NSAID-Induced Mucosal Injury:

Analysis of Gastric Toxicity of New Generation NSAIDs:
Ulcerogenicity Compared with Ulcer Healing

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(Received June 28, 1996; returned for revision October 10, 1996; accepted January 8, 1997)

Introduction: Some non-steroidal anti-inflammatory drugs (NSAIDs) delay healing of experimental gastric ulcers. The two experimental NSAIDs tebufelone and nitrofenac exert relatively low ulcerogenicity in various animal models compared with conventional NSAIDs. In addition, it has been reported that nitrofenac accelerates experimental acute ulcer healing. However, the effects of these new NSAIDs in a reliable chronic ulcer model has not been fully established.

Methods: Ulcerogenicity of tebufelone was compared with vehicle and indomethacin in arthritic female Lewis rats in a single dose and a 5-day dosage study. Interference with ulcer healing of tebufelone and nitrofenac was compared with vehicle, indomethacin, diclofenac, omeprazole, and indomethacin plus omeprazole in Wistar rats with gastric cryo-ulcers. The rats were treated for 15 days and ulcer size was sequentially quantified by video endoscopy. Prostanoid synthesis in stomach and blood were assessed on day 15.

Results: Ulcerogenicity of tebufelone was markedly lower than that of indomethacin using doses with equipotent anti-inflammatory activities. Ulcer healing was accelerated by omeprazole in the first week, but significantly delayed by tebufelone and nitrofenac to a similar extent as indomethacin and diclofenac predominantly during the second week. All NSAIDs decreased prostanoid synthesis.

Conclusion: Tebufelone and nitrofenac delayed gastric ulcer healing to a similar extent as conventional NSAIDs even though tebufelone appears to induce less mucosal damage when determined in standard ulcer assays in rats. Thus there does not appear to be a relationship between ulcerogenicity of these NSAIDs and their behaviour in ulcer healing.

INTRODUCTION

Chronic infection with \textit{Helicobacter pylori} and non-steroidal anti-inflammatory drugs (NSAIDs)\textsuperscript{e} represent the two most important pathogenic factors in peptic ulcer disease [1-3]. Since eradication of \textit{H. pylori} can cure peptic ulcer disease in the majority of \textit{H. pylori}-associated peptic ulcers, increasingly more attention is given to prevention and therapy of NSAID induced ulcers. This is of particular importance since NSAIDs are highly liable to produce severe ulcer complications such as massive gastrointestinal hemorrhage or perforation from complicated peptic ulcers [4, 5]. It is now increasingly recognized that the occurrence of such complications is not restricted to the stomach and duo-
denum but can also result from ulcers localized in the small and large bowel [6]. Chronic administration of NSAIDs produces gastroduodenal mucosal erosions in 35-60 percent of patients, ulcerations in 10-25 percent, and severe gastrointestinal hemorrhage or perforation in less than 1 percent [2, 4, 5]. In experimental and clinical conditions, indomethacin delays gastric ulcer healing [7, 8]. Among the mechanisms proposed for delayed healing are: (1) inhibition of synthesis of prostaglandins that are important for gastroduodenal mucosal defense [9, 10], (2) inhibition of epithelial cell proliferation in the ulcer margin that is critical for re-epithelialization of the ulcer crater [7, 11, 12], (3) inhibition of angiogenesis that is essential for nutrient supply in the ulcer bed [11] and, (4) inhibition of proliferation and function of myofibroblasts involved in remodeling and contraction of the granulation tissue in the ulcer bed [11, 13].

NSAIDs inhibit prostaglandin synthesis by the gastric mucosa and thromboxane production by platelets so impairing platelet aggregation. Within 90 minutes of acute aspirin ingestion in humans extensive intramucosal petechial hemorrhage occurs visibly and this may, in part, be related to promotion of bleeding from the antiplatelet actions of aspirin. With longer term of ingestion, the number of erosions may diminish, possibly by a process of adaptation [14]. However, erosions, petechiae and superficial ulcers are quite common in patients on long-term maintenance treatment with NSAIDs. Moreover, it is not established why a small proportion of patients develop chronic ulcers yet others exhibit little or no mucosal damage.

