British Journal of Pharmacology (2000) 129, 681–686
www.nature.com/bjp

Wound collagen deposition in rats: effects of an NO-NSAID and a selective COX-2 inhibitor

[1]Marcelo N. Muscará, [1]Webb McKnight, [1]Samuel Asfaha & *,[1]John L. Wallace

[1]Department of Pharmacology & Therapeutics, University of Calgary, 3330 Hospital Drive NW, Calgary, AB, T2N 4N1, Canada

1 Selective cyclo-oxygenase (COX)-2 inhibitors and nitric oxide-releasing nonsteroidal anti-inflammatory drugs (NSAIDs) exhibit reduced toxicity in the gastrointestinal tract, but may affect wound healing in other tissues. In this study, we have compared the effects of a selective COX-2 inhibitor (celecoxib), a nitric-oxide releasing derivative of naproxen (HCT-3012) and naproxen in a model of wound collagen deposition in the rat.

2 Polyvinyl alcohol sponges were implanted subcutaneously in rats. The rats were treated daily for 5 days with the test drugs at equieffective anti-inflammatory doses.

3 Naproxen (10 mg kg^{-1}) significantly decreased (45%) collagen deposition at the wound site relative to the vehicle-treated control group. In contrast, HCT-3012 (14.5 mg kg^{-1}) significantly increased (62%) collagen deposition, while celecoxib (10 mg kg^{-1}) had no effect.

4 Naproxen and HCT-3012 suppressed prostaglandin (PG) E_2 levels at the wound site and whole blood thromboxane synthesis to similar degrees. Celecoxib had no significant effect on wound fluid PGE_2 levels, but slightly reduced whole blood thromboxane synthesis (by 17%).

5 COX-1 mRNA and protein were expressed in the wound exudate, the skin surrounding the wound and in normal skin. In contrast, COX-2 mRNA, but not protein, was expressed in wound and normal skin.

6 These results demonstrate that HCT-3012 can significantly enhance collagen deposition at a wound site, despite inhibiting prostaglandin synthesis to the same extent as the parent drug. Nitric oxide-releasing NSAIDs may represent a safer alternative to standard NSAIDs for use as anti-inflammatory and analgesic agents by post-surgery patients.
British Journal of Pharmacology (2000) **129,** 681–686

Keywords: Nitric oxide; nonsteroidal anti-inflammatory drug; wound healing

Abbreviations: COX, cyclo-oxygenase; mRNA, messenger ribonucleic acid; NSAID, nonsteroidal anti-inflammatory drug; PG, prostaglandin

Introduction

Nitric oxide has been implicated as a modulator of wound healing based on several observations. First, the beneficial effects of a diet rich in L-arginine on the healing and survival of injured rats (Seifter *et al*., 1978) have been shown to be mediated, at least in part, through nitric oxide (Schäffer *et al*., 1996; 1997; Konturek *et al*., 1993). Secondly, inhibitors of nitric oxide synthase can retard wound healing (Konturek *et al*., 1993; Schäffer *et al*., 1999). Thirdly, administration of nitric oxide donors or transfection of a wound with the gene for inducible nitric oxide synthase have beneficial effects on wound healing (Elliott *et al*., 1995; Thornton *et al*., 1998; Lund & Scholefield, 1997).

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to interfere with wound healing in several tissues (Armstrong & Blower, 1987; Lu *et al*., 1996; Haws *et al*., 1996; Dvivedi *et al*., 1997). Despite this, these drugs are widely used as post-surgery analgesics. The use of NSAIDs for long-term treatment is mainly limited by their toxicity in the gastrointestinal tract (Wallace, 1997). In recent years, a number of novel approaches have been taken to develop gastrointestinal-sparing NSAIDs. For example, selective inhibitors of cyclo-oxygenase-2 appear to have comparable anti-inflammatory effects to standard NSAIDs in many situations (Hawkey, 1999); however, by not inhibiting the isoform of cyclo-oxygenase that is normally expressed in the stomach (COX-1), these drugs have a reduced capacity to induce gastric ulceration. It should be noted that COX-2 is strongly induced at sites of gastrointestinal ulceration, and in such circumstances, selective inhibition of COX-2 has been shown to exacerbate ulceration and interfere with healing (Reuter *et al*., 1996; Mizuno *et al*., 1997). This raises the possibility that selective COX-2 inhibitors might exert inhibitory effects on wound healing in other tissues. A second approach to the development of gastrointestinal-sparing NSAIDs involves the coupling of a nitric oxide-releasing moiety to standard NSAIDs. These 'NO-NSAIDs' have comparable anti-inflammatory activity to the parent drugs, but have greatly reduced toxicity in the gastrointestinal tract (Wallace *et al*., 1994a,b). Given the beneficial effects of nitric oxide in wound healing, it is possible that a nitric oxide-releasing NSAID might have reduced detrimental effects on the healing of pre-existing wounds. Indeed, in a model of gastric ulceration, a nitric oxide-releasing derivative of diclofenac was found to significantly accelerate ulcer healing (Elliott *et al*., 1995).

