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RESEARCH PAPER

Lack of effects of acemetacin on signalling pathways for leukocyte adherence may explain its gastrointestinal safety

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Background and purpose: Acemetacin is a non-steroidal anti-inflammatory drug which is rapidly bioconverted to indomethacin, but produces significantly less gastric damage than indomethacin. This study was performed to investigate several possible mechanisms that could account for the gastrointestinal tolerability of acemetacin.
Experimental approach: The gastric and intestinal damaging effects of acemetacin and indomethacin were examined in the rat. Effects of the drugs on blood levels of leukotriene B_4 and thromboxane B_2, on leukocyte-endothelial adherence in post-capillary mesenteric venules, and on gastric expression of tumour necrosis factor-α (TNF-α) were determined. The two drugs were also compared for gastric toxicity in rats pretreated with inhibitors of COX-2 and NOS.
Key results: Acemetacin induced significantly less gastric and intestinal damage than indomethacin, despite markedly suppressing COX activity. Indomethacin, but not acemetacin, significantly increased leukocyte adherence within mesenteric venules, and gastric expression of TNF-α. Pretreatment with L-nitro-arginine methyl ester or lumiracoxib increased the severity of indomethacin-induced gastric damage, but this was not the case with acemetacin.
Conclusions and implications: The increased gastric and intestinal tolerability of acemetacin may be related to the lack of induction of leukocyte–endothelial adherence. This may be attributable to the reduced ability of acemetacin to elevate leukotriene-B_4 synthesis and TNF-α expression, compared to indomethacin, despite the fact that acemetacin is rapidly bioconverted to indomethacin after its absorption.
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Keywords: leukocyte adherence; gastrointestinal; NSAID; nitric oxide; COX

Abbreviations: GI, gastrointestinal; LT, leukotriene; L-NAME, L-nitro-arginine methyl ester; NO, nitric oxide; NSAID, non-steroidal anti-inflammatory drug; PG, prostaglandin; TNF-α, tumour necrosis factor-α; TX, thromboxane

Introduction

Adverse gastrointestinal (GI) effects of non-steroidal anti-inflammatory drugs (NSAIDs) remain a significant risk associated with the use of these drugs, even after the introduction of selective COX-2 inhibitors (Scheiman *et al.*, 2006). Gastric damage induced by NSAIDs is due in large part to the suppression of prostaglandin (PG) synthesis (Wallace, 1997). PGs derived from both COX-1 and COX-2 make important contributions to gastric mucosal defence, and suppression of the activity of both isoforms is required for gastric injury to be produced (Wallace *et al.*, 2000). However, the damage that is induced by NSAIDs in the small intestine appears to be less PG dependent, and more related to the secretion of these drugs in bile, their subsequent ability to damage the epithelium and the exacerbating effects of luminal bacteria (Wax *et al.*, 1970; Somasundaram *et al.*, 1995; Reuter *et al.*, 1997). In recent years, several strategies have been employed to develop novel NSAIDs that produce less damage in the GI tract. These include the linking of NSAIDs to nitric oxide (NO)-releasing moieties (Wallace and Cirino, 1994; Wallace and del Soldato, 2003), phosphatidylcholine (Lichtenberger *et al.*, 1995) and hydrogen sulphide (H_2S)-releasing moieties (Wallace, 2007a, b).

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In each case, the modified NSAID produced less GI damage than the parent NSAID, but suppressed GI PG synthesis as effectively as the parent NSAID (Wallace *et al*., 1994, 2007; Lichtenberger *et al*., 1995).

One of the critical events in the pathogenesis of NSAID-induced gastric damage is the adherence of leukocytes to the vascular endothelium within the GI microcirculation (Wallace, 1994). This adherence appears to be related to the suppression of COX-2 by NSAIDs (Muscará *et al*., 2000; Wallace *et al*., 2000). Prevention of NSAID-induced leukocyte adherence with antibodies directed against specific leukocyte or endothelial adhesion molecules (Wallace *et al*., 1991, 1993; McCafferty *et al*., 1995) resulted in a marked reduction of gastric injury. The ability of PGs to reduce the severity of NSAID-induced gastric damage may be attributable, in part, to their ability to suppress NSAID-induced leukocyte adherence (Asako *et al*., 1992). NSAIDs linked to NO or H_2S have been shown to induce much less leukocyte adherence than the parent drugs, which may contribute to their improved GI toxicity profiles (Wallace and del Soldato, 2003; Wallace, 2007b). Both leukotriene (LT)-B_4 and tumour necrosis factor-α (TNF-α) have been implicated as signals mediating NSAID-induced leukocyte-endothelial adhesion (Asako *et al*., 1992; Santucci *et al*., 1994), and inhibition of synthesis of these mediators has been shown to result in a significant reduction of NSAID-induced gastric injury (Vaananen *et al*., 1992; Santucci *et al*., 1994; Appleyard *et al*., 1996).