In the last decade or so, there have been major efforts to reduce the gastroduodenal toxicity of NSAIDs. Much work has concentrated on use of co-prescribed mucosal protective agents. Thus, endoscopic studies have shown that the prostaglandin analogue misoprostol reduces the incidence of gastric and duodenal ulcers and the admission rate to hospital with ulcer complications [10]. Prostaglandins did not, however, meet their expectations in healing of peptic ulcers, they only exert modest ulcer healing at acid inhibitory doses [15]. Profound acid suppression by proton pump inhibitors is, however, highly effective in the treatment of NSAID-induced gastroduodenal ulcerations both in man and experimental models [7, 11, 16, 17] but long term acidity may prove undesirable side-effects (e.g., promotion of bacterial overgrowth).

Numerous strategies have been used in recent years to develop new anti-inflammato-ry and analgesic drugs that spare the gastrointestinal tract. Several groups are attempting to develop NSAIDs that predominantly inhibit effects on lipoxygenase or other particularly non-ulcerogenic pathways of lipid metabolism and/or which have antioxidant activity. Tebufelone has been shown to be gastroprotective in various models of NSAID-induced injury [18]. We reasoned that the unique pharmacological properties of tebufelone having differential effects on lipoxygenase pathways and/or antioxidant effects might influence ulcer healing as part of the overall anti-ulcer effects seen with these compounds.

Another strategy for developing gastrointestinal-sparing NSAIDs is the coupling of a nitric oxide-releasing moiety to a standard NSAID [19-26]. The rationale behind this strategy is that the nitric oxide (NO) released from these derivatives will exert beneficial effects on the mucosa by maintaining gastrointestinal blood flow and inhibiting adherence and activation of white blood cells with the gastrointestinal mucosa [19, 20]. One such NO-releasing NSAID is nitrofenac, this being the NO-donor of the NSAID, diclofenac.

**Hypothesis:** a) Antioxidant activity and/or effects on lipoxygenase or other pathways of lipid metabolism affected by tebufelone might decrease ulcerogenic damage and interferes with gastric ulcer healing to a lesser extent compared with conventional NSAIDs, and b) nitrofenac may interfere with chronic gastric ulcer healing to a lesser extent compared with diclofenac consequent upon NO-donation from nitrofenac.
MATERIALS AND METHODS

I. Comparative ulcerogenicity of tebufelone and reference NSAIDs in arthritic rats with an intact stomach

Animals: The model of adjuvant-induced arthritis was employed as this is known to be more sensitive to the ulcerogenic effects of NSAIDs [27, 28]. Moreover, this is a model of polyarthritis resembling rheumatoid and related arthritic conditions in humans, and thus has direct clinical relevance [27]. Tests of NSAIDs in this animal model can be considered representative of their chronic ulcerogenicity in arthritic disease. Female Lewis rats (Specific pathogen-free, from Charles River) were injected with heat-killed *Mycobacterium butyricum* (Difco) 0.5 mg in 0.05 ml squalene (Sigma) [29] then used at 14-16 day post-induction i.e., after injection of the adjuvant. Those animals exhibiting full manifestation of polyarthritis were selected for subsequent use. Two treatment groups were employed:

Single dose study: Animals were prior fasted for 24 hrs then given a single oral dose of test drugs. They were killed at 2 and 4 hrs (to ensure that the time of peak ulcerogenicity of the drugs related to the peak periods of gastric absorption). The gastric mucosa was removed and the lesion area and numbers quantified as described [31].