Based on these observations, we decided to compare the effects of a standard NSAID (naproxen) to those of a selective COX-2 inhibitor (celecoxib) and a nitric oxide-releasing NSAID (HCT-3012) in a model of wound healing in the rat.

*Author for correspondence; E-mail: wallacej@ucalgary.ca

Methods

Animals

Male Wistar rats weighing approximately 250 g were obtained from Charles River Breeding Farms (Montreal, QC, Canada). They were housed in polypropylene cages in groups of three per cage, and received standard laboratory chow and tap water *ad libitum*. All protocols described below were approved by the Animal Care Committee of the University of Calgary.

Wound induction

The model of wound healing previously described by Barbul *et al*. (1985) was used. Briefly, the rats were anaesthetized by exposure to halothane vapour (5% in oxygen) and a 3 cm dorsal skin incision was made under sterile conditions. Subcutaneous pockets were created on both sides of the incision and three sterile, saline-moistened polyvinyl alcohol sponge disks (10 mm diameter $\times$ 4 mm thickness; Ivalon, KS, U.S.A.) were implanted. The wounds were closed with running 3-0 silk sutures and the rats were randomly assigned to one of the treatment groups, as described below.

Treatments

Groups of rats ($n=5-6$ each) received a daily oral dose of vehicle (1 ml kg^{-1}), naproxen (10 mg kg^{-1}), an equimolar dose of HCT-3012 (14.5 mg kg^{-1}), or celecoxib (10 mg kg^{-1}) for 5 days. The vehicle for the test drugs was 0.5% carboxymethylcellulose:DMSO (95 : 5, vol : vol). The choice of the doses of each test drug was based on their equivalence in terms of suppression of inflammatory prostaglandin production in the carrageenan-airpouch model, as described in detail previously (Wallace *et al*., 1999). Briefly, groups of five rats each were treated with naproxen (10 mg kg^{-1}), HCT-3012 (14.5 mg kg^{-1}), celecoxib (10 mg kg^{-1}) or vehicle orally 1 h prior to the injection of carrageenan (2 ml of a 1% w v^{-1} solution in sterile saline) into the airpouch. The exudate was collected 6 h later and the concentration of PGE_2 in the exudate was measured using an enzyme-linked immunosorbent assay (Wallace *et al*., 1999).

Wound collagen deposition

Five days after induction of the wounds, and 2 h after the last dose of the test drugs or vehicle, the rats were anaesthetized with sodium pentobarbitone (65 mg kg^{-1}; i.p.) and the wounds were opened. The sponges were harvested and squeezed with forceps in order to collect the wound exudate. The samples of exudate fluid were centrifuged (10 min at 2000 $\times$ *g*) and the supernatants frozen at -20°C. The three sponges from each rat were cleared of any adherent tissue and were then homogenized in 1 mL of HPLC-grade water. After centrifugation (10 min at 2000 $\times$ *g*) the liquid phase was stored at -20°C until analysed for hydroxyproline content as an index of collagen deposition (Woessner, 1961). Total protein content was also quantified in these samples by reaction with Coomassie blue (Bradford, 1976), using bovine serum albumin as the standard.

Serum nitrite/nitrate concentrations and whole blood thromboxane synthesis

After removing the sponges and the wound exudates, blood samples (2 mL) were collected from the abdominal descending aorta of each rat. The samples were allowed to clot at room temperature for 45 min, the sera were separated by centrifugation (10 min at 2000 $\times$ *g*) and kept at -20°C until analysed for their nitrite and nitrate concentrations (Muscará & de Nucci, 1996). Thromboxane B_2 levels in the serum were measured using an enzyme-linked immunosorbent assay (Wallace *et al*., 1999).

Wound fluid analysis

The wound exudates were analysed for their PGE_2 concentrations using a commercial enzyme-linked immunosorbent assay kit (Wallace *et al*., 1999). Nitrite and nitrate concentrations were analysed as above.

