British Journal of Pharmacology (1999) 126, 1200–1204
http://www.stockton-press.co.uk/bjp

Limited anti-inflammatory efficacy of cyclo-oxygenase-2 inhibition in carrageenan-airpouch inflammation

*,[1]John L. Wallace, [1]Kevin Chapman & [1]Webb McKnight

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

1 Cyclo-oxygenase-2 (COX-2) is expressed at sites of inflammation and is believed to be the major source of inflammation-associated prostaglandin synthesis. Selective inhibition of COX-2 has been suggested to produce anti-inflammatory effects with reduced toxicity in the gastrointestinal tract. We examined the extent to which suppression of COX-2 led to inhibition of various components of inflammation in the carrageenan-airpouch model in the rat.

2 Indomethacin ($\geqslant$0.3 mg kg^{-1}), nimesulide ($\geqslant$3 mg kg^{-1}) and the selective COX-2 inhibitor, SC-58125 ($\geqslant$0.3 mg kg^{-1}), significantly suppressed the production of prostaglandin E2 at the site of inflammation. At higher doses, indomethacin ($\geqslant$1 mg kg^{-1}) and nimesulide (30 mg kg^{-1}), but not SC-58125 (up to 10 mg kg^{-1}), significantly inhibited COX-1 activity (as measured by whole blood thromboxane synthesis).

3 All three test drugs significantly reduced the volume of exudate in the airpouch, but only at doses greater than those required for substantial (>90%) suppression of COX-2 activity. Similarly, reduction of leukocyte infiltration was only observed with the doses of indomethacin and nimesulide that caused significant suppression of COX-1 activity.

4 SC-58125 did not significantly affect leukocyte infiltration into the airpouch at any dose tested (up to 10 mg kg^{-1}). A second selective COX-2 inhibitor, Dup-697, was also found to suppress exudate PGE_2 levels without significant effects on leukocyte infiltration.

5 These results indicate that selective inhibition of COX-2 results in profound suppression of PGE_2 synthesis in the carrageenan-airpouch, but does not affect leukocyte infiltration. Exudate volume was only reduced with the highly selective COX-2 inhibitor when a dose far above that necessary for suppression of COX-2 activity was used. Inhibition of leukocyte infiltration was observed with indomethacin and nimesulide, but only at doses that inhibited both COX-1 and COX-2.

Keywords: Cyclo-oxygenase; prostaglandins; inflammation; COX-2

Abbreviations: COX, cyclo-oxygenase; NSAIDs, nonsteroidal anti-inflammatory drugs

Introduction

The confirmation of the existence of a second isoform of cyclo-oxygenase (COX) (Kujubu *et al*., 1991; Xie *et al*., 1991) led shortly thereafter to the development of selective inhibitors of this enzyme (Chan *et al*., 1995; Seibert *et al*., 1994).COX-2 inhibitors have been suggested to be as effective as nonsteroidal anti-inflammatory drugs (NSAIDs) in reducing inflammation and pain, but to spare the gastrointestinal tract of injury (Chan *et al*., 1995; Masferrer *et al*., 1994; Seibert *et al*., 1994). This proposal is based on the findings that COX-2 is strongly induced at sites of inflammation, while COX-1 is the predominant isoform of the enzyme expressed in the normal gastrointestinal tract (Kargman *et al*., 1996; Vane *et al*., 1994).

In recent years, a number of studies have suggested that the original description of the roles of COX-1 and COX-2 was overly simplistic. For example, several studies have demonstrated that COX-2 plays an important role in gastrointestinal mucosal defence in certain circumstances, such as when the mucosa is inflamed or ulcerated (Mizuno *et al*., 1997; Reuter *et al*., 1996; Schmassman *et al*., 1998). In these circumstances, COX-2 inhibitors can delay the healing of ulcerated tissue and exacerbate the mucosal inflammation. On the other hand, there is evidence that in some circumstances, COX-1 produces prostaglandins that contribute to inflammation. For example, carrageenan-induced inflammation in the rat or mouse paw is mediated to a considerable extent by COX-1 (Wallace *et al*., 1998), while the prostaglandins produced in human bursitis are derived mainly from COX-1 (Gretzer *et al*., 1998). It is possible that these represent rare examples, and that other models of inflammation and other human inflammatory conditions do not include a COX-1-mediated component.