5-day dosage study: The rats were dosed orally with the drugs each day for a total of 5 days. They were then fasted for approximately 20 hrs on day 4 prior to the final dose and then killed 4 hrs after the final dose of drug. The intestinal tract as well as the stomach of these animals was removed and the area and number of lesions quantified. It was important to establish the ulcerogenicity in the intestinal tract since recent studies suggest that this may be a more significant site of ulcerogenicity in arthritic patients than recognized for many NSAIDs [29].

Test drugs and dosing: All drugs were prepared immediately before dosing in 0.5 percent Tween 20 with 1 percent carboxymethyl-cellulose at doses as follows: Tebufelone 15, 50, 250, or 500 mg/kg *per os* in single dose studies and 15 or 50 mg/kg/day *per os* in 5-day dosage group. Indomethacin 5, 10, or 30 mg/kg *per os* in single dose experiments; 1, or 3.0 mg/kg/day *per os* in 5-day dosage group; high doses are inevitably fatal because of peritonitis from this drug. Control animals received 1 ml 1 percent carboxymethyl-cellulose plus 0.5 percent Tween 20 in water per 200 g body weight *per os*. At least 3-7 animals were employed per group.

These studies were approved by the Annual Research Experimentation Board of McMaster University, Hamilton, Ontario, Canada.

II. Ulcer healing studies with new generation and reference NSAIDs

Ulcer induction: This study was approved by the Animal Study Committee of the University of Bern, Switzerland. A gastric cannula (steel, inner diameter 8 mm, exteriorly fitted with a screwed on cap, Band, Bern, Switzerland [11, 31] allowing video endoscopic examination of the gastric mucosa, was placed into the lumen of female Wistar rats (body weight: 200-220 g). Three weeks later, standardized gastric ulcers were produced by cryoinjury as previously described [31]. In brief: fed rats were anesthetized with ether and the abdomen was opened by median incision. The serosal surface of the posterior wall of the midcorpus was injured by cryoprobe (outside diameter 6.5 mm, cooled by gaseous carbon dioxide to -60°C; Cryoprobe BM 250, Erbokryo 12, Rüegge Medical, Baden, Switzerland) to the wall for 45 s. The lesion was allowed to thaw naturally and then the abdomen was rinsed with isotonic saline solution and closed with catgut and silk sutures: The rats were kept under normal laboratory conditions with free access to water and a standard pelleted rat diet (Naphag, Gossau, Switzerland). At 24 h after cryoinjury, the
ulcer size was measured by video endoscopy [11]. Only those rats with round ulcers with an ulcer diameter of 4.5-6.5 mm were allocated to the trial.

**Study drugs**: Omeprazole was provided by AB Hässle (Mölndal, Sweden), nitrofenac by Pharmaceutical Discovery Service (Monza, Italy), and tebufelone from Procter & Gamble Pharmaceuticals (Cincinnati, Ohio, USA). Indomethacin (Indocid for injection) was obtained from Merck Sharp & Dohme and diclofenac sodium from Sigma.

**Study design**: At 24 h after cryoinjury, 30 rats were randomly assigned to one of the following six groups (5 rats each): (1) placebo (volume: 1 ml) intragastrically (i.g.) and distilled water (volume: 0.25 ml) subcutaneously (s.c.) twice daily, (2) placebo i.g. and indomethacin 0.5 mg/kg s.c. twice daily, (3) placebo i.g. twice daily and omeprazole 40 µmol/kg s.c. once daily, (4) placebo i.g. and indomethacin 0.5 mg s.c. twice daily and omeprazole 40 µmol/kg s.c. once daily, (5) tebufelone 1 mg/kg i.g. and distilled water s.c. twice daily, (6) tebufelone 10 mg/kg i.g. and distilled water s.c. twice daily. Rats were treated for 15 days.

