Effects of Novel Anti-Inflammatory Compounds on Healing of Acetic Acid-Induced Gastric Ulcer in Rats

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ABSTRACT
Nonsteroidal anti-inflammatory drugs often cause development of significant GI lesions. Selective inhibitors of prostaglandin G/H synthase/cyclooxygenase-2 (PGHS-2) enzyme and some dual inhibitors of PGHS-5-lipoxygenase (5-LO) enzymes have been reported to be potent anti-inflammatory compounds that carry a much lower risk of having GI irritating effects. We have evaluated the anti-inflammatory effect and the GI safety profile of three new anti-inflammatory compounds: the selective PGHS-2 inhibitors NS-398 and PD 138387 and the PGHS/5-LO dual inhibitor PD 137968. All the compounds tested showed an anti-inflammatory activity in the carragenan footpad edema test in rats. None of these compounds caused either gastric damage 4 h after p.o. administration of 100 mg/kg in rats or inhibition of PGE2 synthesis in the stomach. However, when administered p.o. at an effective anti-inflammatory dose to rats with pre-existing acetic acid-induced gastric ulcer, NS-398 caused a statistically significant delay of ulcer healing. No impairment of the ulcer healing was observed with the other compounds evaluated. Derivatives of 2,6-di-tert-butylphenol, whose members may act as PGHS-1/PGHS-2 inhibitors, selective PGHS-2 inhibitors or PGHS/5-LO dual inhibitors, are novel anti-inflammatory compounds that are devoid of GI irritating effects and do not affect the rate of pre-existing gastric ulcer healing.

The use of anti-inflammatory compounds such as NSAIDs is often associated with development of GI side effects such as gastric erosions and ulcer, which often limit their widespread clinical use (Wright, 1995; Jones and Tait, 1995; Henry, 1988).

The anti-inflammatory mechanism of NSAIDs is due mainly to inhibition of PGHS (Smith, 1992; Vane, 1971), an enzyme that catalyzes the conversion of arachidonic acid to PGH2, which is then further metabolized to various prostaglandins, prostacyclin and thromboxane A2 (Vane and Botting, 1996; Smith, 1992; Vane, 1971). PGHS exists in a constitutive form (PGHS-1) and in an inducible form (PGHS-2) (Smith, 1992). PGHS-1 is constitutively expressed in almost any tissue, including GI tract (Simmons et al., 1991), whereas expression of PGHS-2 is induced by inflammatory stimuli and growth factors (Smith, 1992; Simmons et al., 1991).

It has been hypothesized that the anti-inflammatory action of NSAIDs is due to inhibition of PGHS-2, whereas the toxic effects on the stomach are due to inhibition of PGHS-1 (Vane, 1994). By this hypothesis, selective inhibitors of PGHS-2 should retain their full anti-inflammatory effect and be associated with much lower toxic effects in the GI tract and stomach. Structural differences between PGHS-1 and PGHS-2 (Jones et al., 1993) have made it possible to design selective PGHS-2 inhibitors (Masferrer et al., 1994). For instance, NS-398 is a selective inhibitor of PGHS-2 that shows a potent anti-inflammatory effect associated with a low risk of significant gastric damage (Futaki et al., 1994; Futaki et al., 1993; Araï et al., 1993). On the other hand, PGHS-1 inhibitors and inhibitors of both PGHS-1/PGHS-2 are much more likely to cause significant gastric lesions (Wallace, 1997; Meade et al., 1993; Michell et al., 1993).