The airpouch model in rodents has been extensively used to assess effects of experimental drugs, including COX-2 inhibitors (Edwards *et al*., 1981; Gilroy *et al*., 1998; Sedgwick *et al*., 1985; Seibert *et al*., 1994). The airpouch lining has been reported to bear histological similarity to synovial membranes (Edwards *et al*., 1981) and when challenged *via* an injection of carrageenan, to mount an inflammatory reaction histologically similar to that observed in chronic synovial inflammation (Sedgwick *et al*., 1985). The prostaglandins that can be recovered from the airpouch following injection of carrageenan are derived primarily from COX-2 (Seibert *et al*., 1994). In the present study, we used the carrageenan-airpouch model in the rat to investigate the effectiveness of a selective inhibitor of COX-2 (SC-58125) to prevent various components of the inflammatory reaction (leukocyte infiltration, exudate volume). SC-58125 has been reported to be 100 fold more potent as an inhibitor, *in vitro*, of COX-2 than of COX-1 (Seibert *et al*., 1994). Furthermore, we measured the effects of this drug on prostaglandin production at the site of inflammation (COX-2 activity) and systemic COX-1 activity. For compar-

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

ison, we also assessed the effects of a nonselective COX inhibitor (indomethacin) and a modestly selective COX-2 inhibitor (nimesulide). Nimesulide has been reported to be 21-times more potent as an inhibitor, *in vitro*, of COX-2 than of COX-1 (Nakatsugi *et al*., 1996).

Methods

Animals

Male, Wistar rats weighing 200–225 g were obtained from Charles River Breeding Farms (Montreal, Canada) and were housed in the Animal Care Service of the University of Calgary. The rats were kept in plastic-bottomed cages and fed standard laboratory chow and water *ad libitum*. For 18 h prior to an experiment, the rats were deprived of food but not water. All experimental procedures described below were approved by the Animal Care Committee of the University of Calgary and were in accordance with the guidelines of the Canadian Council on Animal Care.

Carrageenan-airpouch model

An airpouch was induced as described in detail previously (Edwards *et al*., 1981; Sedgewick *et al*., 1985; Seibert *et al*., 1994). Briefly, 20 ml of air was injected subcutaneously on the back of the rat on the first day. Two days later, another 10 ml of air was injected at the same site. On the fifth day after the first injection, a further 10 ml of air was injected into the pouch. Twenty-four hours later, carrageenan (2 ml of a 1% w v^{-1} solution in sterile saline) was injected into the airpouch. All of the injections were performed after the rats had been anaesthetized with 5% (v v^{-1}) halothane. Six hours after the carrageenan injection, the rats were anaesthetized with 5% (v v^{-1}) halothane and the pouch was carefully opened by a small incision. The exudate was collected and transferred to a sterile tube. The volume of the exudate was measured gravimetrically. An aliquot of the exudate was used for quantification of leukocyte concentration using a Coulter counter (model Z1). An aliquot of the exudate was applied to glass slides and stained with Wright's stain for characterization of the cellular infiltrate. Another aliquot was frozen on dry ice and stored at $-20^{\circ}C$ for subsequent measurement of prostaglandin E_2 concentration using a specific ELISA (Wallace *et al*., 1998). A sample of blood was drawn from the inferior vena cava for measurement of whole blood thromboxane synthesis, as an index of COX-1 activity (Wallace *et al*., 1998). In one group of rats ($n=5$), sterile saline (0.9% w v^{-1}; 2 ml) was injected into the airpouch instead of carrageenan.

Test drugs

One hour prior to injection of carrageenan into the airpouch, the rats ($n=5$ per group) were orally pretreated with vehicle (1% carboxymethylcellulose) or one of the test drugs (indomethacin, nimesulide or SC-58125) at doses ranging from 0.1–30 mg kg^{-1}. The highest dose of SC-58125 tested was 10 mg kg^{-1}, due to a limited supply of this compound. One group of rats was treated with a combination of indomethacin (1 mg kg^{-1}) and SC-58125 (10 mg kg^{-1}). Another series of experiments were performed in which the rats received dexamethasone (1 mg kg^{-1}; $n=5$) or vehicle (sterile saline; $n=5$) subcutaneously 2 h prior to injection of carrageenan into the airpouch.