Additional preliminary ulcer healing studies: At 24 h after cryoinjury, forty-eight rats were randomly assigned to one of the following six groups (eight rats each) and treated twice daily for 15 days: (1) placebo i.g. and distilled water s.c. twice daily, (2) placebo i.g. and indomethacin 0.5 mg/kg s.c. twice daily, (3) placebo i.g. twice daily and omeprazole 40 µmol/kg s.c. once daily, (4) diclofenac 2.5 mg/kg i.g. and distilled water s.c. twice daily, (5) nitrofenac 3.75 mg/kg i.g. and distilled water s.c. twice daily, (6) indomethacin 0.5 mg/kg s.c. twice daily plus omeprazole 40 µmol/kg s.c. once daily. Rats were treated for 15 days.

**Video endoscopy**: Endoscopic examinations were performed on rats with a cannula (implanted into the rumen) through which a video endoscope (arthroscope, outer diameter: 4 mm, 30° side-view, Storz, Tuttingen, Germany; Video camera OTV-F Olympus, Tokyo, Japan) was inserted. In the first part of the study, video endoscopy was performed on days 1, 3, 6, 8, 10, 13, and 15 without fasting. In the additional preliminary part of the study, video endoscopy was performed on days 3, 8, and 15. Rats were immobilized in Bollman cages without anesthesia. They did not show any sign of discomfort during endoscopy. Food present in the stomach was washed out through the gastric cannula. For the ulcer size measurement, a round piece of calibration paper (diameter 4 mm) was placed close to the ulcer crater. Ulcer size was measured and compared with the calibration paper as described [8]. The ulcer diameter was calculated according to the formula: diameter = 2 x square root of (area/(π)). To eliminate the influence of the initial variation in ulcer diameter, we expressed ulcer healing as a percent reduction of the ulcer diameter per day. Ulcer healing rate on a given day was [ulcer diameter on day “x”] - [ulcer diameter on day “y”] / [y - x] in percent.

**Generation of prostanoids**: Prostaglandin (6-keto-PG-F_1α) and thromboxane B_2 generation were measured one hour after drug administration on day 15. Gastric mucosal specimens (approximately 30 g) were obtained at sacrifice, carefully blotted and the wet weight was measured. The tissue specimens were then incubated in 0.6 ml of oxygenated Tyrode’s solution at 37°C for 10 min. Release of 6-keto-PG-F_1α (stable metabolite of prostacyclin) into the incubation medium was determined using a specific radioimmune assay as described previously [32, 33]. In the second part of the study, thromboxane B_2 synthesis by the blood was additionally determined as described previously [34]. To assess changes during treatment, plasma gastrin was measured by radioimmune assay in fed rats before and after treatment as described [35].

**Statistics**: The significance of differences was tested by Mann Whitney U-test or by one-way analysis of variance [36]. Probability values of p < .05 were regarded as significant. Results are expressed as mean + SEM.
RESULTS

I. Comparative ulcerogenicity of tebufelone and reference NSAIDs in arthritic rats with an intact stomach

At the lowest dose of tebufelone (15 mg/kg) no detectable gastric mucosal damage was observed when this was given as either a single dose (Table 1) or even when given as daily doses for 5 days (Table 2).

There did not appear to be a marked difference in the gastric mucosal damage induced by tebufelone, alone, at 2 hr compared with 4 hr following single doses of the drug. In contrast, indomethacin given at doses which are approximately equipotent in

