Acute gastrointestinal permeability responses to different non-steroidal anti-inflammatory drugs

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Abstract

***Background and aims*—Non-steroidal anti-inflammatory drugs (NSAIDs) cause gastrointestinal damage both in the upper and lower gastrointestinal tract. New anti-inflammatory drugs have been developed in an attempt to improve their gastrointestinal side effect profile. Our objective was to compare the effect on gastrointestinal permeability of acute equieffective doses of four different NSAIDs; three were designed to reduce gastrointestinal mucosal injury.**

***Materials*—Healthy volunteers underwent sugar tests in a randomised fashion, 15 days apart, at: (1) baseline; (2) after two days of 75 mg slow release (microspheres) indomethacin; (3) after two days of 7.5 mg oral meloxicam which preferentially inhibits cyclooxygenase 2; and (4) after two days of 750 mg naproxen. A subgroup of subjects was tested after two days of 200 mg celecoxib. In each test, subjects ingested a solution containing sucrose, lactulose, and mannitol and sucralose, to evaluate gastroduodenal, intestinal, and colonic permeability, respectively.**

***Results*—Gastric permeability was significantly affected by naproxen ($p<0.05$) but not by slow release indomethacin, meloxicam, or celecoxib. Intestinal permeability was significantly increased by the first three NSAIDs ($p<0.05$) but not by celecoxib. Abnormal lactulose/mannitol ratios were observed in 42% of meloxicam treatments, in 62% during indomethacin, and in 75% of subjects treated with naproxen. Finally, colonic permeability, as measured by sucralose, was not significantly increased by any of the four drugs.**

***Conclusion*—Our study provides evidence that the newly developed NSAIDs reduce gastric mucosal permeability significantly. However, most produced significant alteration of small intestinal permeability. In contrast, our results suggest that celecoxib seems to exhibit the most desirable gastrointestinal side effect profile.**

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Keywords: permeability; non-steroidal anti-inflammatory drugs; celecoxib; meloxican; small intestine; gastric injury

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Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used, with gastrointestinal toxicity mainly in the form of gastroduodenal mucosal damage.[1] However, it is becoming clear that both the small bowel and colon may also be injured.[2] Small intestinal damage can be observed as ulceration,[3] perforation,[4] and stricture,[5] particularly following long term administration of NSAIDs. Moreover, enteropathy in the form of intestinal inflammation,[6] occult blood loss,[7] protein losing enteropathy,[7] and vitamin B12[8] or bile acid malabsorption[9] are also reported in approximately two thirds of patients receiving these compounds long term. However, overt clinically relevant symptoms of small intestinal injury (distal to the duodenum) seem to be much less common than those observed in the stomach.[10] Although rare, NSAIDs can also induce mucosal damage of the large intestine.[11 12] Reported compromise of the colon includes de novo NSAID induced colitis,[13 14] reactivation of quiescent colitis,[15 16] or lower gastrointestinal bleeding from diverticular disease.[17 18]

How NSAIDs initiate gastrointestinal damage is not completely clear. The prevailing view is that upper gastrointestinal toxicity is mediated by both a non-prostaglandin induced local injury[19] but predominantly by systemic inhibition of cyclooxygenase (COX) enzyme. This leads to a subsequent reduction in the cytoprotective prostaglandins required for effective mucosal defence.[20] The pathogenic mechanism leading to inflammatory changes in the distal gastrointestinal tract is currently unknown. One proposed mode of action is drug induced changes in local eicosanoid metabolism coupled with a topical toxic effect of the drug. Together these induce a subsequent increase in the permeability of the mucosa to toxins and luminal antigens such as bile, pancreatic secretion, and bacteria.[21] Enterohepatic recirculation of NSAIDs may be important for this effect although much controversy exists in the literature.[22]

Recent evidence suggests that NSAIDs differentially inhibit the isoforms of COX and therefore vary in their anti-inflammatory effects and toxicity profile.[23] Two forms of COX appear to be differentially inhibited by NSAIDs. While COX-1 is a constitutive enzyme that is thought to be important in maintaining mucosal integrity, COX-2 is primarily inducible and is related to inflammation.[24] One hypothesis is that selective inhibition of the COX-2 enzyme provides the anti-inflammatory activity of NSAIDs but without those side effects attributable to COX-1 inhibition.[23]

Abbreviations used in this paper: NSAIDs, non-steroidal anti-inflammatory drugs; COX, cyclooxygenase; HPLC, high pressure liquid chromatography.