Chemicals

The ELISA kits for TXB_2 and PGE_2 were obtained from Caymen Chemical Co. (Ann Arbor, MI, U.S.A.). Indomethacin, nimesulide, dexamethasone and lambda carrageenan were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). SC-58125 and DuP-697 were obtained from DuPont-Merck. (Wilmington, DE, U.S.A.). All other reagents were obtained from VWR Scientific (Edmonton, AB, Canada).

Statistical analysis

All data are expressed as the mean $\pm$ s.e.mean. Comparisons among groups of data were made using a one-way analysis of variance followed by the Dunnett Multiple Comparisons test. An associated probability (P value) of less than 5% was considered significant.

Results

Carrageenan-induced inflammation

Injection of carrageenan into the airpouch resulted in accumulation of a mean of $27.4 \pm 4.9 \times 10^6$ cells, significantly ($P<0.001$) greater than that observed when sterile saline was injected ($3.1 \pm 0.6 \times 10^6$ cells). The vast majority of the cells (>90%) were neutrophils. The volume of exudate 6 h following carrageenan injection was also significantly elevated above that observed following saline injection (2.37 ± 0.04 ml versus 0.81 ± 0.05 ml, respectively; $P<0.001$). The mean prostaglandin E_2 concentration in the exudate from rats in which saline was injected into the airpouch was 17 ± 2 ng ml^{-1}. In rats in which carrageenan was injected into the airpouch, the mean PGE_2 concentration was 621 ± 138 ng ml^{-1} ($P<0.001$).

Effects of inhibitors on prostanoid synthesis

Indomethacin significantly reduced exudate PGE_2 concentrations at doses of 0.3 mg kg^{-1} or greater (Figure 1). Significant suppression of whole blood thromboxane synthesis (COX-1 activity) was observed with doses of indomethacin of $\geqslant 1$ mg kg^{-1}. Nimesulide significantly reduced exudate PGE_2 levels when given at 1 mg kg^{-1} or greater, while significant inhibition of COX-1 was only observed with the 30 mg kg^{-1} dose. SC-58125 significantly reduced exudate PGE_2 concentrations at doses of 0.3 mg kg^{-1} or greater, but had no effect on COX-1 activity at any of the doses tested. Dexamethasone pretreatment resulted in almost complete suppression of the exudate PGE_2 concentrations (27 ± 9 ng ml^{-1} versus 621 ± 138 ng ml^{-1} in the vehicle-treated group; $P<0.05$).

Effects of inhibitors on leukocyte infiltration

Indomethacin significantly reduced the numbers of leukocytes in the exudate at doses of $\geqslant 1$ mg kg^{-1} (Figure 1). Nimesulide only produced a significant reduction of leukocyte infiltration at the 30 mg kg^{-1}, while SC-58125 did not significantly affect leukocyte infiltration into the airpouch at any dose tested. Nimesulide and indomethacin, at the highest doses tested, reduced leukocyte infiltration by approximately 50 and 70%. For comparison, dexamethasone (1 mg kg^{-1}) reduced the infiltration of leukocytes from an average of $27.4 \pm 4.9 \times 10^{-6}$ cells in vehicle-treated rats to $5.4 \pm 1.4 \times 10^6$ cells (80% inhibition). The fact that SC-58125 could cause nearly