Another approach to the development of GI-sparing NSAIDs is to formulate them as pro-drugs that require hepatic metabolism to become active as COX inhibitors. This is based on the assumption that these drugs would be unable to inhibit PG synthesis during their presence in the stomach, and therefore would not produce as much gastric damage. However, it is now clear that these drugs, sulindac being one example, do not produce significantly less GI ulceration than conventional NSAIDs (Graham, 1990). One exception is acemetacin, a carboxymethylester derivate of indomethacin (Boltze *et al*., 1980; Jacobi and Dell, 1980). Acemetacin exhibits better gastric tolerability than indomethacin (Bori Segura *et al*., 2002; Chou and Tsai, 2002), and gastric safety similar to that of celecoxib (Leeb *et al*., 2004). In a rat model of zymosan-induced inflammation, acemetacin and indomethacin were equally effective in reducing inflammation (that is, leukocyte infiltration, PGE_2 levels) when compared at equimolar doses (Chavez-Pina *et al*., 2007). The mechanism for the improved gastric tolerance of acemetacin remains unclear. It is rapidly transformed into indomethacin after oral administration. Given this rapid transformation and the fact that the latter can produce extensive small intestinal damage as it undergoes enterohepatic recirculation, it is possible that acemetacin's favourable tolerability may be limited to the stomach. Moreover, it seems likely that acemetacin must exhibit activities distinct from those of indomethacin to account for its favourable gastric safety profile.

In this study, we have compared the effects of acemetacin and indomethacin in terms of some of the early events in the pathogenesis of NSAID gastropathy. These include their effects on LTB_4 synthesis, TNF-α expression and leukocyte–endothelial cell adherence.

Materials and methods

Animals

All experimental protocols were approved by the Animal Care Committee of the University of Calgary, and the experiments were performed in accordance with the guidelines of the Canadian Council on Animal Care. Male Wistar rats (175–200 g) were obtained from Charles River Laboratories (Montreal, Quebec, Canada) and were housed in the Animal Care Facility at the University of Calgary. Rats were fed with standard laboratory chow and tap water *ad libitum*.

Acute gastric damage

Groups of at least five rats were deprived of food for 18–20 h and were then given acemetacin or indomethacin (8, 28 or 56 μmol kg^{-1}) orally. Control rats received the same volume of vehicle orally (5% sodium bicarbonate). Note that across the range of doses tested, the pH of solutions of indomethacin versus acemetacin did not differ (both had a pH of $\sim$9.4). Three hours later, the rats were euthanized with an overdose of sodium pentobarbital. The stomach was removed and the extent of haemorrhagic damage was scored by an observer unaware of the treatments that the rats had received. The length (in mm) of all haemorrhagic lesions was measured and the gastric damage score was calculated for each stomach by summing these values (Wallace *et al*., 2000). A sample of the corpus region of the stomach was removed, weighed and added to a tube containing 1 mL of sodium phosphate buffer (10 mM; pH 7.4). The tissue sample was minced with scissors for 30 s and then placed in a shaking water bath (37 °C) for 20 min. The samples were centrifuged (9000 *g*) for 1 min, the supernatant was snap-frozen and then stored at −80 °C. The concentration of PGE_2 in the supernatants was determined by enzyme-linked immunosorbent assay (Wallace *et al*., 2000).

Other experiments were performed to examine if the effects of indomethacin versus acemetacin on whole blood thromboxane (TX) B_2 and LTB_4 synthesis. In this experiment, groups of five rats that had been fasted for 18–20 h were treated orally with vehicle, indomethacin (28 μmol kg^{-1}) or acemetacin (28 μmol kg^{-1}). One hour later, the rats were anesthetized with halothane and blood was drawn from the inferior vena cava and immediately transferred to a glass vial. The blood was allowed to clot at room temperature for 45 min. After centrifugation (1000 *g*) for 10 min, the serum was removed and transferred into an Eppendorf tube, then stored at −80 °C until assays for TXB_2 and LTB_4 were performed, as described previously (Wallace *et al*., 2000).