Gastric mucosal damage

After removal and processing of the sponges and blood sample collection, the stomachs were examined for macroscopically visible mucosa damage by an observer unaware of the treatment. This involved measuring the lengths (in mm) of all haemorrhagic lesions and calculating a gastric damage score, which was the sum of the lengths of all lesions in a stomach.

Expression of COX-1/COX-2 mRNA and protein

A separate group of five rats was submitted to the wound induction protocol. Five days later, the skin from the wound site was excised after removal of the sutures. In addition, a sample of adjacent, non-wounded skin was removed from each rat. The skin samples were divided in two for further analysis of COX-1 and COX-2 protein and mRNA expression by Western blotting and reverse-transcriptase polymerase chain reaction (RT–PCR), respectively. The wound fluids were collected and centrifuged as described above, and the cell pellets were subsequently lysed for COX-1 and COX-2 protein expression by Western blotting. The skin samples (150–200 mg) were placed in 2 ml of TRIzol reagent (GIBCO BRL, Gaithersburg, MD, U.S.A.). RNA was isolated according to previously described methods (Chomczynski & Sacchi, 1987). The RT–PCR method that we used and the sequences of the primers have been described in detail previously (Ferraz *et al*., 1997). For Western blots, the pellet of the wound exudate and the skin samples homogenized and centrifuged (14,000 $\times$ *g*, 2 min). The supernatants (20 μg protein) were subjected to 10% SDS–PAGE electrophoresis. The protein bands were further electro-transferred to nitrocellulose membrane and analysed for the presence of COX-1 and COX-2 by Western blot. For COX-1, the primary antibody was a polyclonal goat IgG anti-mouse COX-1 (Santa Cruz; dilution: 1 : 500) and the secondary antibody was a polyclonal rabbit anti-goat IgG coupled to horseradish peroxidase (Santa Cruz; dilution 1 : 6000). For COX-2, a monoclonal mouse IgG1 anti-rat COX-2 (Transduction Laboratories; diluted 1 : 250) was used as the primary antibody, and a polyclonal goat anti-mouse IgG coupled to horseradish peroxidase (Jackson Laboratories; diluted 1 : 6,000) as the secondary antibody. Immunoreactive bands were detected by chemiluminescence (ECL kit, Amersham, U.K.).

Statistical analysis

All data are shown as the mean ± s.e.mean. Comparisons among groups were performed using one-way analysis of variance followed by the Dunnett's Multiple Comparison test. Values of probability less than 5% ($P<0.05$) were considered significant.

Materials

Celecoxib and HCT-3012 were kindly provided by NicOx S.A. (Nice, France). The kits for measurement of PGE_2 and TXB_2 were obtained from Cayman Chemical Co. (Ann Arbor, MI, U.S.A.). All other drugs and reagents were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.) or VWR Scientific (Edmonton, AB, Canada).

Results

Carrageenan-induced airpouch inflammation

In order to confirm that the doses of the three test drugs used in the wound healing model were comparable, their effects on inflammatory prostaglandin production in the carrageenan-induced airpouch inflammation model were compared. As shown in Figure 1, naproxen, HCT-3012 and celecoxib each significantly inhibited the production of PGE_2 in the inflamed airpouch, with no significant differences among the three test drugs in terms of the magnitude of the inhibition.

Collagen deposition

Hydroxyproline content of the implanted sponges, an index of collagen deposition, was significantly reduced in rats treated with naproxen (Figure 2). On the other hand, treatment with HCT-3012 significantly increased collagen deposition relative to the vehicle-treated naproxen-treated groups. Treatment with celecoxib did not affect collagen deposition. There were no significant differences among these groups in terms of total protein in the sponges (data not shown).

Eicosanoid synthesis

The PGE_2 concentrations in wound exudates collected 2 h after the final dose of the test drugs were reduced by more than 65% ($P<0.01$) by both naproxen and HCT-3012 relative to the control group (Figure 3). Surprisingly, celecoxib did not significantly affect wound fluid PGE_2 concentrations.