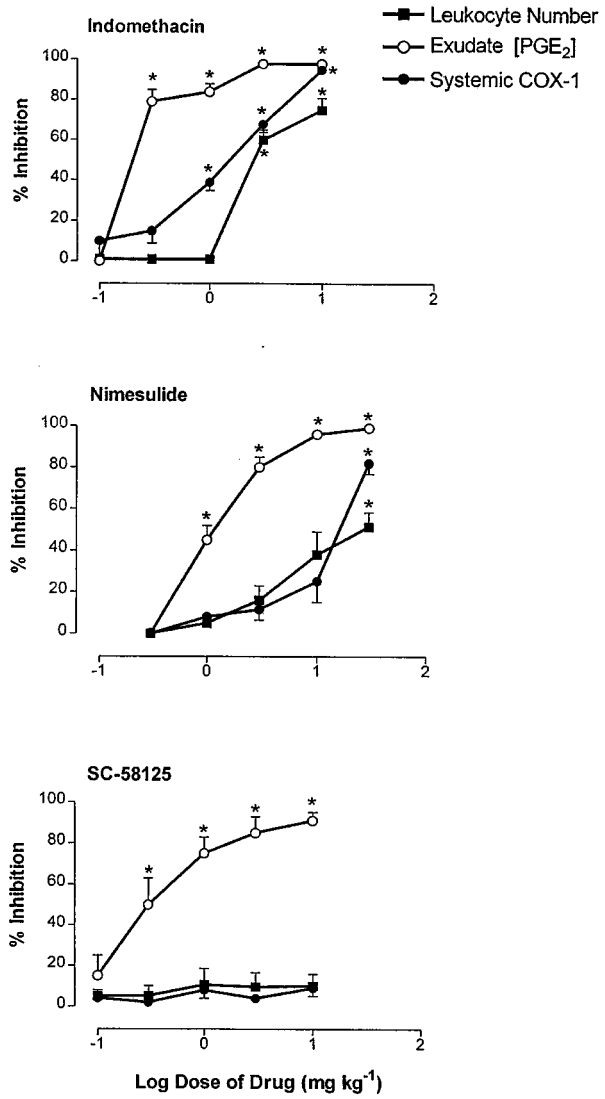

Figure 1 Effects of indomethacin, nimesulide and SC-58125 on carrageenan-induced airpouch inflammation in the rats. Endpoints included the numbers of leukocytes in the exudate, the concentration of prostaglandin E_2 in the exudate and whole blood thromboxane B_2 synthesis, as an index of COX-1 activity. Results are expressed as the mean ± s.e.mean per cent inhibition relative to a vehicle-treated control group. Asterisks signify significant differences ($P<0.05$) from the vehicle-treated control group.

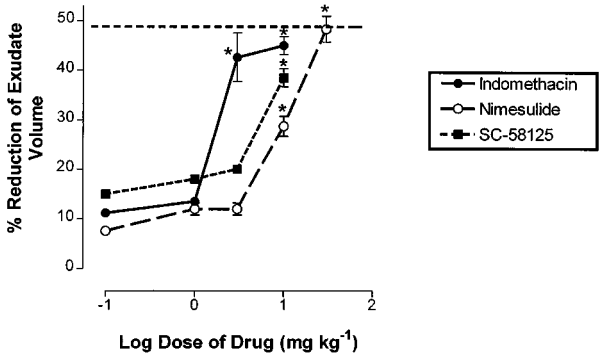

Figure 2 Effects of indomethacin, nimesulide and SC-58125 on exudate volume in carrageenan-induced airpouch inflammation in rats. Results are expressed as the mean ± s.e.mean per cent reduction relative to vehicle-treated controls. The dotted line represents the mean reduction of exudate volume observed in rats pretreated with dexamethasone (1 mg kg^{-1}). Asterisks signify significant differences ($P<0.05$) from the vehicle-treated control group.

complete inhibition of prostaglandin synthesis in the airpouch without affecting COX-1 activity is consistent with a previous report that the production of prostaglandins in this model occurred through COX-2 (Seibert *et al.*, 1994).

Effects of inhibitors on exudate volume

Indomethacin significantly reduced the volume of the exudate recovered from the airpouch at doses of 3 and 10 mg kg^{-1} (Figure 2). Nimesulide significantly reduced the exudate volume at doses of 10 and 30 mg kg^{-1}. SC-58125 significantly reduced the exudate volume at the highest dose tested (10 mg kg^{-1}). The reduction of exudate volume achieved with the highest dose of each test drug was comparable to the approximately 50% reduction observed in rats pretreated with dexamethasone (1 mg kg^{-1}).

Effects of indomethacin plus SC-58125

Figure 3 illustrates the effects of indomethacin (1 mg kg^{-1}) and SC-58125 (10 mg kg^{-1}), alone and in combination, on COX-1 activity, exudate PGE_2 levels and leukocyte infiltration. The combination of indomethacin and SC-58125, unlike either drug given alone, significantly reduced leukocyte infiltration into the airpouch. However, the combination of the two drugs did not produce greater inhibition of exudate PGE_2, nor did the combination produce a greater reduction of systemic COX-1 activity than was observed with indomethacin alone.