Effect of lumiracoxib and NG-L-nitro-arginine methyl ester

Nitric oxide and COX-2-derived PGs and lipoxins have been shown to contribute significantly to gastric mucosal defence (Wallace and Tigley, 1995; Wallace *et al*., 2000; Fiorucci *et al*., 2002). To determine if these mediators may be contributing to the relative gastric safety of acemetacin versus indomethacin, the following series of experiments was performed. Groups of five rats each were fasted for 18–20 h,

then given lumiracoxib (10 mg kg^{-1}), L-nitro-arginine methyl ester (L-NAME) (25 mg kg^{-1}) or vehicle (1% carboxy methylcellulose) intraperitoneally. Thirty minutes later, vehicle, acemetacin (14 μmol kg^{-1}) or indomethacin (14 μmol kg^{-1}) was administered orally. The doses of L-NAME and lumiracoxib that were used have previously been shown to effectively inhibit NOS and COX-2, respectively (Rees *et al*., 1990; Zanardo *et al*., 2006). Three hours later, the extent of gastric damage was scored, as described above.

Leukocyte adherence

Leukocyte–endothelial interactions *in vivo* were examined as described in detail (Wallace *et al*., 1993; Zanardo *et al*., 2006). Post-capillary mesenteric venules with a length of at least 150 μm and diameters ranging from 25 to 40 μm were studied. A video camera mounted on a microscope (Panasonic digital 5000, Tokyo, Japan) projected the image onto a monitor, and the images were recorded for playback, using a videocassette recorder; image analysis was carried out without the knowledge of the treatments. Images of the mesenteric microcirculation were recorded over 5-min periods starting before drug administration and at 15-min intervals thereafter for 90 min. Acemetacin (28 μmol kg^{-1}), indomethacin (28 μmol kg^{-1}) or vehicle was administered intragastrically. Leukocytes were considered adherent if they remained stationary for at least 30 s.

Real-time reverse transcription PCR

Gastric tissues were excised, snap-frozen in liquid nitrogen, and stored at −80 °C until required for processing. RNA extraction was performed using the RNeasy Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. For gene expression studies, two-step real-time reverse transcription PCR (RT-PCR) was utilized. RNA was reverse-transcribed using the QuantiTect Reverse Transcription kit (Qiagen). Next, 1 μg of RNA was incubated with gDNA wipe-out buffer at 42 °C for 2 min to eliminate contaminating genomic DNA. Thereafter, QuantiScript reverse transcriptase primer mix (containing oligo-dT and random primers) and 5 × reaction buffer were added and incubated at 42 °C for 15 min. The QuantiTect SYBR Green PCR kit (Qiagen) and MasterCycler EP Realplex thermal cycler (Eppendorf, Westbury, NY, USA) were used for template amplification and fluorescent detection of SYBR green dye. Validated primer sets for rat TNF-α, COX-2 and β-actin were also obtained from Qiagen. Briefly, 50 ng of template cDNA was combined with 2 × QuantiTect SYBR Green master mix in a 96-well plate. HotStarTaq DNA polymerase activity was initiated by incubation of the reaction at 95 °C for 15 min. Conditions for template amplification are as follows: 94 °C for 15 s, 55 °C for 15 s, 72 °C for 30 s; 45 cycles. All data were recorded and analysed using Realplex software (Eppendorf). The comparative C_t method was used to calculate relative amplification of gene products, with target genes normalized against the housekeeping gene β-actin.