Dual inhibitors are compounds that, in addition to inhibiting PGHS enzyme, also inhibit 5-LO, another major enzyme involved in the metabolism of arachidonic acid. 5-LO is responsible, among other things, for the synthesis of leukotrienes (Smith, 1992), which are also believed to contribute to inflammation and the side effects of NSAIDs (Vaananen et al., 1992; Brain and Williams 1990; Guslandi, 1987). Therefore, dual inhibitors might also be efficacious anti-inflammatory drugs with a better GI safety profile than conventional NSAIDs (Unangst et al., 1994). The development of novel GI-safe anti-inflammatory compounds such as PGHS-2 and dual inhibitors could be a major breakthrough in the treatment of inflammatory conditions such as arthritis. However,
Materials and Methods

Compounds. NS-398, PD137968 (hydrochloride salt) and PD 138387 (choline salt) were all synthesized at Parke-Davis Pharmaceutical Research Division. Indomethacin and bacterial LPS were purchased from Sigma (St. Louis, MO). Immediately before their use, all the compounds were suspended in 1% CMC (Sigma) followed by 10-s sonication.

Inhibition of isolated PGHS isoforms. Inhibitory potencies were determined against purified recombinant human PGHS-2 (rhPGHS-2) isolated from baculovirus-infected Sf9 cells and against commercially obtained ram seminal vesicle PGHS-1 (roPGHS-1, Cayman Chemical, Ann Arbor, MI). Inhibition assays were conducted in 50 mM phosphate buffer, pH 7.5, containing 2 mM epinephrine as cofactor for the PGHS peroxidase reaction and 20 μM [14C]-arachidonic acid as substrate. rhPGHS-2 or roPGHS-1, in phosphate buffer containing Tween-20, was added in sufficient amount to convert 20% to 30% of added substrate to products in a 1-min incubation. Reaction products were separated from unconverted substrate and measured by radiometric HPLC analysis. Inhibition was measured as the percentage reduction of product formation compared with that in inhibitor-free incubations, and the concentration of compound causing 50% inhibition (IC50) was estimated with the software package KaleidaGraph, version 3.0.1 (Synergy Software Reading, PA) on a Macintosh Centris 650 (Apple Computers, Cupertino, CA) running System 7.1. Percent inhibition was fit to the two-parameter equation

\[
\% \text{ Inhibition} = 100/(1 + (\mu M/IC_{50})^{1/3})
\]

and best fits for IC50 and slope coefficient were estimated by least-squares analysis.

Inhibition of isolated rat basophilic leukemia 5-LO. Compounds were evaluated for inhibition of 5-LA activity contained in the 20,000 × g supernatant from rat basophilic leukemia (RBL-2H3) cells. Incubations contained 5% (v/v) RBL 20,000 × g supernatant in assay buffer (10 mM BES, 10 mM PIPES, 1 mM EDTA, 0.7 mM CaCl2, 1 mM ATP, 100 mM NaCl, pH 6.8). Inhibitors or vehicle (2% assay buffer (10 mM BES, 10 mM PIPES, 1 mM EDTA, 0.7 mM CaCl2, 1 mM ATP, 100 mM NaCl, pH 6.8)) were preincubated with the enzyme for 20 min at 37°C before initiating the 5-LA-catalyzed reaction by adding 33 μM [14C]-arachidonic acid (55.8 mCi/mmol (New England Nuclear, Boston, MA) dissolved in 16 mM aqueous NH2OH (3% of the total reaction volume). The reactions were terminated after 5 min of incubation by the addition of three volumes of methanol that contained 100 μg TTP and sufficient HCl to reduce the pH to 3 to 4. These samples were then analyzed by HPLC with radiometric detection for 5-LO reaction products. All treatments were evaluated in duplicate, and percent inhibition was computed by comparing the products formed in treatment incubations with the mean product formation in the uninhibited vehicle control group. The IC50 was estimated as described for the inhibition of PGHS isoforms.