| Treatment         | Time (hours) | No. of lesions (means ± S.E.M.) | Severity of lesions** (means ± S.E.M.) | No. of animals |
|-------------------|--------------|---------------------------------|----------------------------------------|---------------|
| Control           | 1 ml H₂O     | 2                               | 0                                      | 0             |
|                   | 4            | 0                               | 0                                      | 0             |
| Indomethacin†     | 3 mg/kg      | 4                               | 7.7 ± 1.9*                             | 23.0 ± 9.5*   |
|                   | 15 mg/kg     | 2                               | 27.7 ± 3.2*                           | 68.0 ± 13.5*  |
|                   | 30 mg/kg     | 2                               | 39.0 ± 4.0*                           | 156 ± 46.2*   |
| Tebufelone        | 15 mg/kg     | 2                               | 0                                      | 0             |
|                   | 50 mg/kg     | 0                               | 0.8 ± 0.2                              | 0.4 ± 0.18    |
|                   | 250 mg/kg    | 0                               | 13.9 ± 1.1*                           | 47.4 ± 12.0*  |
|                   | 500 mg/kg    | 0                               | 1.2 ± 1.16                            | 4.7 ± 4.6     |
|                   | 15 mg/kg     | 4                               | 0                                      | 0             |
|                   | 50 mg/kg     | 4                               | 10.2 ± 1.0*                           | 34.2 ± 8.9*   |
|                   | 250 mg/kg    | 4                               | 13.1 ± 1.1*                           | 47.4 ± 12.0*  |
|                   | 500 mg/kg    | 4                               | 20.0 ± 3.4*                           | 62.7 ± 20.0*  |

* Denotes statistically significant increase in mucosal damage compared with controls (Mann-Whitney U-test). ** Severity of damage determined from average width of lesions in mm. † Peak lesion development with indomethacin occurs at 4 hr in this model.

anti-inflammatory activity with tebufelone (in the chronic adjuvant arthritis model in rats; S.P. Sirko, K.D. Rainsford, unpublished studies) at a dose of 3 mg/kg produced marked acute mucosal damage (Table 1). Furthermore, the dose response for acute gastric lesions was appreciably lower with the single dose treatment of tebufelone than with indomethacin (Table 1).

Following 5-day treatment there was more damage evident with 50 mg/kg tebufelone than after a single dose of this drug. No intestinal mucosal damage was evident following repeated doses of tebufelone for 5 days whereas indomethacin produced severe injury to the extent that at the high dose of 3 mg/kg/day all animals had died with peritonitis (Table 2).
Table 2. NSAIDs-induced gastric lesions in fasted arthritic rats: repeated daily dosing for 5 days.

| Treatment     | Dose       | N  | No of lesions | Severity* |
|---------------|------------|----|---------------|-----------|
| Tebufelone    | 15 mg/kg day | 6  | 0             | 0         |
|               | 50 mg/kg day | 6  | 4.9 (1.1)     | 33.3 (22.0) |
| Indomethacin  | 1 mg/kg day | 6  | 8.8 (1.4)     | 2.1 (3.0) |
|               | 3 mg/kg day | 6  | **in small intestine all grade 4.0+ dead with peritonitis** |

Values are means (standard errors). † Statistically significant increase in mucosal lesion numbers compared with controls (Mann Whitney U-test, p < .05). * Severity of gastric lesions was determined from the average width of lesions (mm). ** Severity of intestinal lesions graded 0-5.

II. Ulcer healing studies with new generation and reference NSAIDs

The 25 rats remained well during the total study period and had the same weight gain in all groups (290 ± 3 g at start and 315 ± 4 g at day 15).

Sequential analysis of ulcer healing by video endoscopy: The mean ulcer diameter on day 1 was 5.7 ± 0.1 mm. The initial ulcer did not show significant differences between the groups. In the placebo treated rats, healing rate was relatively rapid during days 3-8.

![Figure 1](image-url)

Figure 1. Ulcer healing curve assessed by video endoscopy. (a) The data indicate mean percentage residual ulcer size ± SEM over the 7 observation time points. (b) Residual ulcer size on day 15 in percentage of the initial ulcer size on day 1. Omeprazole (●) treated rats showed significant reduction of ulcer diameter during days 6-15. Compared with placebo (▲), tebufelone and indomethacin (●) significantly increased ulcer size which became progressively apparent during days 13-15 (tebufalone: 2 x 10 mg/kg (●); 2 x 1 mg/kg (●)). Indomethacin with omeprazole (Δ) showed significantly decreased ulcer size compared with placebo on days 8 and 10, but not on days 13 and 15. * p < .05 vs. placebo (ANOVA).
Then, healing velocity decreased during days 8-10, particularly during days 10-15. Omeprazole significantly accelerated ulcer healing rate during days 3-8 and showed a significantly decreased ulcer size compared with placebo from day 6 onward (Figures 1 and 2).