Small intestinal damage

Non-steroidal anti-inflammatory drugs can cause damage to the small intestine, largely related to their excretion in bile and the repeated exposure of the intestinal epithelium to the NSAID as it undergoes enterohepatic recirculation (Wax *et al*., 1970; Somasundaram *et al*., 1995; Reuter *et al*., 1997). Following oral administration, acemetacin is completely converted to indomethacin within ∼1 h and indomethacin is detectable in blood for more than 30 h thereafter (Chavez-Pina *et al*., 2007). To determine if there would be any difference in the ability of acemetacin and indomethacin to induce small intestinal damage, groups of five rats each (not fasted) were given vehicle, indomethacin (28 μmol kg^{-1}) or acemetacin (28 μmol kg^{-1}) orally. After 24 h, the rats were killed with an overdose of sodium pentobarbital. The intestine was removed and the damage was scored (without knowledge of the treatments) using a 0–3 scale (0 = normal, 1 = mild, 2 = moderate and 3 = severe) (Wallace and Whittle, 1986). A separate series of experiments was carried out in an identical manner, except that the rats were subjected to bile duct ligation 12 h before drug or vehicle administration, as described previously (Wax *et al*., 1970; Reuter *et al*., 1997).

Statistical analysis

All data are expressed as mean ± s.e.mean Comparisons among groups were made using a one-way analysis of variance followed by the Newman–Keuls test, except for the intestinal damage scores, which were compared using the Mann–Whitney *U*-test ($P<0.05$ was considered as significant).

Materials

Indomethacin and acemetacin were obtained from Sigma Aldrich (St Louis, MO, USA). The enzyme-linked immunosorbent assay kits for measuring PGE_2, TXB_2 and LTB_4 were obtained from Cayman Chemical Co. (Ann Arbor, MI, USA). Drug and molecular target nomenclature in this paper conforms to the *British Journal of Pharmacology*'s Guide to Receptors and Channels (Alexander *et al*., 2008).

Results

Gastric damage and PG synthesis

Oral administration of indomethacin resulted in the formation of haemorrhagic erosions in the corpus of the stomach that increased in severity in a dose-dependent manner (Figure 1). Acemetacin did not cause any detectable gastric damage at the lowest dose tested and caused significantly less gastric damage than indomethacin at the two higher doses. All doses of indomethacin and acemetacin markedly inhibited gastric PGE_2 synthesis.

Effects of lumiracoxib and L-NAME on indomethacin or acemetacin-induced gastric damage

At a dose of 14 μmol kg^{-1}, neither indomethacin nor acemetacin induced significant gastric damage (Figure 2). However, when rats were pretreated with L-NAME (which alone did not induce damage), gastric damage was increased significantly following indomethacin administration. Gastric

damage was observed in some rats treated with acemetacin, but the mean gastric damage score did not differ significantly from that in rats given acemetacin without L-NAME pretreatment. Previous administration of lumiracoxib also significantly increased the gastric damaging effects of indomethacin (no damage was observed in rats given lumiracoxib alone). In lumiracoxib-pretreated rats, the gastric damage in the indomethacin group was significantly more severe than that in the acemetacin group.

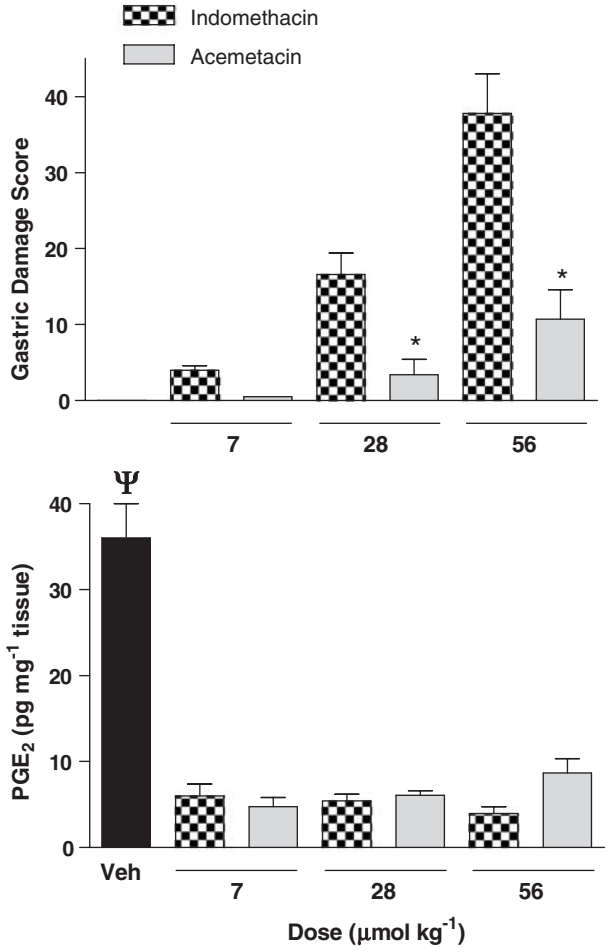

Figure 1 Gastric damage score and PGE_2 synthesis 3 h after oral administration of various doses of indomethacin or acemetacin. Data are expressed as mean ± s.e.mean. $^*P<0.05$ versus the corresponding dose of indomethacin; $^{\Psi}P<0.05$ versus all other groups (5–8 rats per group).