Whole blood thromboxane synthesis was similarly inhibited (>90%; $P<0.001$) by naproxen and HCT-3012 (Figure 4). On the other hand, celecoxib treatment each day for 5 days

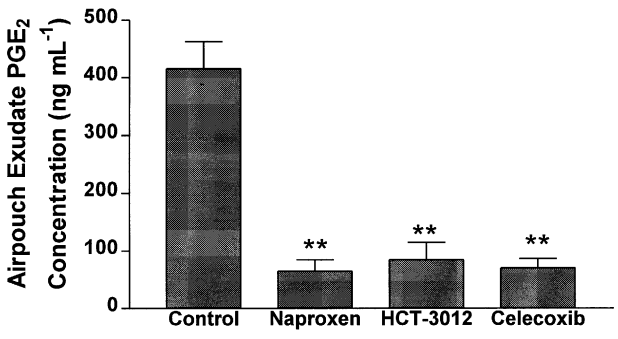

Figure 1 Inhibition of inflammatory prostaglandin E_2 synthesis in the carrageenan-airpouch model by naproxen (10 mg kg^{-1}), HCT-3012 (14.5 mg kg^{-1}) and celecoxib (10 mg kg^{-1}). $^{**}P<0.01$ versus the vehicle-treated group.

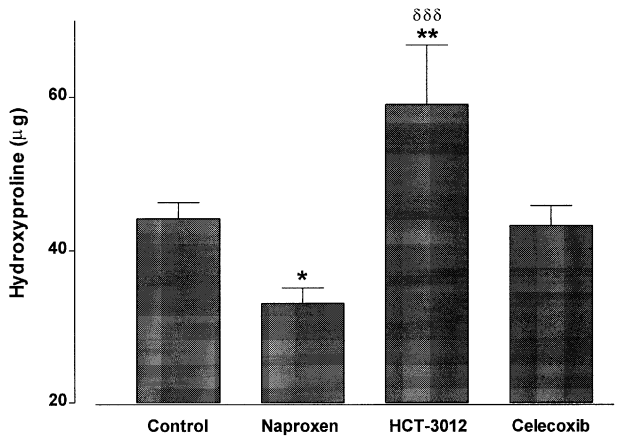

Figure 2 Hydroxyproline content in polyvinyl alcohol sponges harvested 5 days after subcutaneous implantation (2 h after the final dose of the test drug or vehicle). $^{*}P<0.05$, $^{**}P<0.01$ vs the control (vehicle-treated) group. $\delta\delta\delta P<0.001$ versus the naproxen-treated group.

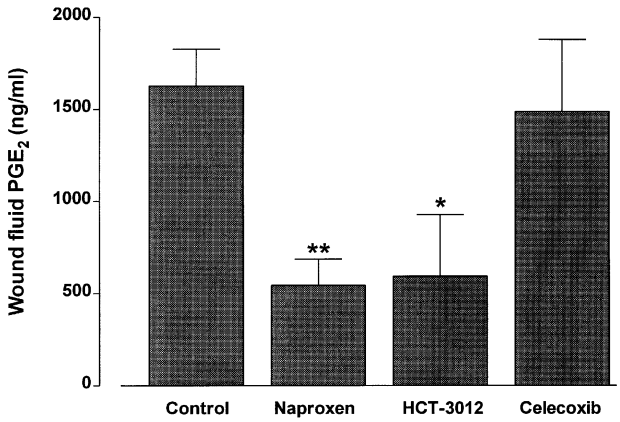

Figure 3 Prostaglandin E_2 concentrations in wound fluid samples obtained 5 days after implantation of polyvinyl alcohol sponges (2 h after the final dose of the test drug or vehicle). $^{*}P<0.05$, $^{**}P<0.01$ versus the control (vehicle-treated) group.

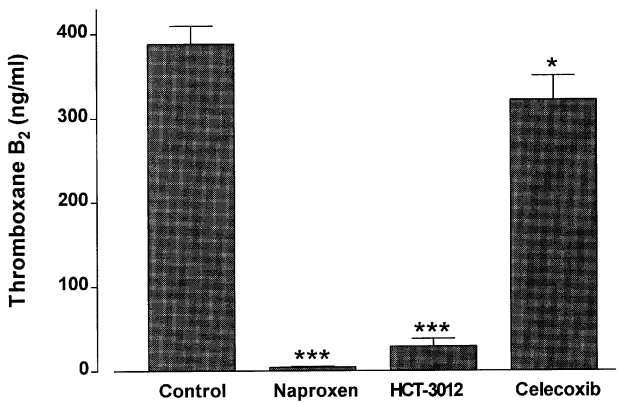

Figure 4 Whole blood thromboxane B_2 synthesis in rats treated for 5 days with vehicle, naproxen, HCT-3012 or celecoxib. The blood samples were taken 2 h after the final dose of the test drug or vehicle. $^{***}P<0.001$ versus the control (vehicle-treated) group.