Inhibition of PGHS-1 and PGHS-2 in PrP or J774A.1 cells. The PGHS-1 assay was based on the method of Grossman et al. (Grossman et al., 1995) and utilized PrP from NSAIID-free normal human volunteers in a culture medium consisting of 3.1% autologous plasma in RPMI-1640 (a 1:32 dilution of PrP). J774A.1 (J7) cells, a murine macrophage cell line, were used as described by Michell et al. (1995) to study PGHS-2 after an overnight induction of PGHS-2 with LPS (1 μg/ml). J7 cells were harvested with a rubber policeman and adjusted to 1 × 106 cells/ml RPMI-1640 without addition of plasma or serum. Assays were performed in flat-bottom 96-well plates (100 μl cell preparation, 100 μl drug dilution). After preincubation of cells and test compounds for 60 min at 37°C in a 5% CO2 incubator, samples were spiked with 3 μg of arachidonic acid (Nu-Chek Prep, Elysian, MN) and incubated for 30 min. Then the reaction was stopped by the addition of 25 μl of 80 μM indomethacin. TxB2 and PGE2 concentrations in clarified cell supernatants were determined by ELISA from Cayman Chemical Co. (Ann Arbor, MI) (TXB2) and Assay Designs (Ann Arbor, MI) (PGE2).

CFE inflammation test. CFE was induced in Wistar rats as previously described (Winter et al., 1982). Briefly, rats were injected in the right hind paw with 0.1 ml of 1% solution of carrageenan in 0.9% saline. Compounds were suspended in 0.5% hydroxypropyl methyl cellulose containing 0.2% Tween 80 and were administered p.o. at different doses in a volume of 10 ml/kg h 1 h before carrageenan injection. Paw volume was measured by mercury plethysmography. Swelling was assessed by subtracting the initial volume (determined immediately after the carrageenan injection) from the final volume (determined 5 h after carrageenan challenge) of the treated paw. Swelling was compared in the compound- and vehicle-treated groups to obtain percent inhibitions. The ED50 values were determined by linear regression analysis.

In vivo inhibition of PGHS-1 and PGHS-2 by NS 398, PD 137968 and PD 138387. In order to evaluate whether, at the effective anti-inflammatory doses, the compounds tested in this study behaved as selective PGHS-2 inhibitors in vivo, NS-398, PD137968 and PD 138387 were administered p.o. to rats at a dose equal to twice their ED50 on CFE, and the effects on whole-blood TxB2 and on whole-blood LPS-stimulated PGE2 were evaluated.

Whole-Blood thromboxane synthesis. Male (Crl:CD(SD)BR) rats were treated p.o. with the following compounds and doses: NS-398, 2 mg/kg; PD 137968, 11.4 mg/kg; PD 138387, 38 mg/kg. Indomethacin was used as a positive control and was administered p.o. at a dose of 2.5 mg/kg. All compounds were administered in a volume of 1 ml. Control animals received the same volume of vehicle (1% CMC) p.o. Three hours after administration of the compounds, animal were lightly anaesthetized, and blood was drawn by cardiac puncture. After a 45-min incubation in a water bath at 37°C, blood samples were centrifuged at 2000 × g for 10 min, and 1 ml of serum was removed and its TXB2 concentration measured with a commercially available ELISA kit (Assays Designs). Because platelets are the predominant source of thromboxane synthesis and contain only the PGHS-1 isozyme (Xie et al., 1992; Meade et al., 1993), this assay served as an index of inhibitory effects of the test drug on PGHS-1 activity.

Whole-blood LPS-stimulated PGE2 synthesis. Rats were treated p.o. with the following compounds and doses: NS-398, 2 mg/kg; PD 137968, 11.4 mg/kg; PD 138387, 38 mg/kg. Indomethacin was used as a positive control and was administered p.o. at a dose of 2.5 mg/kg. All compounds were administered in a volume of 1 ml. Control animals received the same volume of vehicle (1% CMC) p.o. After 30 min, animals were injected i.p. with 1 ml of a 5 mg/kg solution of LPS. Three hours from the time the animals were administered the compounds under study, and under light ether anesthesia, blood was drawn by cardiac puncture and incubated in a water bath at 37°C for 45 min. The blood samples were then centrifuged at 2000 × g for 10 min, and 1 ml of serum was removed and used for PGE2 measurement with a commercially available ELISA kit (Assays Designs). Because LPS administered p.o. induces a diffuse stimulation of PGHS products such as PGE2, this assay served as a measurement of the whole-blood LPS-stimulated PGE2 synthesis in rats.