Effects of acemetacin and indomethacin on leukocyte adherence in mesentery

Basal leukocyte adherence was similar (~3 per 100 μm vessel length) in the three groups of rats studied (Figure 3). Intragastric administration of indomethacin resulted in a progressive increase in the number of adherent leukocytes, reaching approximately five times the basal levels by the end of the 90-min experiment. In contrast, acemetacin administration did not significantly alter leukocyte adherence as compared with vehicle-treated rats.

Effects on whole blood synthesis of LTB_4 and TXB_2

Blood taken 1 h after oral administration of indomethacin had significantly higher levels of LTB_4 than blood from vehicle-treated rats (Figure 4). Acemetacin administration did not significantly affect LTB_4 levels in blood. Both indomethacin and acemetacin completely suppressed TXB_2 synthesis.

Effects on gastric TNF-α and COX-2 expression

Indomethacin administration resulted, within 1 h, in a significant increase (fourfold) in gastric expression of mRNA for TNF-α (Figure 5). In contrast, acemetacin did not significantly change gastric TNF-α expression. Neither indomethacin nor acemetacin significantly affected gastric expression of mRNA for COX-2 (2.1 ± 1.2 versus 1.2 ± 1.1, respectively; expressed as fold change over vehicle-treated rats).

Intestinal damage

Indomethacin (28 μmol kg^{-1}) elicited extensive haemorrhagic damage in the small intestine, with a significantly

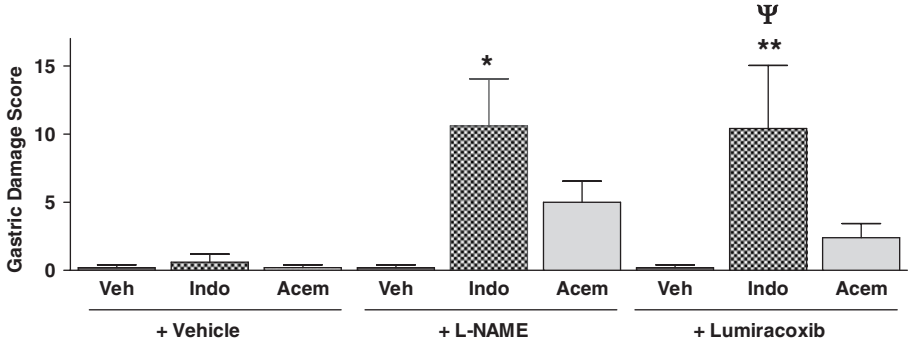

Figure 2 Effect of lumiracoxib (10 mg kg^{-1}) or L-NAME (25 mg kg^{-1}) on the gastric damage induced by indomethacin or acemetacin (both at 14 μmol kg^{-1}, p.o.). Rats were pretreated with vehicle, L-NAME or lumiracoxib, then 30 min later with vehicle, indomethacin or acemetacin. Data are expressed as mean ± s.e.mean. $^*P<0.05$, $^{**}P<0.01$ versus corresponding the vehicle + indomethacin group. $^{\Psi}P<0.05$ versus corresponding acemetacin-treated group (five rats per group).

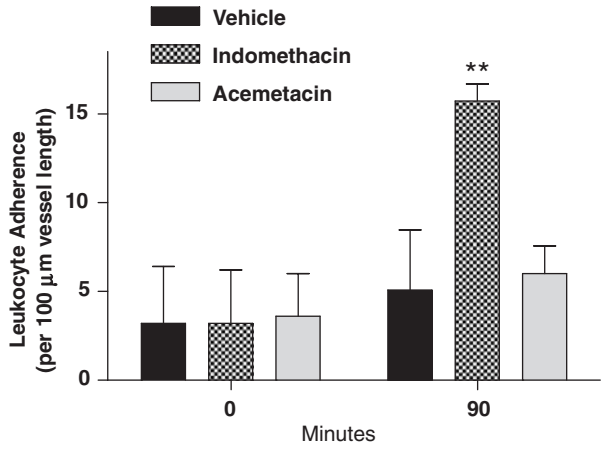

Figure 3 Leukocyte adherence in post-capillary venules of rats before and 90 min after intragastric administration of indomethacin or acemetacin (both at 28 μmol kg^{-1}, p.o.). **$P<0.01$ versus the corresponding vehicle-treated and acemetacin-treated groups (five rats per group).