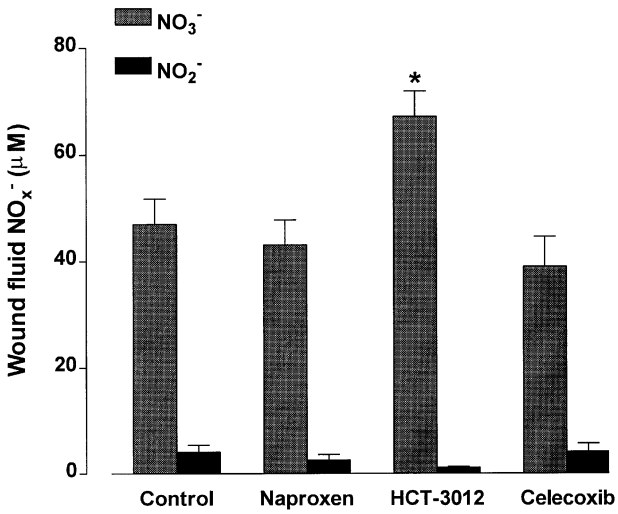

Figure 5 Nitrite (NO_2^-) and nitrate (NO_3^-) concentrations in wound fluid samples obtained 5 days after subcutaneous implantation of polyvinyl alcohol sponges (2 h after the final dose of the test drug or vehicle). $*P<0.05$ versus the control (vehicle-treated) group.

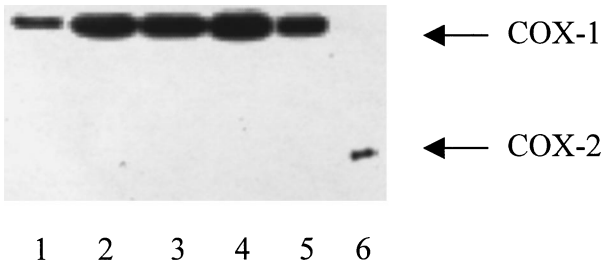

Figure 6 Western blots for COX-1 and COX-2 obtained from wound exudate cell pellet lysates (lanes 1–5; 20 μg protein). Lane 6: COX-2 positive control (LPS-activated macrophage lysate; 2 μg protein).

produced only a small, but statistically significant, reduction of whole blood thromboxane synthesis.

Nitrate and nitrite concentrations

None of the test drugs significantly affected the levels of nitrite in the wound exudate. (Figure 5). However, the levels of nitrate in the wound exudate were significantly increased in the HCT-3012 group. Neither naproxen nor celecoxib significantly affected nitrate levels in the wound exudate. Serum nitrate and nitrate levels were not significantly affected by any of the three test drugs (data not shown).

Gastric damage

No gastric mucosal damage was observed in any of the vehicle- or HCT-3012-treated rats. In contrast, seven of the 12 naproxen-treated rats exhibited haemorrhagic erosions in the corpus region of the stomach (mean damage score of 8.8 ± 3.5). Only one of the seven rats treated with celecoxib exhibited haemorrhagic erosions (mean damage score of 0.2 ± 0.2).

Expression of COX isoforms

Expression of COX-1 and COX-2 mRNA was significantly increased in skin samples from the wound site in comparison to the samples of normal skin (COX-1: 275 ± 23 versus 187 ± 25, respectively; COX-2: 1126 ± 272 versus 275 ± 47, respectively. Data are expressed in arbitrary densitometry units, normalized to GAPDH mRNA expression in each sample).

Western blotting revealed expression of COX-1, but not COX-2, in the wound exudate (Figure 6). Despite the observed increase in COX-2 mRNA expression, COX-2 protein was not detected in the wound or normal skin samples, while COX-1 was detectable in all samples of wound skin and normal skin. There was no significant difference in the expression of COX-1 protein between the samples of skin from the wound and those from normal skin. The lack of detection of COX-2 in the tissue and exudate samples was not due to inadequacies of the Western blot system that was employed. We were able to demonstrate detection of mouse macrophage COX-2 (Figure 6), and we have used the same Western blot system to demonstrate COX-2 protein in the rat colon (data not shown).

