JPP Journal of Pharmacy and Pharmacology

JPP 2003, 55: 229–237

Received August 12, 2002
Accepted October 4, 2002
DOI 10.1211/002235702478
ISSN 0022-3573

Non-steroidal anti-inflammatory drug, nabumetone, prevents indometacin-induced gastric damage via inhibition of neutrophil functions

Yoshiro Ishiwata, Masayuki Okamoto, Shoji Yokochi, Hiroyuki Hashimoto, Takashi Nakamura, Atsushi Miyachi, Yuji Naito and Toshikazu Yoshikawa

Abstract

Nabumetone is a non-steroidal anti-inflammatory drug (NSAID). It works as a prodrug and is extensively metabolized to an active metabolite, 6-methoxy-2-naphthylacetic acid (6MNA). It is well known that neutrophil infiltration and activation are critical in the pathogenesis of NSAID-induced gastric injury, and nabumetone shows less incidence of gastrointestinal irritancy. We examined the effects of nabumetone on neutrophil activation and on indometacin-induced gastric damage. In the indometacin-induced gastric mucosal injury, rats were treated with indometacin and then nabumetone or 6MNA was orally administered. Nabumetone prevented gastric damage accompanied by the reduction of neutrophil infiltration into gastric mucosa, but such an effect was not observed with 6MNA. Nabumetone reduced the formyl methionyl leucyl phenylalanine (fMLP)-induced respiratory burst of human neutrophils to 30% of the control level in-vitro, but 6MNA did not. In addition, nabumetone prevented the fMLP-induced migration of neutrophils. Nabumetone did not inhibit O_2^- generation in the xanthine–xanthine oxidase system. These results suggest that nabumetone prevents gastric damage induced by the active metabolite, 6MNA, via the suppression of neutrophil activation in gastric mucosa.

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are used as anti-inflammatory agents but their use often causes gastric erosions and ulcers, which are among the most serious clinical problems (Griffin et al 1991; Langman et al 1994). NSAID-induced gastric damage has been attributed to suppression of endogenous prostaglandins, direct toxic effect on gastric epithelial cells such as apoptosis or necrosis (Alderman et al 2000) and superoxide or cytokines produced by neutrophils (Ding et al 1998), although the precise mechanisms of NSAID-induced gastric mucosal injury are not clearly understood. Among them, the inhibition of prostaglandin synthesis, which has a cytoprotective effect on gastric mucosa, was thought to be the major mechanism of gastrointestinal problems with NSAIDs (Wallace 1997). Recently, however, increasing evidence has indicated that neutrophil infiltration and activation precede gastric damage and are critical in the pathogenesis of NSAID-induced gastric injury (Wallace 1993; Yoshida et al 1993).

Nabumetone is one of the NSAIDs, and is extensively metabolized mainly to an active metabolite, 6-methoxy-2-naphthylacetic acid (6MNA; Figure 1), an effective inhibitor of prostaglandin synthesis after oral administration (Davies 1997). It is well known that nabumetone causes less incidence of gastrointestinal irritancy than other conventional NSAIDs (Boyle et al 1982; Melarange et al 1992; Huang et al 1999). Nabumetone did not impair ulcer healing at clinical doses, but celecoxib and indometacin delayed healing of cryoprobe-induced gastric ulcers in the rat (Tibble et al 2001). Nabumetone or indometacin decreased the migration of polymorphonuclear leucocytes and mononuclear cells into polyvinyl sponge implants in the rat (Elizabeth et al 1982). In normal subjects treated with nabumetone or indometacin, neutrophil chemotaxis also decreased, but 6MNA and indometacin did not have any effect

Central Research Laboratory, Sanwa Kagaku Kenkyusho Co. Ltd, 363 Shiosaki Hokusei-cho, Inabe-Gun, Mie 511-0406, Japan

Yoshiro Ishiwata, Masayuki Okamoto, Shoji Yokochi, Hiroyuki Hashimoto, Takashi Nakamura, Atsushi Miyachi

First Department of Medicine, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto, 602-8566, Japan

Yuji Naito, Toshikazu Yoshikawa

Correspondence: Y. Ishiwata, Central Research Laboratory, Sanwa Kagaku Kenkyusho Co. Ltd, 363 Shiosaki Hokusei-cho, Inabe-Gun, Mie 511-0406, Japan. E-mail: y_ishiwata@mb4.skk-net.com

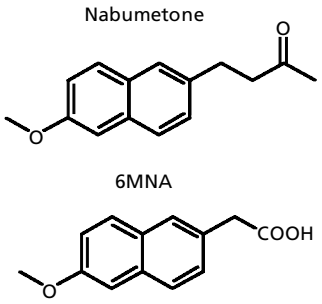

Figure 1 Chemical structure of nabumetone and an active metabolite, 6-methoxy-2-naphthylacetic acid (6MNA).

in-vitro on the chemotactic response of neutrophils isolated from healthy subjects (Ip et al 1990). However, these results did not explain the low incidence of gastrointestinal damage with nabumetone. Some NSAIDs have been shown to inhibit polymorphonuclear leucocyte (PMN) function, and these effects are different from the effects on cyclooxygenase (COX) (Anthony et al 1996; Angelis-Stoforidis et al 1998; Ding et al 1998; Parij et al 1998).

In this study, we evaluated the effects of nabumetone on neutrophil activation and showed that nabumetone, unlike other NSAIDs such as loxoprofen or diclofenac, prevented gastric damage via inhibition of neutrophils.

Materials and Methods

This experiment was reviewed by the ethics committee on animal experiment at the Sanwa Kagaku Kenkyusho Co. Ltd and was carried out in accordance with the guidelines for animal experiment at the Sanwa Kagaku Kenkyusho and the Law and Notification of the Japanese Government.