Tebufelone and indomethacin showed significantly increased ulcer size compared with placebo on day 15. Omeprazole reversed indomethacin-induced delay of ulcer healing to placebo healing speed (Figure 1).

![Figure 2. Ulcer healing rate-time curve.](image)

In addition to the ulcer healing curve, we assessed the ulcer healing rate in different phases of ulcer healing. These analyses showed that omeprazole accelerated ulcer healing in the early phase (days 3-8) while indomethacin and tebufelone delayed ulcer healing in the late phase (days 10-15) (Figure 2).

**Plasma gastrin level:** Gastrin plasma levels in fed rats were 160 ± 7.1 pmol/l before treatment. Gastrin plasma levels were not affected by treatment with any of the tested NSAIDs. Compared with placebo, plasma gastrin levels were increased by 3.4-fold in rats treated with omeprazole.

Additional preliminary ulcer healing studies: In placebo group, ulcer diameters on days 3, 8, and 15 were 3.8 ± 0.2, 2.2 ± 0.1, and 0.9 ± 0.1 mm, respectively. On day 15, ulcer size was significantly smaller in omeprazole and larger in indomethacin, diclofenac, and nitrofenac groups (Figure 1/Table 3); ex-vivo 6-keto-PG-F1α (prostaglandin generation in gastric mucosa and thromboxane B2 synthesis by blood) were significantly reduced by indomethacin, diclofenac, and nitrofenac (Table 3).
Figure 3. Gastric prostaglandin generation (6-keto-PG-F\textsubscript{2alpha}) Data are expressed as mean ± SEM. Compared with placebo, prostaglandin generation was significantly decreased by tebufalone and indomethacin. * p < .05 vs. placebo (ANOVA).

Table 3. Effects of NSAIDs on videoendoscopic ulcer size, prostanoid synthesis, and histologic healing parameters

|                         | Indomethacin | Indomethacin + Glyceryl Trinitrate | Diclofenac | Nitrofenac |
|-------------------------|--------------|-----------------------------------|------------|------------|
| Percentage change compared with placebo * |
| Videoendoscopic ulcer size on day 15 | + 81 % | + 77 % | +111 % | + 91 % |
| Gastric 6-keto-PG\textsubscript{F2alpha} | - 42 % | - 45 % | - 88 % | - 59 % |
| Thromboxane B\textsubscript{2} in blood | - 76 % | - 71 % | - 67 % | - 53 % |
| Angiogenesis in ulcer bed | - 55 % | - 44 % | - 59 % | - 53 % |
| Thickness of ulcer base | + 78 % | + 75 % | + 94 % | + 84 % |
| Gap between musc. mucosae | + 65 % | + 68 % | + 72 % | + 63 % |

* All values differ significantly from the placebo: p < .05 (ANOVA)

Ex vivo 6-keto-PG\textsubscript{F2alpha} prostaglandin generation in gastric mucosa and thromboxane B\textsubscript{2} synthesis by blood were significantly reduced by indomethacin, diclofenac, and nitrofenac.

DISCUSSION

The observations in this study on rats with an intact stomach suggest that tebufalone is less ulcerogenic than indomethacin when given at doses with equipotent anti-inflammatory activity. This is not only evident in the gastric mucosa but particularly for the small intestinal mucosa. The small bowel toxicity of indomethacin is likely to be related to the property of this drug to undergo enterohepatic recirculation since it is well established that this enhances ulcerogenicity in the small intestine [37]. In contrast, ulcer healing was significantly delayed by tebufalone both at daily doses of 2 and 20 mg/kg tebufalone with the high dose to a similar degree as by 1 mg/kg indomethacin, which is the highest dose with
can be repeatedly administered to rats, since higher doses almost invariably induce small bowel perforation [11]. In the ulcerogenicity studies, tebufelone 15 mg/kg did not show any evidence of mucosal damage in either the stomach or the intestinal tract after single or repeated daily dosing of the drug in a very sensitive model of NSAID-induced gastrointestinal damage [27].