Efforts to generate safe and efficacious NSAIDs have followed different strategies including the development of prodrugs,[25] enteric coated,[26] or modified release formulations.[27] In recent years selectively targeting inhibition of the COX-2 isoenzyme has become common practice.[28] Meloxicam and celecoxib are both new agents with different COX-2 inhibition profiles relative to COX-1.[29 30] Preliminary uncontrolled data evaluating their tolerability and efficacy are encouraging.[28] However, most studies which evaluated the tolerability of new agents were aimed at assessing gastric or duodenal damage. Whether these formulations have differential toxicity in the mid or distal gastrointestinal tract is currently unknown.

Gastrointestinal permeability tests have been demonstrated to be useful in both basic and clinical research for investigation of gastrointestinal damage induced by NSAIDs.[31] Different permeability tests have been shown to be highly sensitive for recognition of mucosal permeability disturbance in subjects receiving NSAIDs.[31] Recently, administration of site specific permeability probes detecting permeability defects at different levels of the gastrointestinal tract allowed a single screening test for assessment of the functional integrity of the gastrointestinal mucosa.[32] Thus simultaneous use of sucrose, lactulose/mannitol, and sucralose probes allows non-invasive detection of gastric, enteric, or colonic damage, respectively.[32] Our objective in this prospective randomised study was to compare the acute effects of four different NSAIDs, in equieffective doses, on regional gastrointestinal permeability. Two of these drugs were designed to reduce mucosal injury, one by selective COX-2 inhibition (celecoxib) and the other by formulation as slow release microspheres (indomethacin). Meloxicam may demonstrate some COX-2 preferential inhibiting activity.

Material and methods

SUBJECTS AND STUDY DESIGN

We evaluated 19 adult healthy volunteers (10 females; median age 32 years (range 22–50)) among the staff of the Clinical Department of the Gastroenterology Hospital. Subjects participating in the study underwent sugar tests in a randomised fashion 15 days apart, at: (1) baseline; (2) after two days of 75 mg of slow release (microspheres) indomethacin (IM75; Montpellier, Argentina); (3) after two days of 7.5 mg of oral meloxicam (Mobic; Boehringer Ingelheim, Argentina); and (4) after two days of 750 mg of oral naproxen (Naprontag; Rontag, Argentina). The second part of the study comprised nine patients from this population (four females; median age 37 years (range 27–50)) who were included in an additional study and evaluated after two days of 200 mg/day celecoxib (Celebrex, Searle, Argentina). At the time of enrolment, all subjects were interviewed and only those who denied gastrointestinal symptoms and had not taken NSAIDs, acetylsalicylic acid, or alcoholic beverages for the two weeks prior to the study were included.

PERMEABILITY PROTOCOL

Subjects came to the laboratory after an overnight fast, ingested the sugar probes, and collected all urine passed over the ensuing 24 hours into a preweighed container with 5 ml of 10% thymol in isopropanol. Urine was vigorously mixed, total volume recorded, and aliquots rapidly frozen for subsequent transport and analysis.