Effects of DuP-697

It is possible that the lack of effect of SC-58125 on leukocyte infiltration into the airpouch is not a generalized feature of selective COX-2 inhibitors; rather, this may be a feature unique to this particular COX-2 inhibitor. To test this hypothesis, studies were performed in which rats were pretreated with another selective COX-2 inhibitor, DuP-697, at doses of 1 or 10 mg kg^{-1} (i.p.; $n=5$ per group) 1 h prior to carrageenan administration. DuP-697 has been reported to be 120 fold more potent as an inhibitor of COX-2 than of COX-1 (Leblanc *et al.*, 1995). As was the case with SC-58125, DuP-697 was able to significantly inhibit exudate PGE_2 concentrations without any effect on leukocyte infiltration (Figure 4). Neither dose of DuP-697 significantly affected whole blood thromboxane synthesis (data not shown).

Discussion

There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation and leukocyte infiltration represent two such components of inflammation. In the carrageenan-airpouch model, both of these could be significantly suppressed by a glucocorticoid (dexamethasone) or by a standard NSAID (indomethacin). In contrast, SC-58125, a selective inhibitor of COX-2 (Seibert *et al.*, 1994), had no effect on leukocyte infiltration and only reduced exudate volume at the highest dose tested. However, SC-58125 was very effective at reducing prostaglandin synthesis at the site of inflammation, significantly reducing exudate PGE_2 concentrations at a dose as low as 0.3 mg kg^{-1}. The selectivity of this compound for COX-2 was confirmed by the lack of detectable effects on systemic COX-1 activity (whole blood thromboxane synthesis) at doses of up to 10 mg kg^{-1}. Nimesulide, a modestly selective