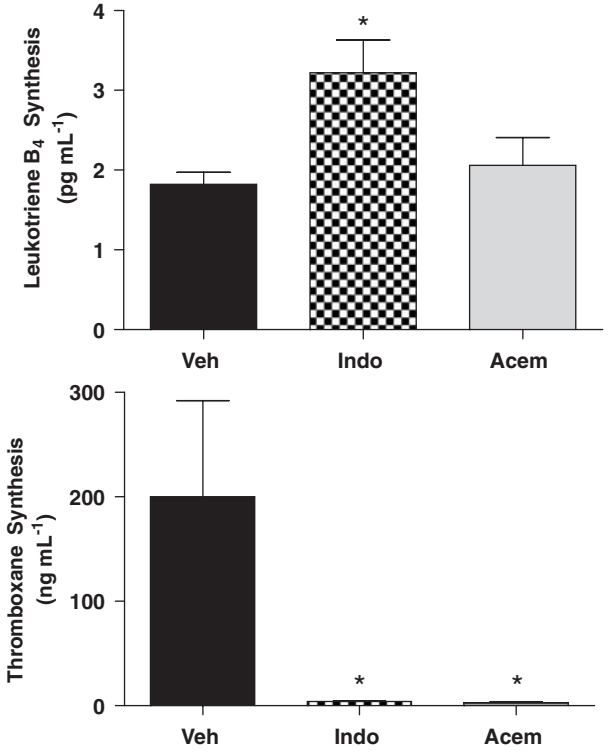

Figure 4 Whole-blood synthesis of leukotriene B_4 and thromboxanes B_2 1 h after oral administration of indomethacin or acemetacin (both at 28 μmol kg^{-1}). *$P<0.05$ versus the vehicle-treated group (five rats per group).

higher damage score than that for vehicle-treated rats (2.3 ± 0.3 and 0 ± 0, respectively; $P<0.05$; $n=5$ per group). Acemetacin administration caused mild damage in some rats, but the mean damage score (1.1 ± 0.3; $n=5$) was not significantly different from that in vehicle-treated rats (as some acemetacin-treated rats did not exhibit any damage). In bile duct-ligated rats, intestinal damage was not observed in any rat treated with indomethacin or acemetacin.

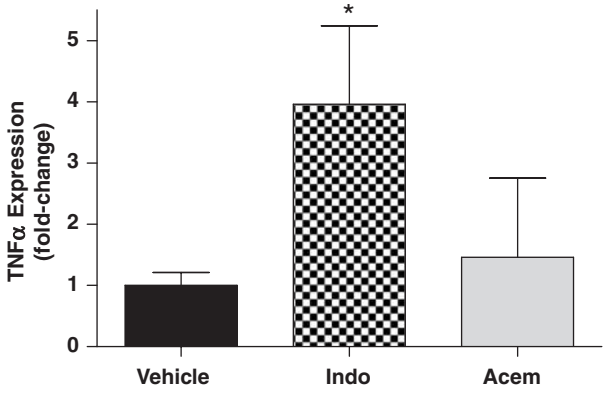

Figure 5 Gastric expression of mRNA for tumour necrosis factor-α. Real-time PCR was performed on tissue samples taken 1 h after oral administration of vehicle, indomethacin (28 μmol kg^{-1}) or acemetacin (28 μmol kg^{-1}). Results are expressed as fold-changes in expression relative to the vehicle-treated group, and corrected for β-actin expression in each sample. *$P<0.05$ versus the vehicle-treated group (3–4 rats per group).