To evaluate gastroduodenal, intestinal, and colonic permeability, subjects ingested a solution containing: 100 g sucrose, 5 g lactulose (Technilab, Montreal, Quebec, Canada), and 2 g mannitol (Sigma, St Louis, Missouri, USA) in 450 ml of water (osmolality approximately 1800 mosmol/l). In addition, subjects ingested capsules containing 2 g sucralose (McNeil Consumer Products Co, Guelph, Ontario, Canada).[32]

ANALYTICAL METHODS

Fractional excretion of lactulose and mannitol, and the total mass of sucrose and sucralose excreted were calculated from urinary concentrations determined by high pressure liquid chromatography (HPLC).[31]

Probe assays

Samples of urine (10 ml) were obtained for analysis. Cellobiose was added as an internal standard. Samples were deionised by adding 1 g of a 1:1.5 (weight:weight) mixture of Amberlite IR-120 and IRA-400 resin (BDH chemicals, Toronto, Ontario, Canada). The supernatant was then filtered through a 45 μm millipore filter (Millipore, Bedford, Massachusetts, USA). Samples were separated on a Dionex Carbopac MA-1 anion exchange column (Dionex, Ontario, Canada) in a Dionex HPLC using 520 mM NAOH as the isocratic mobile phase. Peak identification was performed using pulsed amperometric electrochemical detection on a gold electrode. Quantitation was performed using known standards at multiple concentrations, with linear interpolations between concentrations. As electrochemical detection of carbohydrates is sensitive, samples were diluted after addition of the internal standard. If these dilutions were not satisfactory for proper analysis, adjustments to the dilutions were performed so that sucrose, mannitol, and lactulose concentrations fell within the range of the standards. Fractional excretion of lactulose and mannitol was calculated from urinary concentrations of these sugars; the lactulose/mannitol ratio is reported. The total mass of sucrose excreted in overnight urine sample was calculated from its urinary concentration and total volume of urine produced.

Sucralose was also assayed by HPLC. However, it cannot be detected under the conditions used for other sugars. Separation was achieved using a Dionex Ionpac NS1 column and acetonitrile/water as the eluent at a flow rate of 1 ml/min. A gradient run was used beginning with acetonitrile in water increasing from 0% to 20% over the course of the run. Detection was performed with an electrochemical detector in a fashion identical to the

Table 1 Urinary excretion of probes induced by non-steroidal anti-inflammatory drugs. Results illustrate urinary excretion of sucrose, lactulose/mannitol (lac/man), and sucralose in 19 subjects at baseline, and after two days of 7.5 mg/day meloxicam, 75 mg/day slow release (SR) indomethacin, and 750 mg/day naproxen

Probe	*Baseline*	*Meloxicam*	*SR indomethacin*	*Naproxen*
Sucrose (mg)	78.4 (51.4–105.4)	95.9 (57.3–134.4)	78.7 (52.2–105.2)	107 (82.9–138.5)*
Lac/man (ratio)	0.022(0.017–0.026)	0.034(0.023–0.046)*	0.041(0.028–0.054)*	0.032(0.025–0.039)*
Sucralose (mg)	41.1 (26.6–55.5)	29.1 (19.4–38.9)	28.6 (19.3–37.9)	38.8 (23.3–52.9)

Values are median (95% confidence intervals).
*p<0.05 versus baseline values.

other sugars. Because this only works at a high pH, postcolumn addition of 300 mmol/l NaOH at a constant flow rate of 0.5 ml/min was used. For these assays, the internal standard used was phenyl-Beta-d-thiogalactoside (Sigma Chemical Co.) added to the initial urine sample at a concentration of 0.1 mg/ml. This compound is stable in urine for at least one week at room temperature and indefinitely when frozen. Calibration and peak authentication were performed in a manner similar to that described above.

STATISTICAL ANALYSIS

Results are presented as median (95% confidence intervals). Statistical comparison between baseline and post-treatment results was performed using the Friedman test and the Wilcoxon signed rank test.

Results

Data obtained from the permeability experiments are shown in tables 1 and 2. As celecoxib was tested separately with a repeat baseline and repeat ingestion of naproxen, these data are shown separately. Figures 1 and 2 also illustrate this information, displaying excretion of sucrose, lactulose/mannitol ratio, and excretion of sucralose, respectively.