Materials

Nabumetone and 6MNA were provided by SmithKline Beecham (SKB, UK). Indometacin and formyl methionyl leucyl phenylalanine (fMLP) were purchased from Sigma (St Louis, MO). Meloxicam (Mobic) was purchased from Daiiti Pharmaceutical Co. Ltd (Tokyo, Japan). Ferricytochrome C, NADPH and recombinant human superoxide dismutase (SOD) were obtained from Sigma. Guanosine 5′-3-*O*-(thio) triphosphate (GTPγS) was obtained from Calbiochem Co. (Darmstadt, Germany). Flavine-adenine dinucleotide (FAD) was purchased from Wako (Osaka, Japan). Troglitazone was synthesized by Sanwa Kagaku Kenkyusho Co. Ltd (Nagoya, Japan).

NSAID-induced gastric injury

Male Lewis rats, 200–250 g, were fasted for 24 h with free access to water. The rats were kept in cages with mesh bottoms to avoid coprophagia in a light-controlled environment. Five rats were randomly sorted to each treatment group, not to be significantly different, and orally treated with each NSAID. Five hours after the NSAID treatment, the rats were killed and rapidly exsanguinated. Their stomachs were removed, fixed with 5% formalin for 20 min, opened along the small curvature, rinsed with phosphate-buffered saline (PBS) and examined for gastric mucosal haemorrhagic lesions using an image scanner system IPAP-Win (Sumika Technoservice, Takarazuka, Japan) (Nygard et al 1994). In this study, indometacin was given to rats at a dose of 20 mg kg^{-1}. This dose of indometacin was chosen to induce gastric damage in the rats. The dose of nabumetone (25 and 100 mg kg^{-1}) given to the rats corresponds to the maximum recommended dose in man (2000 mg daily).

Assay of myeloperoxidase activity

Gastric mucosa (100 mg) was homogenized in 1.6 mL of 0.02 M ethylenediamine tetra-acetic acid (EDTA) (pH 4.7). The homogenate was centrifuged at 20 000 rev min^{-1} for 15 min at 4 °C and the pellet was then homogenized in an equivalent volume of 0.05 M potassium phosphate buffer (pH 5.4) containing 0.5% cetyltrimethylammonium bromide. A sample was added to 0.01 M phosphate buffer (pH 7.0), 3 mM H_2O_2 and 0.02% *o*-dianisidine and incubated at 25 °C. The myeloperoxidase (MPO) activity was assessed by measuring the H_2O_2-dependent oxidation of *o*-dianisidine. One unit of enzyme activity was defined as the amount of MPO that caused a change in absorbance (Akar et al 1999).

Induction of respiratory burst

Human PMNs were isolated from whole blood of healthy subjects with an anticoagulant, EDTA, using Polymorphprep (Nycomed, Oslo, Norway). PMN suspension (50 μL; 2×10^7 cells/mL) was incubated with 125 μL of test compound solution for 10 min at 37 °C and then 75 μL of nitroblue tetrazolium (NBT) (0.4% in Ca,Mg-free Hanks solution) was added. The reaction was started by the addition of 50 μL fMLP solution (6 mM in Ca,Mg-free Hanks) and the mixtures were incubated for 30 min at 37 °C. The reaction was stopped by adding cold Ca,Mg-free Hanks solution (300 μL) and cells were separated by centrifugation. Blue formazan formed in this incubation was dissolved in a mixture of 50% isobutyl alcohol, 12.5% sodium dodecyl sulfate (SDS) and 0.04% HCl and absorbance at 540 nm was determined (Manna et al 1997).

O_2^- generation in xanthine–xanthine oxidase system

The generation of O_2^- in xanthine–xanthine oxidase system was determined by the reduction of ferricytochrome C (Wang et al 1997). Absorbance change of ferricytochrome C at 550 nm was monitored in the presence of nabumetone.

Assay of NADPH oxidase activity

NADPH oxidase activity was assayed by cytochrome C reduction (Hua et al 2000). Neutrophil cytosol and

membrane fraction, 10 μM FAD, 3 μM GTPγS and 1.5 mg mL^{-1} of ferricytochrome C were pre-incubated with nabumetone in the presence of 50 μM NADPH for 3 min. The reaction was started by the addition of 100 mM arachidonate and O_2^- generation was measured by detecting the absorbance change of ferricytochrome C.

fMLP-induced migration of neutrophils

Neutrophil migration by fMLP was assayed using a 48-well microchemotaxis chamber (Neuro Probe, Cabin John, MD) and 3-μm-pore-size PVP-free polycarbonate filter membranes (Neuro Probe). Twenty-six microlitres of the medium (RPMI 1640 medium supplemented with 0.5% bovine serum albumin (BSA), 25 mM HEPES, pH 7.4) containing 1 μM fMLP was added to the lower chamber. The filter membrane was sandwiched between the lower and upper chamber, and 50 μL of neutrophil suspension (10^6 cells/mL) was added to the upper chamber. After incubation for 1 h, the migrated cells on the filter membrane were fixed, stained with Diff-Quik solution (International Reagents, Kobe, Japan) and counted by microscopic observation in high power fields (×600).

Concentration of nabumetone and 6MNA in gastric mucosa and serum

Nabumetone was administered to male Lewis rats fasted for 24 h, and then killed to obtain the gastric mucosa and serum at various time points. Nabumetone was extracted from the homogenate of gastric mucosa or serum with n-hexane under basic condition with NaOH. Organic phase was evaporated to dryness, and the residue was dissolved in 5 mM ammonium acetate–acetonitrile (2:8 v/v). Active metabolite, 6MNA, was extracted with ethyl acetate under acidic condition with HCl. All LC-MS/MS experiments were performed on a TSQ7000 triple quadrupole mass spectrometer (Finnigan MAT, USA) equipped with an ESI source, a divert valve and Interactive Chemical Information System (ICIS). The HPLC system was a Waters 2690 Alliance (Waters, USA). A YMC-Pack ProC18 (2.0 × 75 mm, 5 μm; YMC, Japan) column was used for the determination of nabumetone in homogenate or serum. The mobile phase composition consisted of 2/8 5 mM ammonium acetate–acetonitrile mixture. For the determination of 6MNA in homogenate or serum, two HPLC columns, an OASIS column (1.0 × 50 mm, 30 μm; Waters, USA) and a Cadenza CD-C18 column (4.6 × 50 mm, 3 μm; Imtakt, Japan), were used for on-line purification column-switching system (Jemal et al 1998). As the mobile phases, 5 mM ammonium acetate and acetonitrile were used and gradient elution was run.