Discussion and conclusions

Acemetacin is a carboxymethylester derivative of indomethacin that, in both clinical and laboratory studies, exhibited comparable anti-inflammatory efficacy to indomethacin, but with better gastric tolerability (Bori Segura *et al*., 2002; Chou and Tsai, 2002; Leeb *et al*., 2004; Chavez-Pina *et al*., 2007). The gastric-damaging effects of NSAIDs are largely related to the inhibition of gastric COX activity by these drugs. As acemetacin is rapidly bioconverted to indomethacin, and suppresses COX-1 and COX-2 *in vivo* to the same extent as indomethacin (Chavez-Pina *et al*., 2007), the reasons for the lower gastric toxicity of acemetacin have been unclear. The results of this study shed some light on possible mechanisms for the improved safety of acemetacin versus indomethacin. Indomethacin increases plasma levels of TNF-α (Appleyard *et al*., 1996) and LTB_4, and provokes the adherence of leukocytes to the vascular endothelium in the GI microcirculation (Asako *et al*., 1992). All of these have been shown to contribute to the generation of gastric mucosal injury (Wallace *et al*., 1991; Asako *et al*., 1992; Vaananen *et al*., 1992; Wallace *et al*., 1993; Santucci *et al*., 1994; Appleyard *et al*., 1996). These effects of indomethacin were confirmed in this study. Moreover, we found that acemetacin behaved very differently: it did not increase gastric expression of TNF-α mRNA, did not significantly elevate blood levels of LTB_4, did not induce leukocyte adherence to the vascular endothelium and produced significantly less gastric and intestinal damage than indomethacin. Importantly, acemetacin suppressed whole-blood TXB_2 synthesis and gastric PG synthesis as effectively as indomethacin.

Acemetacin is rapidly absorbed and bioconverted to indomethacin. Following oral administration to the rat, this transformation is essentially complete within an hour. This raises the question: what are the effects of acemetacin, before its conversion to indomethacin that can account for its markedly different effects on gastric mucosal defence? We

attempted to answer this question by examining the possibility that acemetacin may influence some of the key elements of gastric mucosal defence, including PGs, NO and lipoxin A_4. We focused on these mediators because they have been shown to reduce the severity of NSAID-induced damage and to inhibit NSAID-induced leukocyte adherence (MacNaughton *et al*., 1989; Asako *et al*., 1992; Wallace *et al*., 1999; Fiorucci *et al*., 2002, 2003; Souza *et al*., 2003). Moreover, PGs, NO and lipoxins have been shown to reduce the synthesis and/or release of LTB_4 and TNF-α (Ham *et al*., 1983; Kunkel *et al*., 1988; Hogaboam *et al*., 1993; Ariel *et al*., 2003; Fiorucci *et al*., 2004).

Nitric oxide has potent gastroprotective effects and contributes significantly to mucosal defence throughout the GI tract (Wallace and Miller, 2000). In this study, inhibition of NO synthesis (with L-NAME) did not, in itself, cause gastric damage. However, it did significantly increase the severity of indomethacin-induced damage. Although some rats treated with L-NAME and acemetacin exhibited mild haemorrhagic damage in the stomach, the mean gastric damage score in this group was not significantly increased above that in rats given acemetacin alone. These results suggest that the relative safety of acemetacin versus indomethacin is unlikely to be solely related to stimulatory effects of the former on gastric NO synthesis.

Prostaglandins from both COX-1 and COX-2 contribute to gastric mucosal defence (Wallace *et al*., 2000). Rapid upregulation of COX-2 can be detected following administration of aspirin or a selective COX-1 inhibitor (Davies *et al*., 1997; Tanaka *et al*., 2002), or following a short period of ischaemia (Maricic *et al*., 1999). In this study, we did not observe any significant change in the expression of mRNA for COX-2 in the stomach within an hour of administering acemetacin (or indomethacin), and gastric PG synthesis was markedly inhibited. However, this does not completely rule out an enhanced function of COX-2 in mucosal defence following acemetacin administration. Acemetacin bears some similarity to aspirin, in that it has an acetyl group that could potentially interact with the same serine residue in COX-2 that is acetylated by aspirin. Although aspirin-induced acetylation of COX-2 results in inhibition of PG synthesis, arachidonic acid can still be metabolized to 15-*R*-hydroxyeicosatetraenoic acid, and then further metabolized (through 5-lipoxygenase) to produce 15-*R*-epi-lipoxin A_4 (Serhan *et al*., 2007). We have previously shown that lipoxin A_4 is a very potent gastroprotective substance, and its synthesis can be detected in the stomach following aspirin administration (Fiorucci *et al*., 2002). Inhibition of aspirin-induced lipoxin synthesis by co-administration of a selective COX-2 inhibitor leads to a significant exacerbation of gastric damage. To determine if acemetacin's gastric safety was dependent on COX-2 activity, we tested the effects of co-administration of lumiracoxib, a selective COX-2 inhibitor (Esser *et al*., 2005). Whereas lumiracoxib itself did not induce gastric damage, when given together with a non-damaging dose of indomethacin, extensive haemorrhagic erosions formed in the stomach. This confirms the importance of COX-2 as a source of gastroprotective substances. In contrast, administration of lumiracoxib together with acemetacin did not result in significant gastric damage. These results therefore suggest that acemetacin's gastric safety is not related to the generation of gastroprotective substances, such as PGs or lipoxin A_4, from COX-2.