Indeed both the new generation NSAIDs tebufelone and nitrofenac delayed ulcer healing to a similar degree as the conventional NSAIDs, indomethacin and diclofenac.

The ulcer healing studies were performed in a well validated chronic model. This is the only published ulcer healing model which allows direct assessment of the entire healing dynamics using the video endoscopy technique. The reference drugs, omeprazole and indomethacin, clearly and reproducibly modified the ulcer healing curve in an opposite manner. Profound acid inhibition by omeprazole predominantly accelerates ulcer healing during in the early healing phase, such as during days 3-8 [11, 38, 39], a finding which is consistent with the observation that histamine-induced hyperacidity delays ulcer healing in pigs only in the early phase [40]. Similarly profound acid inhibition with omeprazole accordingly accelerates ulcer healing predominantly during the early (first two weeks) healing phase [16]. Indomethacin interferes with ulcer healing predominantly in the early phase, but by contrast with omeprazole this modifies the ulcer healing curve only after a time-lag of 7-10 days [11, 39].

The reason why ulcerogenicity and interference with healing diverge with some NSAIDs is not entirely clear. The degree of inhibition of prostanoid synthesis did not significantly differ between tebufelone and the two conventional NSAIDs, indomethacin and diclofenac. Modification of tissue prostaglandin levels, however, predominantly affects mucosal protective mechanisms which are predominantly mediated by preservation of the microvascular system and/or stimulation of mucus-bicarbonate production. In contrast, ulcer healing is predominantly affected by the triad of interference with epithelial cell proliferation, angiogenesis, and proliferation and function of myofibroblasts. Indomethacin has been shown to delay ulcer healing by decreasing epithelial cell proliferation, angiogenesis in the ulcer bed, and interference with remodelling of the granulation tissue in the ulcer bed [11]. Since both drugs affect the late phase of ulcer healing, it seems likely that similar mechanisms are responsible for the delaying effect of tebufelone and indomethacin on ulcer healing.

Elliott et al. [20] have reported that the nitrite-oxide releasing compound, nitrofenac, accelerated healing of gastric ulcers in the rat using the acetic-acid model. It is, however, of particular interest that diclofenac which was administered at the same dose as used in this study did not delay ulcer healing in their model [39]. This, however, comes to no surprise since both diclofenac and nitrofenac were only applied in the second week after ulcer induction only for 7 days in a time period when NSAIDs interferes less with healing dynamics, while the delay is four-fold if the application is initiated shortly after ulcer induction and maintained for a two week period [39]. In our model indomethacin, diclofenac and nitrofenac similarly decreased gastric prostaglandin synthesis and thromboxane synthesis by the blood, delayed ulcer healing predominantly in the second week, and interfered with angiogenesis and maturation of granulation tissue in the ulcer base. Overall the deleterious effects in our model of both diclofenac alone or as in the esterified nitro-donor, nitrofenac, is more pronounced than the possible beneficial effects of nitric oxide.

In conclusion, in this reproducible chronic ulcer model, where reference drugs clearly affect the ulcer healing curve, the two new generation NSAIDs tested negatively interfere with gastric ulcer healing to a similar extent as indomethacin. The better performance of an NSAID in ulcerogenicity studies does not allow extrapolation of reduced interference with ulcer healing.
ACKNOWLEDGEMENTS: This study was sponsored by a joint grant from the Swiss National Science Foundation (No. 83BC-041834) and the British Council. The costs were covered in part by Procter & Gamble Pharmaceuticals, Cincinnati, Ohio, USA.

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