GASTRODUODENAL PERMEABILITY

Using a large group of controls maintained by our reference laboratory, the upper limit of urinary sucrose excretion for normal subjects is 180 mg, defined as mean sucrose excretion +2 SDs of the mean (n=520).[33] None of our subjects excreted abnormal amounts of sucrose at baseline. However, following ingestion of naproxen, there was a significant increase in mean excretion of sucrose (table 1) suggesting the presence of gastroduodenal damage. In contrast, following ingestion of either meloxicam or slow release indomethacin, there was no significant increase in gastroduodenal permeability (sucrose excretion). In table 2, data are shown from the repeat study and it is apparent that baseline sucrose excretion was unchanged as was the significant increase in permeability following another two day course of naproxen. In contrast with these observations, administration of celecoxib did not increase sucrose permeability. These data are presented in figs 1A and 2A, respectively.

Table 2 Urinary excretion of probes induced by celecoxib. Results illustrate urinary excretion of sucrose, lactulose/mannitol (lac/man), and sucralose in nine subjects at baseline, and after two days of 200 mg/day celecoxib and 750 mg/day naproxen

Probe	*Baseline*	*Celecoxib*	*Naproxen*
Sucrose (mg)	84.7 (12.6)	55.7 (5.4)	126.4 (21.3)
Lac/man (ratio)	0.023 (0.003)	0.023 (0.005)	0.027 (0.002)*
Sucralose (mg)	31.1 (3.4)	29.1 (4.2)	29.7 (5.6)

Values are mean (SEM).
*p<0.05 versus baseline values.

SMALL INTESTINAL PERMEABILITY

The upper limit of lactulose/mannitol urinary excretion for normal subjects is 0.025 in our laboratory, as defined previously using five

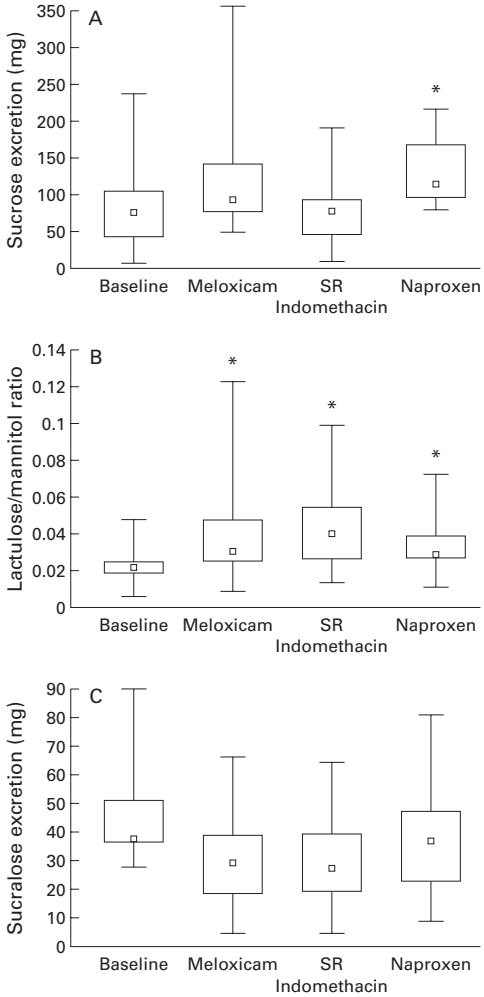

*Figure 1 Gastrointestinal permeability following meloxicam, slow release (SR) indomethacin, and naproxen in healthy subjects. Results obtained from 19 subjects at baseline, after two days of 7.5 mg/day meloxicam, 75 mg/day SR indomethacin, and 750 mg/day naproxen. (A) Excretion of sucrose. (B) Lactulose/mannitol ratio. (C) Excretion of sucralose. Data are presented as boxplots with the small boxes representing median values, the large boxes 50% of values, and the whiskers the range of values. Fractional excretion of sucrose was increased after naproxen administration; all non-steroidal anti-inflammatory drugs caused a significant increase in the lactulose/mannitol ratio. *p<0.05 versus baseline values.*