Statistical analysis

Data are expressed as the mean ± s.e. Analysis of variance followed by Dunnett's method was used to determine significant differences among groups. Statistical significance was assessed at the $P < 0.05$ level.

Results

Effect of nabumetone on indometacin-induced gastric mucosal damage

First of all, we investigated the action of nabumetone and its active metabolite, 6MNA, on the gastric mucosa and compared it with indometacin and loxoprofen. Figure 2 shows the gastric mucosa of NSAID-treated rats and the calculated area of gastric damage obtained by image scanner. Oral administration of indometacin at 20 mg kg^{-1} induced heavy gastric damage. Loxoprofen (40 mg kg^{-1}) and 6MNA (100 mg kg^{-1}) also caused gastric damage, but to a lesser extent than indometacin. In contrast, nabumetone did not induce gastric damage at 100 mg kg^{-1}. We next examined the effect of nabumetone and 6MNA on indometacin-induced gastric damage. Nabumetone or 6MNA were administered 30 min after the indometacin-treatment, to adjust to the incidence of neutrophil infiltration induced by indometacin (Anthony et al 1996). Nabumetone significantly reduced the gastric damage induced by indometacin (Figure 3). However, neither 6MNA, the active metabolite of nabumetone, nor loxoprofen improved the gastric damage.

Furthermore, to examine the action of nabumetone on gastric mucosa, we administered nabumetone 30 min before or after indometacin treatment. As shown in Figure 4, administration of nabumetone 30 min after indometacin treatment showed the protective effect, whereas its administration 0 or 30 min before indometacin treatment did not. Meloxicam, a COX-2 selective NSAID, did not exert the protective effect on mucosal injury.

Effect on neutrophil infiltration into gastric mucosa induced by indometacin

We determined the MPO activity as a marker of the infiltration of neutrophils in the gastric mucosa. Oral administration of indometacin increased the MPO activity 26 times in the gastric mucosa. The administration of nabumetone significantly suppressed the increase in MPO activity induced by indometacin (Figure 5). This result indicates that nabumetone suppressed the indometacin-induced infiltration of neutrophils in gastric mucosa.

Effect on the respiratory burst of fMLP-stimulated neutrophils

The respiratory burst of neutrophils was determined by blue formazan formation in the fMLP-stimulated cells in the presence of NBT. In this experiment, the ratio of formazan-positive cells in the absence of any stimulant was 15 ~ 20% and increased to 70% in the fMLP-stimulated neutrophils. Absorbance at 540 nm of the dissolved formazan reflected these values, and therefore we used these values as the data. Figure 6A shows that nabumetone significantly reduced the respiratory burst at 12.5 ~ 50 μM in a dose-dependent manner. In

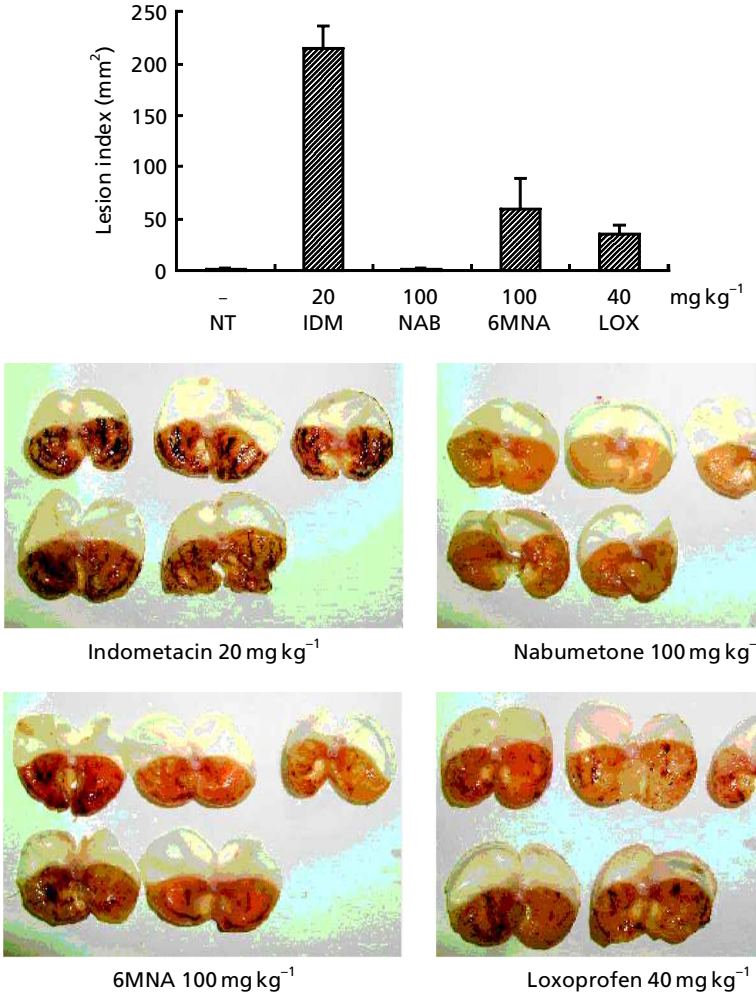

Figure 2 Evaluation of gastric damage induced by nabumetone (NAB), 6MNA, loxoprofen (LOX) or indometacin (IDM) in rats. Five rats were orally treated with each NSAID. Five hours later, their stomachs were removed. Upper figure, gastric haemorrhagic lesions calculated with an image scanner; lower pictures, macroscopic appearance of the rat gastric mucosa. NT, non-treatment.

contrast, 6MNA was not effective even at 200 μM. Troglitazone, a peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist, which has been reported to inhibit aspirin-induced gastric mucosal injury (Naito et al 2001a), did not reduce the respiratory burst at 50 μM, but showed cytotoxicity at 100 μM. Indometacin was not effective at 100 μM.