Development of gastric damage and inhibition of PGE2 synthesis in rats. Male (Crl:CD(SD)BR) rats were administered 100 mg/kg of the compounds under study or indomethacin in a volume of 1 ml. Four hours later the animals were sacrificed, the stomach was removed and opened along the greater curvature and its image was digitized and stored on an optical disk using a 486-
based computer equipped with CUE3 system imaging analysis software (Olympus Corp., Marietta, GA). Two 6-mm biopsies were taken from a constant region located in each side of the glandular portion of the stomach, and their PGE2 content was measured with a commercially available ELISA kit (Assay Designs). The presence of gastric damage was determined with the retrieved electronic image, and the extent measured was determined with the CUE3 imaging software. Data were expressed as ulcer index, which was obtained by multiplying the percent frequency of the gastric damage by the extent of the damage.

**Induction of gastric ulcer.** After a 24-h food fast, male (Crl:CD(SD)BR) rats were anesthetized with an i.m. injection of a ketamine/xylazine mix. Under sterile conditions, the abdomen of the animals was opened, the stomach exposed and 20 μl of 100% acetic acid was injected into the gastric wall near the antral portion of the stomach using a Hamilton syringe with a 30-gauge needle. Control animals received no surgical modifications. The abdomen was then sutured, and the animals were allowed to recover and returned to their cages with food and water *ad libitum*. Injection of 20 μl of acetic acid causes the development of a single deep ulceration, which, after reaching its maximum size, starts to heal. The healing process starts around day 3 and is completed in about 4 weeks (Baragi *et al.*, 1997). To evaluate the relationship between development of acetic acid gastric ulcer and gastric content of prostaglandins, we sacrificed acetic acid-injected animals 72 h after acetic acid administration, removed the ulcer area and a nonulcerated area of the stomach and measured their PGE2 content with a commercially available ELISA kit (Assay Designs). In order to study the effect of the anti-inflammatory compounds on the healing process and to avoid any interference with the formation phase of acetic acid ulcer, the compounds used in this study were administered p.o. to rats starting 3 days after acetic acid injection. For each compound, an effective anti-inflammatory compound on the formation phase of acetic acid ulcer, the compounds were used in this study were administered p.o. to rats starting 3 days after acetic acid injection. For each compound, an effective anti-inflammatory dose equal to twice the ED50 on CFE was used. NS-398 was administered at a dose of 2.0 mg/kg, PD 137968 at a dose of 11.4 mg/kg and PD 138387 at a dose of 38 mg/kg. This treatment regimen was repeated daily for the duration of the study.

**Measurement of ulcer area.** Groups of animals were sacrificed at weeks 2, 3 and 4. The stomach was removed, opened along the greater curvature, rinsed in saline and pinned out on a flat surface. The image of the stomach was then digitized with CUE3 image analysis software (Olympus Corp.) installed on a 486-based computer equipped with a color camera and stored on a magneto-optical disk. Measurement of the ulcer crater was performed on the retrieved image. Briefly, the edges of the ulcer crater were traced, and the area of the ulcer was automatically calculated by the computer and expressed in square millimeters.

**Statistics.** Data on CFE were analyzed by paired *t* test, and the results were considered significant at a level of *P* < .05. All the other data were analyzed by Kruskal-Wallis one-way ANOVA followed by Dunnett's test. Results were considered significant at a level of *P* < .05.