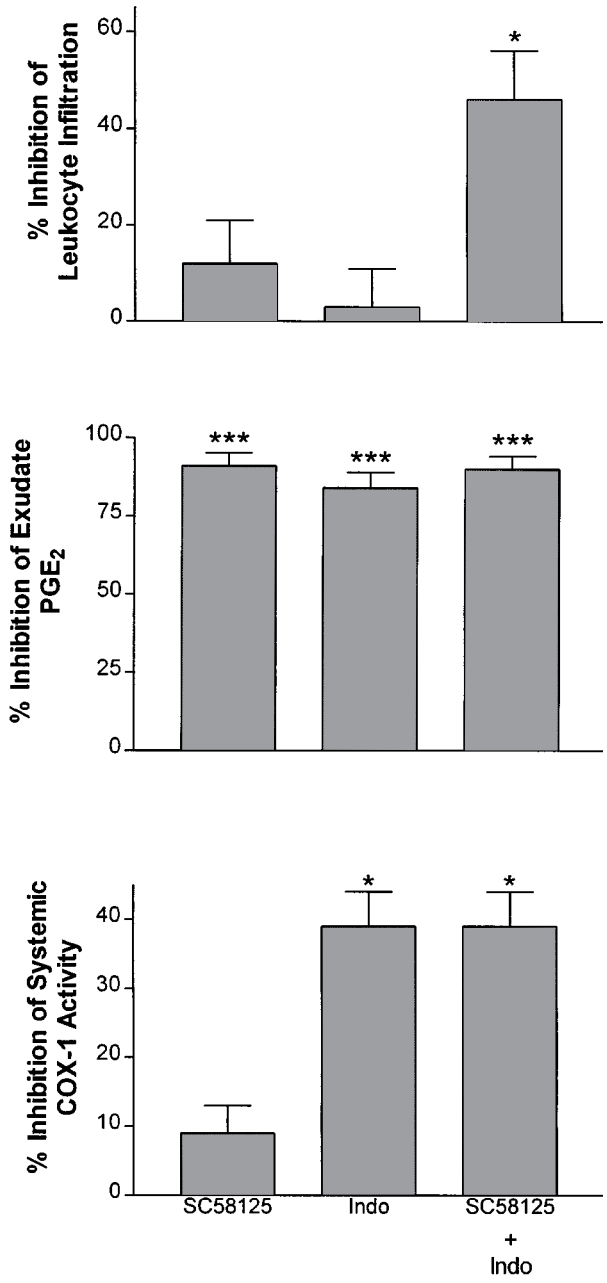

Figure 3 Effects of indomethacin (1 mg kg^{-1}) and SC-58125 (10 mg kg^{-1}), alone or in combination, on leukocyte infiltration, exudate PGE_2 concentration and systemic COX-1 activity in carrageenan-induced airpouch inflammation in rats. Results are expressed as the mean$\pm$s.e.mean per cent reduction relative to vehicle-treated controls. *$P<0.05$, ***$P<0.001$ compared to vehicle-treated controls.

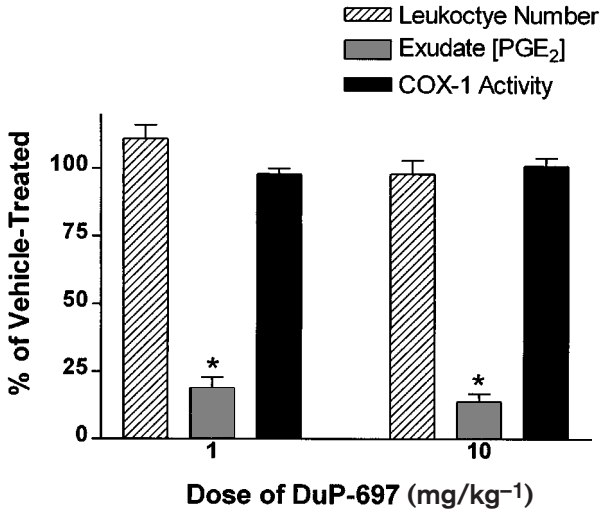

Figure 4 Effects of DuP-697 (1 or 10 mg kg^{-1} i.p.) on leukocyte infiltration, exudate PGE_2 concentration and systemic COX-1 activity in carrageenan-induced airpouch inflammation in rats. Results are expressed as the mean$\pm$s.e.mean per cent reduction relative to vehicle-treated controls. *$P<0.05$ compared to the vehicle-treated group.

COX-2 inhibitor (Famaey, 1997), was found to inhibit both leukocyte infiltration and oedema formation (exudate volume) in the carrageenan-airpouch. However, these effects were only observed at doses above those necessary for inhibition of COX-2, where significant suppression of COX-1 was also observed. The inability of selective COX-2 inhibition to result in suppression of leukocyte infiltration was confirmed with a second selective COX-2 inhibitor, DuP-697.

These results suggest that suppression of COX-2 is insufficient to produce the full range of anti-inflammatory effects in the carrageenan-airpouch model observed with standard NSAIDs. Moreover, the results suggest that COX-1 contributes to inflammation in this model. COX-1 has been suggested to contribute to inflammation in other experimental models (Gilroy *et al*., 1998; Wallace *et al*., 1998), and in human bursitis (Gretzer *et al*., 1998). Of course, NSAIDs may exhibit anti-inflammatory activities unrelated to their effects on prostaglandin synthesis (Abramson *et al*., 1994). In this regard, it is noteworthy that a combination of indomethacin (1 mg kg^{-1}) and SC-58125 (10 mg kg^{-1}) was effective in reducing leukocyte infiltration into the airpouch, while neither drug was effective when given alone at these doses. The observation that the combination of these two drugs did not produce greater suppression of COX-1 or COX-2 than that achieved with the individual drugs suggests that the inhibition of leukocyte infiltration may have been due to COX-independent effects of one or both of these drugs.

The development of selective COX-2 inhibitors is based on the notion that the prostaglandins produced at sites of inflammation are derived exclusively from this isoenzyme. In some studies there is evidence that selective COX-2 suppression is sufficient for anti-inflammatory and analgesic effects (Boyce *et al*., 1994; Chan *et al*., 1995). In other studies, however, the doses of the COX-2 inhibitor required for anti-inflammatory and/or analgesic effects were found to be much greater than those required for suppression of COX-2 activity (Penning *et al*., 1997; Seibert *et al*., 1994; Wallace *et al*., 1998). Of course, this raises the possibility that the ability of the COX-2 inhibitor to reduce pain or inflammation was due to suppression of COX-1 or to COX-unrelated activities of the compounds. Such findings also raise concerns about the efficacy of highly selective COX-2 inhibitors and about the potential toxicity of modestly selective COX-2 inhibitors when used at doses where suppression of COX-1 is produced. It is noteworthy that both nimesulide and NS-398 were shown to cause gastric damage in the rat when given at doses required for significant anti-inflammatory activity in the carrageenan-induced paw oedema model (Wallace *et al*., 1998).