These findings may reflect the lower gastrointestinal irritancy of nabumetone.

Nabumetone prevented the migration of neutrophils induced by fMLP

To investigate the effect of nabumetone on the chemotaxis of neutrophils, the cells were incubated with nabumetone for 30 min at 37 °C and applied to migration assay. As shown in Figure 6B, nabumetone significantly reduced the fMLP-induced migration of neutrophils up to 50 μM. The active metabolite of nabumetone, 6MNA, did not affect the migration.

Effect on O_2^- generation and on NADPH oxidase activity in cell free system

The scavenging ability of nabumetone was assessed using xanthine–xanthine oxidase system. In this system, superoxide dismutase (SOD) markedly attenuated O_2^- generation, but nabumetone or 6MNA did not (data not shown). Furthermore, the NADPH oxidase activity that generates O_2^- was not affected by nabumetone or 6MNA in the cell-free system (data not shown). These results indicated that nabumetone did not interact directly with NADPH oxidase or attenuate O_2^-.

Concentration of nabumetone in gastric mucosa

From these data described above, nabumetone may act on the neutrophils in the gastric mucosa and inhibit infiltration and activation of the neutrophils, resulting in improving mucosal damage. Therefore, we determined the gastric mucosal concentration of nabumetone after oral administration

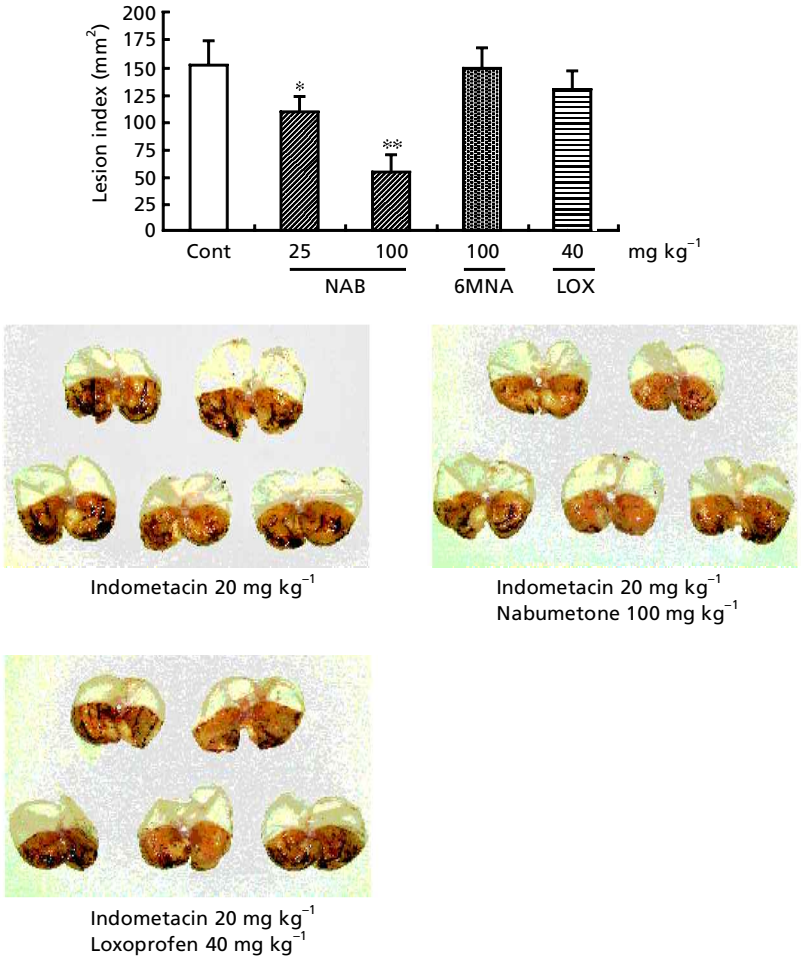

Figure 3 Effect of nabumetone on indometacin-induced damage of the gastric mucosa in rats. Nabumetone (NAB), 6MNA or loxoprofen (LOX) were given 30 min after the administration of indometacin. Five hours after the indometacin treatment, their stomachs were removed. Upper figure, gastric haemorrhagic lesions calculated with an image scanner; lower pictures, macroscopic appearance of the rat gastric mucosa. Cont, indometacin only. The data are expressed as mean ± s.e. (n = 5). $*P < 0.05$, $**P < 0.01$, compared with the control group.

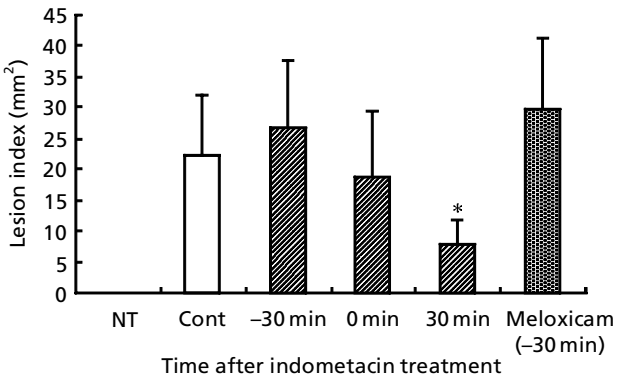

Figure 4 Nabumetone shows the protective effect when administered after indometacin treatment. Nabumetone was administered at the indicated time before or after indometacin treatment. Five hours after the indometacin treatment, gastric mucosal haemorrhagic lesions were determined. NT, non-treatment; Cont, indometacin only. The data are expressed as mean ± s.e. (n = 5). $*P < 0.05$ compared with the control group.