**Results**

**In vitro inhibition of isolated PGHS-1 and PGHS-2.** The IC50 values of the compounds evaluated in this study against isolated isoforms of PGHS and 5-LO are reported in table 1. NS-398 behaved as a selective PGHS-2 inhibitor with weak inhibitory activity against PGHS-1 isoform and no significant inhibitory activity against 5-LO. PD 138387 behaved as a dual inhibitor of 5-LO and PGHS-2 with no activity against PGHS-1. PD 137968, on the other hand, inhibited PGHS-1 and PGHS-2 equally well (although 50% inhibition was not reached) and had more potent inhibitory activity against 5-LO. As expected, indomethacin inhibited both PGHS-1 and PGHS-2 but failed to produce any significant inhibition of 5-LO.

**Inhibition of PGHS-1 or PGHS-2 in platelets or J774A.1 cells.** The selectivity of NS-398 and PD 138387 for the PGHS-2 isoform was confirmed by measuring the inhibitory activity of these compounds on the PGHS-1 and PGHS-2 enzymes present in human platelets and J774A.1 cells, respectively. In these cell-based assays, in addition to the potent inhibitory effect against PGHS-2, NS-398 also displayed a less potent inhibitory activity against PGHS-1, whereas PD 138387 was unable to cause a 50% inhibition against the same enzyme at the highest concentration tested (20 μM). PD 137968, on the other hand, potently inhibited the PGHS-1 enzyme in platelets but had no effect on the PGHS-2 isoform in J774A.1 cells. Indomethacin was able to inhibit PGHS-1 and PGHS-2 enzymes equally well.

**Effect of CFE.** All the compounds used in this study showed an anti-inflammatory effect in the CFE test and reduced the swelling of the paw caused by injection of carrageenan (fig. 1). The order of potency of the compounds tested, based on the ID40, was NS-398 ≫ PD 137968 ≫ PD 138387.

**Effect of indomethacin.** NS-398, PD 137968 and PD 138387 on *in vivo* inhibition of PGHS-1 and PGHS-2. Oral administration of 2 mg/kg NS-398, 11.4 mg/kg PD 137968 and 38 mg/kg PD 138387 significantly inhibited LPS-stimulated PGE2 synthesis but did not significantly affect the synthesis of TxB2 in serum (fig. 2). Because the former assay serves as an indicator of PGHS-2 inhibition, whereas the latter indicates inhibitory activity against PGHS-1, our results suggest that these compounds, at the effective anti-inflammatory doses used throughout the study, act as selective PGHS-2 inhibitors *in vivo*. As expected, when a known inhibitor of both PGHS-1 and PGHS-2, such as indomethacin, was used in the same study, significant inhibitory synthesis of both TxB2 (PGHS-1-dependent) and PGE2 (PGHS-2-dependent) was observed (fig. 2).

**Development of gastric damage and the effect on PGE2 synthesis in the stomach.** Four hours after administration of 100 mg/kg NS-398, PD 137968 and PD 138387, animals did not show any macroscopic gastric damage (ulcer index = 0). Conversely, animal treated with the same dose of indomethacin had diffuse areas of gastric damage (ulcer index = 13.36) (table 2). Indomethacin strongly inhibited the synthesis of PGE2 in the stomach. Much weaker inhibition or

**TABLE 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>oPGHS-1</th>
<th>rhPGHS-2</th>
<th>oPGHS-1/rhPGHS-2</th>
<th>RBL 5-LO</th>
</tr>
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<tbody>
<tr>
<td>NS-398</td>
<td>NC100</td>
<td>0.19 ± 0.04 μM</td>
<td>&gt;526</td>
<td>NC100</td>
</tr>
<tr>
<td>PD 137968</td>
<td>NC100</td>
<td>0.19 ± 0.14 μM</td>
<td>NC</td>
<td>3.5 μM</td>
</tr>
<tr>
<td>PD 138387</td>
<td>NC100</td>
<td>1.7 ± 0.7 μM</td>
<td>&gt;58.9</td>
<td>14 μM*</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.012 ± 0.0006 μM</td>
<td>5.0 ± 0.07 μM</td>
<td>0.0020</td>
<td>NC32</td>
</tr>
</tbody>
</table>