Discussion

NSAIDs are widely used for their analgesic and anti-inflammatory properties, but such use carries significant risks. This class of agents has well characterized inhibitory effects on wound healing in various tissues (Lu *et al*., 1996; Haws *et al*., 1996; Dvivedi *et al*., 1997), and in particular, can significantly retard the healing of ulcers in the stomach and duodenum (Armstrong & Blower, 1987), and exacerbate inflammation in the colon (Wallace *et al*., 1992). These effects of NSAIDs are most likely attributable to their ability to suppress prostaglandin synthesis, since exogenous prostaglandins can accelerate healing of wounds (Hatana *et al*., 1998; Talwar *et al*., 1996) and gastrointestinal ulcers (Roth *et al*., 1989; Allgayer *et al*., 1989). Interestingly, it is the "inducible" isoform of cyclooxygerase (COX-2) that appears to be the major source of prostaglandin synthesis in ulcerated and inflamed gastrointestinal tissue (Reuter *et al*., 1996; Mizuno *et al*., 1997). One of the aims of the present study, therefore, was to determine if a selective COX-2 inhibitor would alter wound healing, as measured by collagen deposition into subcutaneously implanted sponges. The results presented herein demonstrate that the selective COX-2 inhibitor, celecoxib, did not significantly affect collagen deposition, despite being given at a dose that was shown to markedly suppress inflammatory prostaglandin production in the carrageenan-airpouch model.

The lack of effect of celecoxib on collagen deposition may be attributable to the fact that it did not suppress prostaglandin synthesis at the site of the wound. Thus, it would appear that prostaglandin synthesis at the wound site occurred primarily *via* COX-1. The Western blot results are consistent with this conclusion, since there was no detectable COX-2 protein at the wound site, despite the observed increase in COX-2 mRNA expression in the skin at the wound sites. The results of the present study contrast with studies of gastric ulcer healing, where selective COX-2 inhibitors have been found to inhibit healing (Mizuno *et al*., 1997; Schmassmann *et al*., 1998). The divergent findings may be due to differences in the levels of bacterial colonization of the gastric ulcer, which is exposed to the substances ingested by the rat, versus the skin lesion that was produced under aseptic conditions. We have previously reported that gastric ulcers in the rat are rapidly colonized within hours of their induction (Elliott *et al*., 1998). It is possible that COX-2 would play a much more important role in wound healing in a setting where infection of the wound occurs.

Another objective of this study was to examine the effects of a nitric oxide-releasing NSAID on wound healing. HCT-3012

is a derivative of naproxen that has previously been shown to have comparable anti-inflammatory activity and enhanced analgesic activity relative to the parent drug (Davies *et al*., 1997). However, HCT-3012 has greatly reduced ulcerogenic activity in the stomach and small intestine (Davies *et al*., 1997). While naproxen was found to significantly decrease collagen deposition in the wound model (by 45%), HCT-3012 markedly increased collagen deposition (by 62%). Differences in the ability of naproxen and HCT-3012 to suppress cyclo-oxygenase activity did not explain the divergent effects on wound healing, as both drugs suppressed prostaglandin synthesis at the wound site to the same extent, as well as inhibiting whole blood thromboxane synthesis to the same extent. Moreover, these drugs were shown to suppress prostaglandin synthesis in the carrageenan-airpouch model to a similar degree as was seen with celecoxib. The beneficial effects of HCT-3012 in the wound healing model are most likely attributable to the nitric oxide that is released from this compound. Nitric oxide generation from NO-NSAIDs has been documented previously (Wallace *et al*., 1994a; Muscará *et al*., 1998), and the increased levels of nitrate at the wound site of rats treated with HCT-3012 are consistent with such release. Acceleration of wound healing by nitric oxide donors has been demonstrated previously (Lund & Scholefield, 1997). Indeed, accelerated healing of gastric ulcers was demonstrated in rats treated with a nitric oxide-releasing derivative of diclofenac, and a similar effect could be observed by treating the rats with glyceryl trinitrate (Elliott *et al*., 1995). The ability of nitric oxide to accelerate wound healing is further demonstrated by a recent report from Thornton *et al*. (1998). They demonstrated that collagen deposition was enhanced in wounded rats transfected *in vivo* with the gene for inducible nitric oxide synthase, and the increased nitric production within the wound milieu preceded the observed increase in collagen synthesis. Nitric oxide could contribute to wound healing in a number of ways, including stimulation of angiogenesis and stimulation of fibroblast synthetic function (Schaeffer *et al*., 1997).

NSAIDs are frequently used by patients in the period immediately following surgery. Clearly, the inhibitory effects of these drugs on wound healing represent a potential impediment to the patient's recovery. HCT-3012, and possibly other nitric oxide-releasing NSAIDs, might be useful alternatives to standard NSAIDs for use by patients in a post-surgical situation by virtue of their positive effects on collagen deposition, in addition to their increased gastrointestinal safety.

Dr Muscará is supported by a Merck Pharmacology Fellowship. Dr Wallace is a Medical Research Council of Canada (MRC) Senior Scientist and an Alberta Heritage Foundation for Medical Research Senior Scientist. This work was supported by a grant from the Medical Research Council of Canada.