The ability of NSAIDs to induce damage in the small intestine has been well established in humans and animals (Wax *et al*., 1970; Bjarnason *et al*., 1986; Somasundaram *et al*., 1995; Reuter *et al*., 1997). There are several pathogenic factors involved in the development of NSAID-induced lesions of the small bowel. These include direct cytotoxic effects of the drugs on enterocytes, bile, luminal bacteria, Toll-like receptors and leukocyte–endothelial interactions (Somasundaram *et al*., 1995; Reuter *et al*., 1997; Wallace, 1997; Watanabe *et al*., 2008). Unlike upper GI toxicity, suppression of PG synthesis by NSAIDs does not appear to be a major contributor to the production of mucosal injury in the small intestine (Reuter *et al*., 1997; Sigthorsson *et al*., 2002). NSAID-induced intestinal damage develops more slowly than that in the stomach (Reuter *et al*., 1997), and likely involves direct damage to the epithelial cells, possibly as a consequence of uncoupling of oxidative phosphorylation (Somasundaram *et al*., 1995). The NSAIDs that are most likely to cause small intestinal damage are those that are excreted in bile. The intestinal epithelium is repeatedly exposed to the NSAID as it undergoes enterohepatic recirculation. Evidence to support this hypothesis includes the demonstration that previous bile duct ligation largely prevents NSAID-induced small intestinal damage (Wax *et al*., 1970; Reuter *et al*., 1997). In this study, acemetacin produced substantially less small intestinal damage than indomethacin. Also, the intestinal damage caused by acemetacin, similar to that elicited by indomethacin, was completely prevented by previous ligation of the bile duct. Considering that acemetacin is completely bioconverted to indomethacin within 1 h of administration to rats (Chavez-Pina *et al*., 2007), this suggests that events during that first hour can have a significant impact in terms of the extent of intestinal damage that will eventually develop. It is possible that the same events, as we observed to be important in the stomach after acemetacin/indomethacin administration, are important in the pathogenesis of small intestinal injury. Indeed, LTB_4-driven leukocyte adherence within the mesenteric microcirculation has been suggested to have an important function in NSAID-induced small intestinal damage (Miura *et al*., 1991). TNF-α production is increased in the small intestine after administration of an NSAID (Bertrand *et al*., 1998; Watanabe *et al*., 2008), but a clear contribution of TNF-α to the pathogenesis of NSAID-enteropathy has not been firmly established (Reuter and Wallace, 1999).

In summary, despite its rapid bioconversion to indomethacin, acemetacin produced significantly less GI damage than equimolar doses of indomethacin, as has been observed in previous laboratory and clinical studies (Bori Segura *et al*., 2002; Chou and Tsai, 2002; Leeb *et al*., 2004; Chavez-Pina *et al*., 2007). A previous study from our laboratory demonstrated that acemetacin and indomethacin exhibited comparable anti-inflammatory effects in a rat model of acute inflammation; that is, at equimolar doses, the two drugs suppress leukocyte infiltration and PGE_2 synthesis to the same extent (Chavez-Pina *et al*., 2007). The reduced GI toxicity of acemetacin may be related to its lack of

stimulation of leukocyte adherence, a key event in the pathogenesis of NSAID gastropathy. This may in turn be a consequence of the absence of an increase in levels of LTB_4 and/or TNF-α expression following acemetacin administration, in contrast to that following indomethacin administration. The results of this study suggest that events occurring within the first hour after acemetacin administration profoundly effect the development of damage in the stomach and small intestine.

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