loxoprofen and was also reproduced by the combined administration of SC-560 and rofecoxib, suggesting the inhibition of both COX-1 and COX-2 as part of the pathogenic mechanism. These results are consistent with the findings that the gastric ulcerogenic properties of NSAIDs are not accounted for solely by the inhibition of COX-1 and requires the inhibition of COX-2 as well (Wallace et al., 2000; Tanaka et al., 2001). As clearly shown in this study, indomethacin produced nonhemorrhagic lesions consisting of mostly superficial damage, but paroxetine aggravated these lesions, causing deep hemorrhagic damage with severe edema in the submucosa. These results support clinical reports suggesting an increased risk of gastric bleeding with the concomitant use of SSRIIs and NSAIDs (Dalton et al., 2003; De Jong et al., 2003; Mort et al., 2006; Lewis et al., 2008; Ahsberg et al., 2010; Dall et al., 2010).

Second, we found that the aggravating effect of paroxetine was mimicked by exogenous 5-HT, suggesting the involvement of endogenous 5-HT in this action. In addition, the effect of paroxetine was reproduced by another SSRI, fluvoxamine, and a SNRI, milnacipran, but not by imipramine or maprotiline. It is assumed that the aggravating action on antral lesions is shared by antidepressants showing selective inhibition of the reuptake of 5-HT or 5-HT/norepinephrine. SSRIIs may increase the 5-HT concentration in tissue, be-
and worsened by drugs that impair the antioxidative system (Ogino et al., 1992). In the present study, the severity of antral lesions induced by indomethacin plus paroxetine was significantly reduced by pretreatment with allopurinol, an inhibitor of oxyradical production, as well as SOD. Furthermore, a marked reduction in SOD activity and GSH content was observed in the antral mucosa after the administration of indomethacin plus paroxetine, and these responses were significantly reverted by pretreatment with ondansetron at the dose that prevented the aggravating effect of paroxetine. These results suggest that the pathogenesis of the antral lesions is accounted for partly by the impairment of the mucosal antioxidative system. This idea was also supported by the experiment using DDC, an inhibitor of Cu^{2+}-dependent SOD activity; this agent further reduced mucosal SOD activity in the presence of indomethacin and markedly increased the severity of antral lesions, although by itself it caused no damage. These findings support the involvement of the impaired antioxidative system in the aggravation by paroxetine of indomethacin-induced antral lesions.

The mucosal protective drugs used in the present study, such as rebamipide, teprenone, and irsogladine, are used to treat gastritis and gastric ulcers in Japan. Among them, rebamipide suppresses inflammatory cell infiltration and the generation of free radicals, exhibits radical-scavenging action, and exerts a potent anti-inflammatory effect (Ogino et al., 1992; Sakurai et al., 2005). Indeed, this agent significantly reduced the severity of antral lesions produced by indomethacin plus paroxetine, together with the suppression of increased MPO activity as well as the restoration of decreased SOD activity and GSH content. These results strongly support the pathogenic importance of the impaired antioxidative system in the development of antral lesions induced by indomethacin plus paroxetine. Irsogladine is also reported to protect the gastric mucosa by enhancing the mucosal integrity of the stomach through the facilitation of gap junctional intracellular communication and increasing the intracellular levels of 3,5′-cAMP (Ueda et al., 1995; Kyo et al., 2004). On the other hand, teprenone exhibits a protective effect in various models by stimulating the secretion of mucus and the intracellular levels of cAMP (Ueda et al., 1995; Kyoi et al., 2005). Kaplowitz et al. (1980) showed that indomethacin significantly suppresses ethanol-induced hepatic glutathione in the rats. J Pharmocol Exp Ther 212:240–245.

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