References

ALLGAYER, H., DESCHRYVER, K. & STENSON, W.F. (1989). Treatment with 16,16′ dimethylprostaglandin E_2 before and after induction of colitis with trinitrobenzenesulfonic acid in rats decreases inflammation. *Gastroenterology,* **96,** 1290 – 1300.

ARMSTRONG, C.P. & BLOWER, A.L. (1987). Non-steroidal anti-inflammatory drugs and life threatening complications of peptic ulceration. *Gut,* **28,** 527 – 532.

BARBUL, A., FISHEL, R.S., SHIMAZU, S., WASSERKRUG, H.L., YOSHIMURA, N.N., TAO, R.C. & EFRON, G. (1985). Intravenous hyperalimentation with high arginine levels improves wound healing and immune function. *J. Surg. Res.,* **38,** 328 – 334.

BRADFORD, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.,* **72,** 248 – 254.

CHOMCZYNSKI, P. & SACCHI, N. (1987). Single-step method of RNA isolation by acid guanidiium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.,* **162,** 156 – 159.

DAVIES, N.M., ROSETH, A.G., APPLEYARD, C.B., MCKNIGHT, W., DEL SOLDATO, P., CALIGNANO, A., CIRINO, G. & WALLACE, J.L. (1997). NO-naproxen vs naproxen: ulcerogenic, analgesic and anti-inflammatory effects. *Aliment. Pharmacol. Ther.,* **11,** 69 – 79.

DVIVEDI, S., TIWARI, S.M. & SHARMA, A. (1997). Effect of ibuprofen and diclofenac sodium on experimental wound healing. *Indian J. Exp. Biol.,* **35,** 1243 – 1245.

ELLIOTT, S.N., BURET, A., MCKNIGHT, W., MILLER, M.J.S. & WALLACE, J.L. (1998). Bacteria rapidly colonize and delay the healing of gastric ulcers in rats. *Am. J. Physiol.,* **275,** G425 – G432.

ELLIOTT, S.N., MCKNIGHT, W., CIRINO, G. & WALLACE, J.L. (1995). A nitric oxide-releasing nonsteroidal anti-inflammatory drug accelerates gastric ulcer healing in rats. *Gastroenterology,* **109,** 524 – 530.

FERRAZ, J.P., SHARKEY, K.A., REUTER, B.K., ASFAHA, S., TIGLEY, A.W., BROWN, M.L., MCKNIGHT, W. & WALLACE, J.L. (1997). Induction of cyclooxygenase 1 and 2 in the rat stomach during endotoxemia: role in resistance to damage. *Gastroenterology,* **113,** 195 – 204.

HATANA, T., YOSHIDA, E., KAWANO, J., SUGIKI, M., ONITSUKA, T. & MARUYAMA, M. (1998). Prostaglandin I_2 analog enhances the expression of urokinase-type plasminogen activator and wound healing in cultured human fibroblast. *Biochim. Biophys. Acta.,* **1403,** 189 – 198.

HAWKEY, C.J. (1999). COX-2 inhibitors. *Lancet,* **353,** 307 – 314.

HAWS, M.J., KUCAN, J.O., ROTH, A.C., SUCHY, H. & BROWN, R.E. (1996). The effects of chronic ketorolac tromethamine (toradol) on wound healing. *Ann. Plast. Surg.,* **37,** 147 – 151.

KONTUREK, S.J., BRZOZOWSKI, T., MAJKA, J., PYTKO-POLONCZYK, J. & STACHURA, J. (1993). Inhibition of nitric oxide synthase delays healing of chronic gastric ulcers. *Eur. J. Pharmacol.,* **239,** 215 – 217.

LU, K.L., WEE, W.R., SAKAMOTO, T. & MCDONNELL, P.J. (1996). Comparison of in vitro antiproliferative effects of steroids and nonsteroidal antiinflammatory drugs on human keratocytes. *Cornea,* **15,** 185 – 190.

LUND, J.N. & SCHOLEFIELD, J.H. (1997). Glyceryl trinitrate is an effective treatment for anal fissure. *Dis. Colon Rectum,* **40,** 468 – 470.

MIZUNO, H., SAKAMOTO, C., MATSUDA, K., WADA, K., UCHIDA, T., NOGUCHI, H., AKAMATSU, T. & KASUGA, M. (1997). Induction of cyclooxygenase 2 in mucosal lesions and its inhibition by the specific antagonist delays healing in mice. *Gastroenterology,* **112,** 387 – 397.