Average ± S.E. oPGHS-1 = ram seminal vesicles PGHS1; rhPGHS-2 = recombinant human PGHS-2; RBL-5-LO = rat basophilic leukemia cell 5-LO. NC means inhibitory activity did not reach 50% inhibition, and IC50 or ratio could not be calculated. Values in parentheses represent the maximum inhibitory activity observed at the highest concentration used (indicated in the subscript). Solubility problems preventing testing of PD 137968 at higher concentration. * Single-run determination.
no inhibition of PGE2 synthesis was observed with NS-398, PD 137968 and PD 138387 (table 2).

Efffect on healing of acetic acid gastric ulcer and on PGE2 synthesis in the stomach.

Development of acetic acid gastric ulcer was associated with an elevation of PGE2 in the area of ulceration but not in nonulcerated gastric areas (fig. 3). To assess the effects on the healing rate of acetic acid ulcer, daily p.o. treatment with the novel anti-inflammatory compounds under study was initiated at day 3 after administration of acetic acid, when the healing phase had already started. Rats treated with PD 137968 or PD 138387 showed a healing rate comparable to that of vehicle-treated control animals. On the other hand, treatment with NS-398 signifi-

cantly slowed the healing process (fig. 4). At week 4, animals treated with NS-398 had an average crater size of 9.56 ± 1.56 mm² compared with 1.79 ± 0.49 mm² for control animals.

Discussion

Development of efficacious anti-inflammatory drugs devoid of gastric irritating effects would represent a major breakthrough in the treatment of chronic inflammatory conditions such as arthritis. In this regard, PGHS-2 inhibitors seem to be promising agents that combine a potent anti-inflammatory activity with a low risk of inducing significant gastric side effects. In addition, compounds that inhibit both PGHS and 5-LO may also represent anti-inflammatory compounds with a better GI safety profile than NSAIDs.

When tested on isolated enzymes, all the compounds evaluated in this study behaved as either selective inhibitors of PGHS-2 (NS-398) or inhibitors of 5-LO and of either both isoforms of the PGHS enzyme (PD137968) or PGHS-2 alone (PD 138387) (table 1). Cell-based assays largely confirmed the selectivity of NS-398 and PD 138387 for PGHS-2, whereas, contrary to what was observed on the isolated enzyme assay, PD 137968 showed a more potent activity against PGHS-1 (table 3). These differences can be explained by the more complex environment of a whole-cell assay. Be-

![Fig. 1](image1.png)

Anti-inflammatory effect of NS-398, PD 137968 and PD 138387 on CFE. Compounds were administered p.o., and the inhibition of foot pad edema was measured 5 h later. n = 10 rats/dose/group. * P < .05 based on paired t test.

![Fig. 2](image2.png)

Effect of indomethacin, NS-398, PD 137968 and PD 138387 on inhibition of synthesis of TxB2 in whole blood and on LPS-induced synthesis of PGE2 in vivo. Rats were administered the compounds under study p.o. at the doses indicated. For TxB2 measurement, blood was drawn 3 h after the administration of the compound. For PGE2 measurement, 1 ml of LPS (5 mg/ml) was injected i.p. and followed 30 min later by administration of the compound. In both cases, blood was drawn 3 h after administration of the compound, incubated at 37°C for 45 min and assayed for TxB2 and PGE2 content using a specific ELISA kit. Numbers in parentheses indicate the n value for each group. * P < .05 based on one-way ANOVA followed by Dunnett’s test and compared with control group.

![Fig. 3](image3.png)

PGE2 content in gastric tissue in ulcerated and nonulcerated areas of the stomach. Animals received 20 μl of 100% acetic acid in the gastric wall. Animals were sacrificed 72 h after acetic acid administration, and the ulcerated and a nonulcerated area of the glandular mucosa were removed and assayed for PGE2 content with a commercially available ELISA kit. Control animals were injected with vehicle and did not develop any ulceration. Numbers in parentheses represent the n value for each group. * P < .05 based on one-way ANOVA followed by Dunnett’s test and compared with vehicle-treated group.