In conclusion, the results of the present study demonstrate that selective suppression of COX-2, with either SC-58125 or DuP-697, does not result in significant inhibition of leukocyte infiltration in the carrageenan-airpouch model. In contrast, a standard NSAID or a modestly selective COX-2 inhibitor were

capable of inhibiting leukocyte infiltration, but only when given at doses where significant suppression of COX-1 was observed. These studies suggest that COX-1 contributes to inflammation in this model, and that effects of NSAIDs unrelated to suppression of COX could also contribute to their anti-inflammatory activity. Selective inhibition of COX-2 may not be sufficient to produce the full range of anti-inflammatory activities associated with standard NSAIDs.

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

References

ABRAMSON, S.B., LESZCZYNSKA-PIZIAK, J., CLANCY, R.M., PHILIPS, M. & WEISSMANN, G. (1994). Inhibition of neutrophil function by aspirin-like drugs (NSAIDs): Requirement for assembly of heterotrimeric G proteins in bilayer phospholipid. *Biochem. Pharmacol.,* **47,** 563 – 572.

BOYCE, S., CHAN, C.-C., GORDON, R., LI, C.S., RODGER, I.W., WEBB, J.K., RUPNIAK, N.M.J. & HILL, R.G. (1994). L-745,337: a selective inhibitor of cyclooxygenase-2 elicits antinociception but not gastric ulceration in rats. *Neuropharmacology,* **33,** 1609 – 1611.

CHAN, C.-C., BOYCE, S., BRIDEAU, C., FORD-HUTCHINSON, A.W., GORDON, R., GUAY, D., HILL, R.G., LI, C.S., MANCINI, J., PENNETON, M., PRASIT, P., RASORI, R., REINDEAU, D., ROY, P., TAGARI, P., VICKERS, P., WONG, E. & RODGER, I.W. (1995). Pharmacology of a selective cyclooxygenase-2 inhibitor, L-745,337: A novel nonsteroidal anti-inflammatory agent with an ulcerogenic sparing effect in rat and nonhuman primate stomach. *J. Pharmacol. Exp. Ther.,* **274:** 1531 – 1537.

EDWARDS, J.C.W., SEDGWICK, A.D. & WILLOUGHY, D.A. (1981). The formation of a structure with features of synovial lining by subcutaneous injection of air: an in vivo tissue culture system. *J. Pathol.,* **134:** 147 – 156.

FAMAEY, J.P. (1997). In vitro and in vivo pharmacological evidence of selective cyclooxygenase-2 inhibition by nimesulide: an overview. *Inflamm. Res.,* **46,** 437 – 446.

GILROY, D.W., TOMLINSON, A. & WILLOUGHBY, D.A. (1998). Differential effects of inhibition of isoforms of cyclooxygenase (COX-1, COX-2) in chronic inflammation. *Inflamm. Res.,* **47,** 79 – 85.

GRETZER, B., KNORTH, H., CHANTRAIN, M., BARBERA, L., WILLBURGER, R.E., WITTENBERG, R.H. & PESKAR, B.M. (1998). Effects of diclofenac and L-745,337, a selective cyclooxygenase-2 inhibitor, on prostaglandin E_2 formation in tissue from human colonic mucosa and chronic bursitis. *Gastroenterology,* **114,** A139.

KARGMAN, S., CHARLESON, S., CARTWRIGHT, M., FRANK, J., RIEANDEAU, D., MANCINI, J., EVANS, J. & O'NEILL, G. (1996). Characterization of prostaglandin G/H synthase 1 and 2 in rat, dog, monkey, and human gastrointestinal tracts. *Gastroenterology,* **111,** 445 – 454.