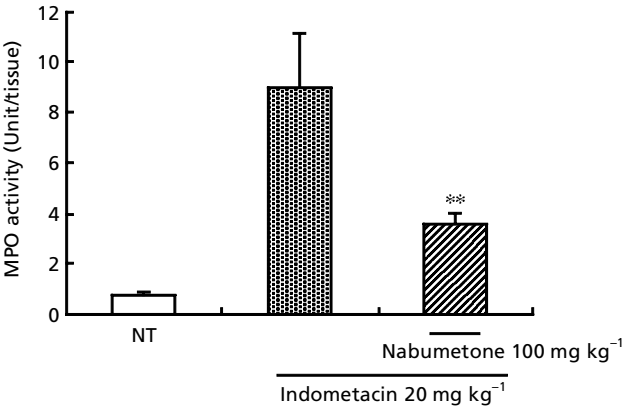

Figure 5 Nabumetone reduced the neutrophil infiltration into the gastric mucosa induced by indometacin. Rats were treated with NSAIDs and the gastric mucosa was collected 3 h after the indometacin treatment. MPO was extracted and the activity was determined as described in the text. NT, non-treatment. The data are expressed as mean ± s.e. $**P < 0.01$ (n = 5) compared with the control group.

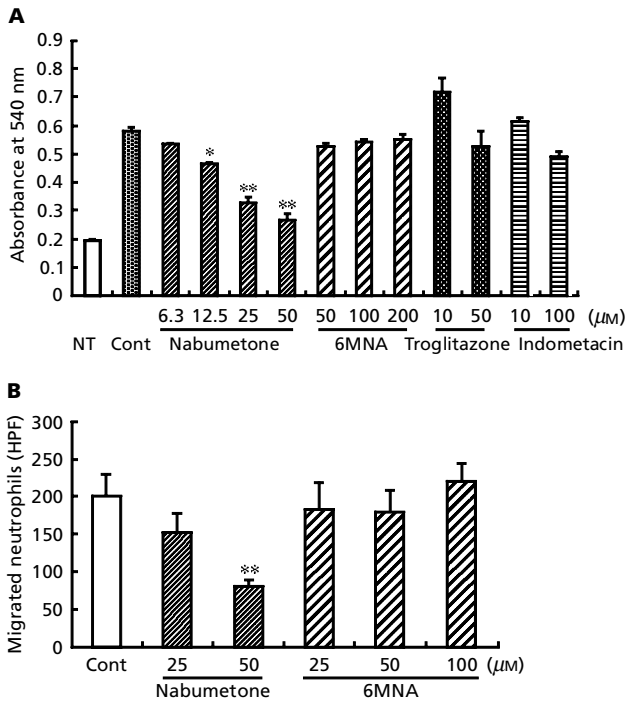

Figure 6 Nabumetone reduced the oxidative burst and the migration of neutrophils induced by fMLP. A. Nabumetone reduced the oxidative burst. Human neutrophils were incubated with nabumetone or the other compounds for 10 min at 37 °C and then NBT and fMLP were added. Blue formazan formed was determined. NT, non-treatment; Cont, control group. The data are expressed as mean ± s.e. (n = 3). $*P < 0.05$, $**P < 0.01$ (n = 4) compared with the control group. B. Nabumetone prevented the migration of neutrophils induced by fMLP. Human neutrophils were treated with the indicated concentrations of nabumetone at 37 °C for 15 min, and subjected to fMLP-induced chemotaxis. NT, non-treatment; Cont, control group; HPF, high power fields. The data are expressed as mean ± s.e. $**P < 0.01$ (n = 4) compared with the control group.

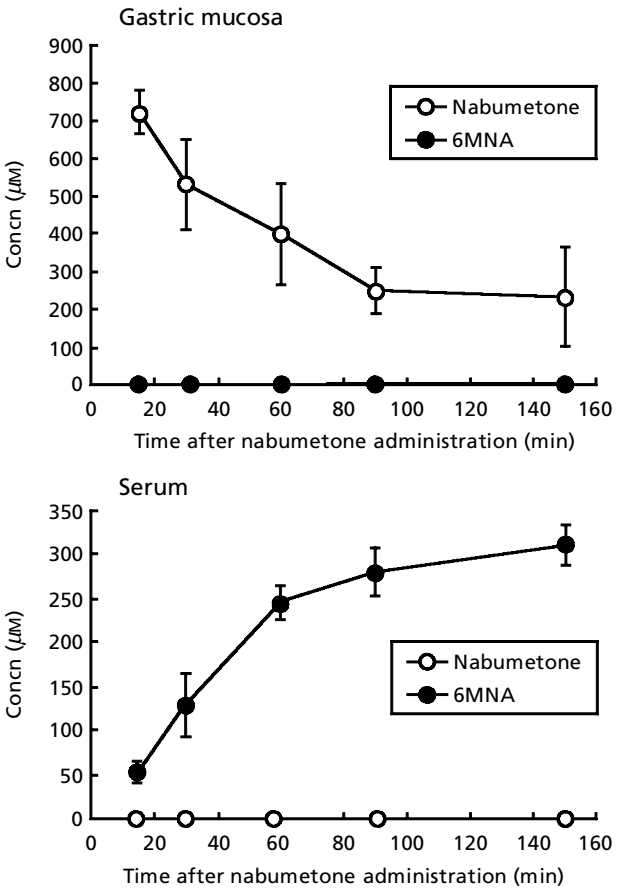

Figure 7 Concentration of nabumetone and 6MNA in the gastric mucosa and the serum of the rat after administration of nabumetone. Nabumetone was orally administered to rats and serum and gastric mucosa were collected at the indicated time. The concentration of nabumetone and 6MNA was determined as described in the text (n = 3).

in rats. As shown in Figure 7, the level of nabumetone in the mucosa reached more than 700 μM and gradually decreased, whereas 6MNA was not detected. In contrast, the concentration of nabumetone in the serum was only trace level, and the level of 6MNA increased to 250 μM 60 min after oral administration. This result is coincident with the results that nabumetone protects gastric mucosal injury by the administration 30 min after indometacin treatment.