MUSCARÁ, M.N. & DE NUCCI, G. (1996). Simultaneous determination of nitrite and nitrate anions in plasma, urine and cell culture supernatants by high-performance liquid chromatography with post column reactions. *J. Chromatogr. B. Biomed. Appl.,* **686,** 157 – 164.

MUSCARÁ, M.N., MCKNIGHT, W., DEL SOLDATO,P. & WALLACE, J.L. (1998). Effect of a nitric oxide-releasing naproxen derivative on hypertension and gastric damage induced by chronic nitric oxide inhibition in the rat. *Life Sci.,* **62,** PL235 – PL240.

REUTER, B.K., ASFAHA, S., BURET, A., SHARKEY, K.A. & WALLACE, J.L. (1996). Exacerbation of inflammation-associated colonic injury in rat through inhibition of cyclooxygenase-2. *J. Clin. Invest.,* **98,** 2076 – 2085.

ROTH, S., AGRAWAL, N., MAHOWALD, M., MONTOYA, H., ROBBINS, D., MILLER, S., NUTTING, E., WOODS, E., CRAGER, M. & NISSEN, C. (1989). Misoprostol heals gastroduodenal injury in patients with rheumatoid arthritis receiving aspirin. *Arch. Intern. Med.,* **149,** 775–779.

SCHÄFFER, M.R., EFRON, P.A., THORNTON, F.J., KLINGEL, K., GROSS, S.S. & BARBUL, A. (1997). Nitric oxide, an autocrine regulator of wound fibroblast synthetic function. *J. Immunol.,* **158,** 2375–2381.

SCHÄFFER, M.R., TANTRY, U., GROSS, S.S., WASSERKRUG, H.L. & BARBUL, A. (1996). Nitric oxide regulates wound healing. *J. Surg. Res.,* **63,** 237–240.

SCHÄFFER, M.R., TANTRY, U., THORNTON, F.J. & BARBUL, A. (1999). Inhibition of nitric oxide synthesis in wounds: pharmacology and effect on accumulation of collagen in wounds in mice. *Eur. J. Surg.,* **165,** 262–267.

SCHMASSMANN, A., PESKAR, B.M., STETTLER, C., NETZER, P., STROFF, T., FLOGERZI, B. & HALTER, F. (1998). Effects of inhibition of prostaglandin endoperoxide synthase-2 in gastrointestinal ulcer models in rats. *Br. J. Pharmacol.,* **123,** 795–804.

SEIFTER, E., RETTURA, G., BARBUL, A. & LEVENSON, S.M. (1978). Arginine: an essential amino acid for injured rats. *Surgery,* **84,** 224–230.

TALWAR, M., MOYANA, T.N., BHARADWAJ, B. & LAN, L.K. (1996). The effect of a synthetic analogue of prostaglandin E_2 on wound healing in rats. *Ann. Clin. Lab. Sci.,* **26,** 451–457.

THORNTON, F.J., SCHÄFFER, M.R., WITTE, M.B., MOLDAWER, L.L., MACKAY, S.L.D., ABOUHAMZE, A., TANNAHILL, C.L. & BARBUL, A. (1998). Enhanced collagen accumulation following direct transfection of the inducible nitric oxide synthase gene in cutaneous wounds. *Biochem. Biophys. Res. Commun.,* **246,** 654–659.

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

WALLACE, J.L., CHAPMAN, K. & MCKNIGHT, W. (1999). Limited anti-inflammatory efficacy of cyclooxygenase-2 inhibition in carrageenan-airpouch inflammation. *Br. J. Pharmacol.,* **126,** 1200–1204.

WALLACE, J.L., KEENAN, C.M., GALE, D. & SHOUPE, T.S. (1992). Exacerbation of experimental colitis by NSAIDs is not related to elevated leukotriene B_4 synthesis. *Gastroenterology,* **102,** 18–27.

WALLACE, J.L., REUTER, B.K., CICALA, C., MCKNIGHT, W., GRISHAM, M.B. & CIRINO, G. (1994a). Novel nonsteroidal anti-inflammatory drug derivative with markedly reduced ulcerogenic properties. *Gastroenterology,* **107,** 173–179.

WALLACE, J.L., REUTER, B.K., CICALA, C., MCKNIGHT, W., GRISHAM, M. & CIRINO, G. (1994b). A diclofenac derivative without ulcerogenic properties. *Eur. J. Pharmacol.,* **257,** 249–255.

WOESSNER, J.F. (1961). The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. *Arch. Biochem. Biophys.,* **93,** 440–447.

(Received November 1, 1999
Accepted November 22, 1999)