![Table 2](image4.png)

Development of gastric damage 4 h after p.o. administration of NS-398, CI-1004, PD 137968, 138387 and indomethacin in rats

<table>
<thead>
<tr>
<th>Compound</th>
<th>n</th>
<th>Frequency (%)</th>
<th>% Gastric Area Damaged</th>
<th>Ulcer Index</th>
<th>PGE2 (% inhibition)</th>
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<tr>
<td>NS-398</td>
<td>8</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>30</td>
</tr>
<tr>
<td>PD-137968</td>
<td>8</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>-22</td>
</tr>
<tr>
<td>PD-138387</td>
<td>8</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>32</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>16</td>
<td>75.0</td>
<td>0.18</td>
<td>13.36</td>
<td>96</td>
</tr>
</tbody>
</table>

Animals were dosed p.o. at 100 mg/kg, and the presence of gastric damage (frequency), its extent and the inhibition of PGE2 were determined 4 h later. The ulcer index was calculated by multiplying the frequency of the damage in a group by the average extent of the damage.
cause in vitro inhibition of PGHS-1 and PGHS-1 is not always indicative of in vivo inhibition, we also evaluated the selectivity of NS-398, PD 137968 and PD 138387 for the PGHS-2 isoform by measuring the effect of these compounds, at the effective anti-inflammatory dose used throughout this study, on TxB2 and LPS-stimulated PGE2 levels in serum after p.o. administration of the compounds. All compounds that we tested significantly inhibited LPS-stimulated PGE2 synthesis but not TxB2 synthesis, which suggests that also in vivo, at the doses used in this study, these compounds act as selective PGHS-2 inhibitors. Interestingly, PD 137968 showed a better selectivity for PGHS-2 in vivo than in vitro. On the other hand (and as expected), indomethacin in the same assays inhibited TxB2 and LPS-stimulated PGE2 equally well, which confirms the ability of this compound to inhibit both PGHS-1 and PGHS-2.

In results compatible with their in vitro and in vivo activity against the PGHS-2 isoform, all the compounds tested in this study showed an anti-inflammatory effect on the CFE assay (fig. 1). The selective PGHS-2 compound NS-398 showed the most potent anti-inflammatory activity, followed by the PGHS/5-LO inhibitor PD137968. A somewhat weaker anti-inflammatory activity was observed with the other selective PGHS-2 inhibitor tested, PD 138387, despite the fact that this compound also showed some inhibitory activity against 5-LO in vitro (table 1). This may be explained by its weaker PGHS-2-inhibitory activity compared with NS-398, which is not compensated by its weaker inhibitory activity on 5-LO. When administered to rats, none of the compounds tested except indomethacin caused gastric damage, even at doses several-fold above those required for an anti-inflammatory effect (table 2). The development of gastric toxicity of anti-inflammatory compounds has been correlated to their ability to inhibit PG synthesis in the stomach. It is consistent with this relationship that NS-398, PD 137968 and PD 138387 did not significantly inhibit PGE2 synthesis. On the other hand, as expected, significant inhibition of PGE2 synthesis was observed with the damaging agent indomethacin.

The compounds we tested differed widely in their ability to interfere with the healing of pre-existing ulcers. The PGHS-2 inhibitor NS-398 significantly delayed the healing of acetic acid-induced ulcer. After 4 weeks of treatment, healing of the acetic acid-induced ulcers in control animals was almost complete, whereas animals treated with NS-398 still had large ulcerations in their stomachs. None of the other compounds tested in this study affected the healing of acetic acid-induced ulcers, and rats treated with the compounds showed a healing rate similar to that of control animals.