Discussion

In this study, we showed that nabumetone, a pro-drug form that does not have cyclooxygenase-inhibiting activity, inhibited the fMLP-induced migration and respiratory burst of human neutrophils, but 6MNA, an active metabolite of nabumetone, did not. In addition, nabumetone inhibited the fMLP-induced migration of neutrophils without showing cytotoxicity against neutrophils at up to 200 μM. We could exclude the possibility of scavenging ability of nabumetone, because nabumetone did not attenuate O_2^- generation in the xanthine–xanthine oxidase system. Furthermore, nabumetone did not affect the NADPH oxidase activity that induces O_2^- generation in the cell-free system. These observations indicate that the inhibitory effect of nabumetone on the respiratory burst of neutrophils does not take place via a reduction of the entire cell function.

6MNA has been described, by Allegrezza-Giulietti et al (1993), as being protective against fMLP-induced reactive oxygen species production. However, they used fMLP at 10^{-7} M and detected reactive oxygen species with luminol, whereas the results described here were observed with 10^{-4} M of fMLP. We could not detect O_2^- generation from neutrophils treated with 10^{-7} M fMLP. Furthermore, we detected O_2^- generation with blue formazan formed from NBT. The difference in the amount of fMLP used may cause the difference in the results.

Interestingly, administration of nabumetone significantly prevented indometacin-induced gastric mucosal damage and infiltration of neutrophils to the gastric mucosa in rats, but 6MNA did not. In these experiments,

the prevention of gastric damage was most remarkable when nabumetone was administered 30 min after the indometacin treatment. This is a characteristic difference from other gastro-protective drugs (Akar et al 1999; Naito et al 2001b; Sener-Muratoglu et al 2001) or the PPAR-γ agonist, pioglitazone (Naito et al 2001a). Sodium salicylate protected against aspirin-induced gastric damage when given in high doses 30–60 min before induction of gastric damage (Ezer et al 1976, 1984; Robert 1981). Sodium salicylate increased the generation of mucosal prostaglandin-like material (Konturek et al 1982). In contrast, nabumetone did not increase the prostaglandin synthesis (Tibble et al 2001) and did not protect against gastric damage when it was administered 30 min before indometacin treatment. These results suggest that the action of nabumetone on gastric mucosa is different from that of sodium salicylate.

In NSAID-induced gastric damage models, the neutrophils begin to infiltrate gastric corpus lesions 15–30 min after dosing (Anthony et al 1996). Studies on the oral administration of nabumetone in rats showed that the concentration of nabumetone in the gastric mucosa was over 700 μM 15 min after administration. 6MNA was slightly detectable. This result suggests that the high concentration of nabumetone in mucosa affected the infiltrated neutrophils that had been induced by indometacin. However, a concentration of 200 μM of nabumetone within the gastric mucosa, which is sufficient to be able to prevent the activation of neutrophils (Figure 6), was detected longer than 120 min after administration of nabumetone. This is contradictory to the result that nabumetone given 30 min after, but not 30 min before, indomethacin protected against gastric mucosal damage (Figure 4). It may be that the local concentration of 6-MNA, derived from the systemic circulation, increases sufficiently to inhibit cyclooxygenase and impair the gastric damage. Administration of nabumetone before or concurrent with indometacin may decrease the concentration of prostaglandin by inhibiting COX and affect the mucosal protective effect of nabumetone.

Recent studies have demonstrated that neutrophil-dependent microvascular injury may be an important prime event that leads to indometacin- or aspirin-induced mucosal injury. Our study showed that MPO activity, an index of tissue-associated neutrophil accumulation, significantly increased in the gastric mucosa 3 h after indometacin treatment in the rat and that the increased MPO activity was significantly inhibited by oral treatment with nabumetone, but not by 6MNA. In-vivo studies have demonstrated that NSAIDs reduce the gastric mucosal blood flow at the site of ulceration (Main & Whittle 1975) or promote leucocyte adherence along the microvascular endothelium of the gastric mucosa (Yoshida et al 1993; Wallace et al 1991, 1993) preceding mucosal contraction. Additionally, the severity of gastric damage induced by NSAIDs can be reduced by neutrophil depletion (Wallace et al 1990; Ding et al 1998) or using monoclonal antibodies against leucocyte and endothelial adhesion molecules (Wallace et al 1991; Yoshida et al 1993). Wallace (1997) proposed that the adherence of neutrophils to the vascular endothelium, resulting in partial occlusion of the gastric microcirculation and free radical generation, is a critical event in the pathogenesis of NSAID-induced gastropathy. Nabumetone, the pro-drug form of 6MNA, at high concentrations in the gastric mucosa, may prevent the accumulation and activation of the neutrophils induced by 6MNA, resulting in less incidence of gastrointestinal irritation.

Recently, selective inhibition of COX-2 has been found not to be associated with the inhibition of prostaglandin synthesis in gastric mucosa and to show significantly less inhibition of prostaglandin synthesis and gastric damage than that seen with anti-inflammatory doses of conventional NSAIDs (Tibble et al 2000; Wight et al 2001). However, selective COX-1 and COX-2 inhibitors delayed the healing of pre-existing ulcers (Mizuno et al 1997; Brzozowski et al 2001) and this suggests that both COX isoforms are important sources of prostaglandins that appear to contribute to ulcer healing. Neutrophil infiltration contributes to delayed ulcer healing (Yamada et al 1999). Based on the results in these studies, nabumetone might promote the healing of ulcers accompanied with anti-inflammatory effects at inflammatory sites. These experiments are in progress.

Conclusions

Nabumetone is an NSAID and is extensively metabolized mainly to an active metabolite, 6MNA, an effective inhibitor of prostaglandin synthesis after oral administration.