KUJUBU, D.A., FLETCHER, B.S., VARNUM, B.C., LIM, R.W. & HERSCHMAN, H.R. (1991). TIS10, a phorbol ester tumor promoter-inducible mRNA from swiss eTe cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue. *J. Biol. Chem.,* **266,** 12866 – 12872.

LEBLANC, Y., GAUTHIER, J.Y., ETHIER, D., GUAY, J., MANCINI, J., RIENDEAU, D., TAGARI, P., VICKERS, P., WONG, E. & PRASIT, P. (1995). Synthesis and biological evaluation of 2,3-diarylthiophenes as selective COX-2 and COX-1 inhibitors. *Bioorg. Med. Chem. Letters,* **5,** 2123 – 2128.

MASFERRER, J.L., ZWEIFEL, B.S., MANNING, P.T., HAUSER, S.D., LEAHY, K.M., SMITH, W.G., ISAKSON, P.C., SEIBERT, K. (1994). Selective inhibition of inducible cyclooxygenase 2 in vivo is antiinflammatory and nonulcerogenic. *Proc. Natl. Acad. Sci. U.S.A.,* **91,** 3228 – 3232.

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

NAKATSUGI, S., TERADA, N., YOSHIMURA, T., HORIE, Y. & FURUKAWA, M. (1996). Effects of nimesulide, a preferential cyclooxygenase-2 inhibitor, on carrageenan-induced pleurisy and stress-induced gastric lesions in rats. *Prost. Leuko. Essent. Fatty Acids,* **55,** 395 – 402.

PENNING, T.D., TALLEY, J.J., BERTENSHAW, S.R., CARTER, J.S., COLLINS, P.W., DOCTER, S., GRANETO, M.J., LEE, L.F., MALECHA, J.W., MIYASHIRO, J.M., ROGERS, R.S., ROGIER, D.J., YU, S.S., ANDERSON, G.D., BURTON, E.G., COGBURN, J.N., GREGORY, S.A., KOBOLDT, C.M., PERKINS, W.E., SEIBERT, K., VEENHIZEN, A.W., ZHANG, Y.Y. & ISAKSON, P.C. (1997). Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase 2 inhibitors: identification of 4-[5-94-methylphenyl)-3-9 trifluoromethyl)-1H-pyrazol-1-yl]benzensulfonamide (SC 58635, celecoxib). *J. Med. Chem.,* **140,** 1347 – 1365.

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.

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 chronic gastro-intestinal ulcer models in rats. *Br. J. Pharmacol.,* **123,** 795 – 804.

SEDGWICK, A.D., MOORE, A.R., AL-DUAIJ, A.Y., EDWARDS, J.C. & WILLOUGHBY, D.A. (1985). Studies into the influence of carrageenan-induced inflammation on articular cartilage degradation using implantation into air pouches. *Br. J. Exp. Pathol.,* **66,** 445 – 453.

SEIBERT, K., ZHANG, Y., LEAHY, K., HAUSER, S., MASFERRER, J., PERKINS, W., LEE, L. & ISAKSON, P. (1994). Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. *Proc. Natl. Acad. Sci. U.S.A.,* **91,** 12013 – 12017.

VANE, J.R., MITCHELL, J.A., APPLETON, I., TOMLINSON, A., BISHOP-BAILEY, D., CROXTALL, J. & WILLOUGHBY, D.A. (1994). Inducible isoforms of cyclooxygenase and nitric-oxide synthase in inflammation. *Proc. Natl. Acad. Sci. U.S.A.,* **91,** 2046 – 2050.

WALLACE, J.L., BAK, A., MCKNIGHT, W., ASFAHA, S., SHARKEY, K.A. & MACNAUGHTON, W.K. (1998). Cyclooxygenase 1 contributes to inflammatory responses in rats and mice: Implications for gastrointestinal toxicity. *Gastroenterology,* **115,** 101 – 109.

XIE, W., CHIPMAN, J.G., ROBERTSON, D.L., ERIKSON, R.L. & SIMMONS, D.L. (1991). Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. *Proc. Natl. Acad. Sci. U.S.A.,* **88,** 2692 – 2696.

(Received October 7, 1998
Revised November 23, 1998
Accepted December 15, 1998)