It is unclear at the present time whether the impairment of healing rate observed with NS-398 is a consequence of the selective inhibition of PGHS-2, or a characteristic of the chemical structure of NS-398. Mizuno et al. (1997) also reported that NS-398 i.p. administered at a dose of 1 mg/100 g in mice also impairs healing of acetic acid-induced gastric ulcer, and the moderate selective PGHS-2 inhibitor nabumetone was reported to exacerbate the TNBS-induced colonic damage in rats (Reuter et al., 1996). These data suggest that PGHS-2 inhibition might be responsible for the impairment of ulcer healing throughout the GI tract. However, this hypothesis is weakened by the observation that in our study, no delay of healing rate was observed with PD 138387, a selective PGHS-2 inhibitor that belongs to a different chemical class than NS-398. However, PD138387 is also a 5-LO inhibitor. Therefore, it is possible that inhibition of 5-LO activity somehow counteracts the impairment of ulcer healing caused by PGHS-2 inhibitors. Interestingly, inhibition of gastric ulcer healing and exacerbation of TNBS-induced colonic damage have also been reported with another PGHS-2 inhibitor, namely L-745,337 (Schmassmann et al., 1996), which belongs to the same chemical class as NS-398.

Taken together, these data suggest that N-substituted methanesulfonamides PGHS-2 inhibitors (such as NS-398 and L-745,337) might be responsible for the impairment of GI ulcer healing. On the other hand, PD 138,387, in addition to selectively inhibiting PGHS-2, also has some inhibitory activity on 5-LO, and no impairment of ulcer healing was observed with the PGHS/5-LO inhibitor PD 137,968. This raises the possibility that, independently of the chemical

### TABLE 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>n</th>
<th>PGHS-1 IC50</th>
<th>PGHS-2 J774A.1 IC50</th>
<th>PGHS-1/PGHS-2 Ratio</th>
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<tbody>
<tr>
<td>NS 398</td>
<td>16</td>
<td>3.3 μM ± 0.73</td>
<td>0.022 μM ± 0.0077</td>
<td>150</td>
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<tr>
<td>PD 137968</td>
<td>3</td>
<td>1.7 μM ± 0.21</td>
<td>NC</td>
<td>&lt;0.085</td>
</tr>
<tr>
<td>PD 138387</td>
<td>4</td>
<td>0.22 μM ± 0.095</td>
<td>0.005 μM ± 0.0012</td>
<td>&gt;91</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>20</td>
<td>0.008 μM ± 0.00075</td>
<td>1.6</td>
<td></td>
</tr>
</tbody>
</table>

n = number of experiments. IC50 represents the average value of n experiments ± S.E. NC means inhibitory activity did not reach 50% inhibition at the highest concentration tested (20 μM), and IC50 could not be calculated.
structure, inhibition of PGHS-2 may delay ulcer healing if 5-LO activity remains undisturbed.

The impairment of ulcer healing with NS-398 was observed at an effective anti-inflammatory dose (twice the ID$_{50}$ on CFE), so a decrease in the rate of ulcer healing is likely to be observed during treatment of inflammatory processes with this drug at the dose used in this study. Therefore, this effect may be of clinical importance, especially in the case of patients affected by chronic inflammatory conditions who were previously treated with NSAIDs or other ulcerogenic compounds. On the other hand, because a dose of 2 mg/kg of NS-398 produces a near-maximum anti-inflammatory effect on CFE, it is possible that certain mild inflammatory processes may be treated with lower anti-inflammatory doses of the drug, at which the impairment of the ulcer healing may not occur.

In conclusion, our data suggest that the impairment of the healing process observed with NS-398 may be a characteristic of PGHS-2 inhibitors belonging to the same chemical class and that it is not necessarily shared by other PGHS-2 inhibitors. Concurrent inhibition of 5-LO may also play an important role. On the other hand, derivatives of the 2,6-di-tert-butyphenol class are potent anti-inflammatory compounds that are devoid of gastric irritating effects and do not cause any delay of healing of pre-existing gastric ulcers.

References

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