Nabumetone shows less incidence of gastrointestinal irritancy than other conventional NSAIDs. This study showed that nabumetone affects neutrophil function but 6MNA dose not. We found that nabumetone remains in gastric mucosa at high concentration after oral administration and modifies the function of neutrophils that have migrated to the mucosa in response to NSAIDs. Taking these things into consideration, we think that nabumetone acts in the gastric mucosa, resulting in the prevention of the gastric damage induced by an active metabolite, 6MNA, via inhibition of neutrophil function.

References

Akar, F., Uydes-Dogan, B. S., Buharalioglu, C. K., Abban, G., Heinemann, A., Holzer, P., Van de Voorde, J. (1999) Protective effect of cromakalim and diazoxide, and proulcerogenic effect of glibenclamide on indomethacin-induced gastric injury. *Eur. J. Pharmacol.* **374**: 461–470

Alderman, B. M., Cook, G. A., Familari, M., Giraud, A. S. (2000) Resistance to apoptosis is a mechanism of adaptation of rat stomach to aspirin. *Am. J. Physiol. Gastrointest. Physiol.* **278**: G839–G846

Allegrezza-Giulietti, A., Serretti, R., Peroni, M., Cervini, C. (1993) Polymorpohonuclear leucocytes activation: in vitro modulation by 6-methoxy-2-naphthylacetic acid, the major active metabolite of nabumetone. *Pharmacol. Res.* **28**: 163–173

Angelis-Stoforidis, P., Vajda, F. J. E., Christophidis, N. (1998) Effect of non-steroidal anti-inflammatory drugs (NSAIDs) on

human polymorphonuclear leukocyte function in buffer and plasma. *Clin. Exp. Rheumatol*. **16**: 703–708

Anthony, A., Sim, R., Dhillon, A. P., Pounder, R. E., Wakefield, A. J. (1996) Gastric mucosal contraction and vascular injury induced by indomethacin precede neutrophil infiltration in the rat. *Gut* **39**: 363–368

Boyle, E. A., Freeman, P. C., Mangan, F. R., Thomson, M. J. (1982) Nabumetone (BRL 14777, 4-[6-methoxy-2-naphthyl]-butan-2-one: a new anti-inflammatory agent. *J. Pharm. Pharmacol*. **34**: 562–569

Brzozowski, T., Konturek, P. C., Konturek, S. J., Sliwowski, Z., Pajdo, R., Drozdowicz, D., Ptak, A., Hahn, E. G. (2001) Classic NSAID and selective Cyclooxygenase (COX)-1 and COX-2 inhibitors in healing of chronic gastric ulcers. *Microscopy Res. Tech*. **53**: 343–353

Davies, N. M. (1997) Clinical pharmacokinetics of nabumetone. *Clin. Pharmacokinet*. **33**: 403–416

Ding, S. Z., Lam, S. K., Yuen, S. T., Wong, B. C., Hui, W. M., Ho, J., Guo, X., Cho, C. H. (1998) Prostaglandin, tumor necrosis factor α and neutrophils: causative relationship in indomethacin-induced stomach injuries. *Eur. J. Pharmacol*. **348**: 257–263

Elizabeth, A., Boyle, E. A., Mangan, F. R. (1982) The effect of a novel, non-steroidal anti-inflammatory compound, nabumetone (BR 14777), on cellular infiltration into 24-hour polyvinyl sponge implants in the rats, compared with some steroidal and non-steroidal anti-inflammatory drugs. *J. Pharm. Pharmacol*. **34**: 570–575

Ezer, E., Palosi, E., Hajos, G., Szporny, L. (1976) Antagonism of the gastrointestinal ulcerogenic effect of some non-steroidal anti-inflammatory agents by sodium salicylate. *J. Pharm. Pharmacol*. **28**: 655–656

Ezer, E., Matuz, J., Pallagi, K., Szporny, L. (1984) Gastrointestinal cytoprotection by indomethacin + sodium salicylate combination in rats. *Acta Physiol. Hung*. **64**: 355–359

Griffin, M. R., Pipper, J. M., Daugherty, J. R., Snowden, M., Ray, W. A. (1991) Non-steroidal anti-inflammatory drug use and increased risk for peptic ulcer disease in elder person. *Ann. Intern. Med*. **114**: 257–263

Hua, J., Hasebe, T., Someya, A., Nakamura, S., Sugimoto, K., Nagaoka, I. (2000) Evaluation of the expression of NADPH oxidase components during maturation of HL-60 cells to neutrophil lineage. *J. Leukocyte Biol*. **68**: 216–224

Huang, J. Q., Sridhar, S., Hunt, R. H. (1999) Gastrointestinal safety profiles of nabumetone: a meta-analysis. *Am. J. Med*. **107**: 55S–61S

Ip, M., Lomas, D. A., Shaw, J., Burnett, D., Stockley, R. A. (1990) Effect of non-steroidal anti-inflammatory drugs on neutrophil chemotaxis–an in vitro and in vivo study. *Br. J. Rheumatol*. **29**: 363–367

Jemal, M., Xia, Y. Q., Whigan, D. B., Yuan, Q. (1998) The use of high-flow high performance liquid chromatography coupled with positive and negative ion electrospray tandem mass spectrometry for quantitative bioanalysis via direct injection of the plasma/serum samples. *Rapid Commun. Mass Spectrom*. **12**: 1389–1399

Konturek, S. J., Brzozowski, T., Piastucki, I., Radecki, T. (1982) Prevention of ethanol and aspirin-induced gastric mucosal lesions by paracetamol and salicylate in rats: role of endogenous prostaglandins. *Gut* **23**: 536–540

Langman, M. J. S., Weil, J., Wainwright, P., Lawson, D. H., Rawlins, M. D., Logan, R. F. A., Murphy, M., Vessey, M. P., Colin-Jones, D. G. (1994) Risk of bleeding peptic ulcer associated with individual non-steroidal anti-inflammatory drugs. *Lancet* **343**: 1075–1078

Main, I. H., Whittle B. J. (1975) Investigation of the vascular and antisecretory role of prostaglandins in the rat gastric mucosa by use of nonsteroidal anti-inflammatory drugs. *Br. J. Pharmacol*. **53**: 217–224

Manna, S. K., Samanta, S., Samanta, A. K. (1997) Hamycin inhibits IL-8-induced biologic response by modulating its receptor in human polymorphonuclear neutrophils. *J. Immunol*. **159**: 5042–5052

Melarange, R., Gentry, C., O'Connel, C., Blower, P. R., Neil, C., Kelvin, A. S., Toseland, C. D. N. (1992) Anti-inflammatory and gastrointestinal effects of nabumetone or its active metabolites, 6MNA (6-methoxy-2-naphthylacetic acid): comparison with indomethacin. *Agents Actions* Special Conference Issue: C82–C83

Mizuno, H., Sakamoto, C., Matsuda, K., Wada, K., Uchida, T., Noguchi, H., Akamatsu, T., Kasuga, T. (1997) Induction of cyclooxygenase 2 in gastric mucosal lesions and its inhibition by specific antagonist delays healing in mice. *Gastroenterology* **112**: 387–397

Naito, Y., Takagi, T., Yoshida, N., Yoshikawa, T. (2001a) Pioglitazone, specific PPAR-gamma ligand, inhibits aspirin-induced gastric mucosal injury in rats. *Aliment. Pharmacol. Ther*. **15**: 865–873

Naito, Y., Yoshikawa, T., Yagi, N., Matsuyama, K., Yoshida, N., Seto, K., Yoneta, T. (2001b) Effect of polaprezine on lipid peroxidation, neutrophil accumulation, and TNF-α expression in rats with aspirin-induced gastric mucosal injury. *Dig. Dis. Sci*. **46**: 845–851

Nygard, G., Anthony, A., Piasecki, C., Trevethick, M. A., Hudson, M., Dhillon, A. P., Pounder, R. E., Wakefield, A. J. (1994) Acute indomethacin-induced jejunal injury in the rats: early morphological and biological changes. *Gastroenterology* **106**: 567–575

Parij, N., Nagy, A. M., Fondu, P., Neve, J. (1998) Effect of non-steroidal anti-inflammatory drugs on the luminol and lucigenin amplified chemiluminescence of human neutrophils. *Eur. J. Pharmacol*. **352**: 299–305

Robert, A. (1981) Gastric cytoprotection by sodium salicylate. *Prostaglandins* **21** (Suppl.): 139–146

Sener-Muratoglu, G., Paskaloglu, K., Arbak, S., Hurdag, C., Ayanoglu-Dulger, G. (2001) Protective effect of famotidine, omeprazole, and melatonin against acetylsalicylic acid-induced gastric damage in rats. *Dig. Dis. Sci*. **46**: 318–330

Tibble, J. A., Sigthorsson, G., Foster, R., Bjarnason, I. (2000) Comparison of the intestinal toxicity of celecoxib, a selective COX-2 inhibitor, and indomethacin in the experimental rat. *Scand. J. Gastroenterol*. **35**: 802–807

Tibble, J. A., Sigthorsson, G., Caldwell, C., Palmer, R. H., Bjarnason, I. (2001) Effect of NSAIDs on cryoprobe-induced gastric ulcer healing in rats. *Aliment. Pharmacol. Ther*. **15**: 2001–2008

Wallace, J. L. (1993) Gastric ulceration: critical events at the neutrophil-endothelium interface. *Can. J. Physiol. Pharmacol*. **71**: 98–102

Wallace, J. L. (1997) Nonsteroidal anti-inflammatory drugs and gastroenteropathy: the second hundred years. *Gastroenterology* **112**: 1000–1016

Wallace, J. L., Keenan, C. M., Granger, D. N. (1990) Gastric ulceration induced by nonsteroidal anti-inflammatory drugs is a neutrophil-dependent process. *Am. J. Physiol*. **259**: G462–G467

Wallace, J. L., Arfors, K. E., McKnight, G. W. (1991) A monoclonal antibody against the CD18 leukocyte adhesion molecule prevents indomethacin-induced gastric damage in rabbit. *Gastroenterology* **100**: 878–883

Wallace, J. L., McKnight, W., Miyasaka, M., Tamatani, T., Paulson, J., Anderson, D. C., Granger, D. N., Kubes, P. (1993) Role of endothelial adhesion molecules in NSAID-induced gastric mucosal injury. *Am. J. Physiol.* **265**: G993–G998

Wang, J. P., Tsao, L. T., Raung. S. L., Hsu, M. F., Kuo, S. C. (1997) Inhibition by HAJ11 of respiratory burst in neutrophils and the involvement of protein tyrosine phosphorylation and phospholipase D activation. *Br. J. Pharmacol.* **120**: 79–87

Wight, N. J., Gottesdiener, K., Garlick, N. M,. Atherton, C. T., Novac, S., Gertz, B., Calder, N. A., Cote, J., Wong, P., Dallob, A., Hawkey, C. J. (2001) Rofecoxib, a COX-2 inhibitor, does not inhibit human gastric prostaglandin production. *Gastroenterology* **120**: 867–873

Yamada, H., Takahashi, S., Fujita, H., Kobayashi, N., Okabe, S. (1999) Cytokine-induced neutrophil chemoattractants in healing of gastric ulcers in rats: expression of >40-kDa chemoattractant in delayed ulcer healing by indomethacin. *Dig. Dis. Sci.* **44**: 889–895

Yoshida, N., Takemura, T., Granger, D. N., Anderson, D. C., Wolf, R. E., McIntire, L. V., Kvietys, P. R. (1993) Molecular determinants of aspirin-induced neutrophil adherence to endothelial cells. *Gastroenterology* **105**: 715–724