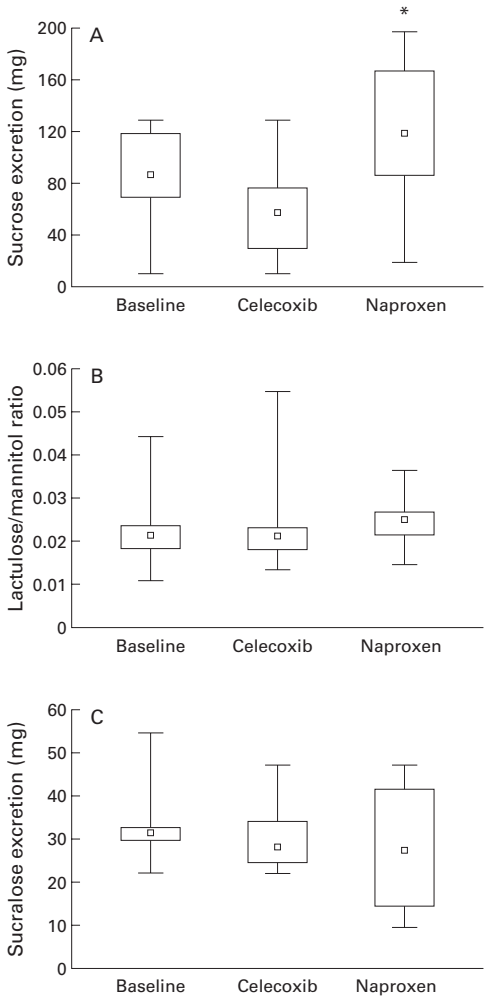

*Figure 2 Gastrointestinal permeability following celecoxib. Results obtained from nine subjects at baseline, after two days of 200 mg/day celecoxib, and after two days of 750 mg/day naproxen. (A) Excretion of sucrose. (B) Lactulose/mannitol ratio. (C) Excretion of sucralose. Data are presented as boxplots with the small boxes representing median values, large boxes 50% of values, and whiskers the range of values. Fractional excretion rates of sucrose were only increased after naproxen administration. *$p<0.05$ versus baseline values.*

hour urine collection.[34] Only minimal excretion of these probes is found at longer collection times. At baseline, the mean lactulose/mannitol ratio for our study population was within the normal range. Two subjects had mildly increased lactulose/mannitol ratios in the absence of any recognised disease. Following ingestion of naproxen, meloxicam, or slow release indomethacin there was a striking and significant increase in small intestinal permeability (table 1). In the case of naproxen ingestion, 75% of subjects had abnormal small intestinal permeability (>0.025). Similar data were apparent for patients after ingestion of meloxicam (42% >0.025) and indomethacin (61% >0.025).

The second study (table 2) corroborated these results. Again ingestion of naproxen significantly increased small intestinal permeability. However, following ingestion of celecoxib, there was no increase in small intestinal permeability.

COLONIC PERMEABILITY

In contrast with the data presented above, colonic permeability (sucralose excretion) was not affected by any of the NSAIDs tested.

Discussion

The present study was designed to compare gastrointestinal mucosal side effects of four different NSAIDs in an acute setting. While one was an agent linked to the development of classical gastrointestinal damage (naproxen), meloxicam shows some preference for the COX-2 enzyme, celecoxib was specifically designed to selectively inhibit COX-2, while a modified release formulation of indomethacin was designed to reduce gastric toxicity. There are several published clinical studies examining NSAID induced intestinal permeability[31]; however, to our knowledge this is the first description of the effect of these different NSAIDs on regional gastrointestinal permeability of the entire gut.

The study demonstrated that acute doses of NSAIDs had differential toxicity depending on the type of drug used and the level of the gastrointestinal tract evaluated. In this regard, we will discriminate between the effects of the drugs at the three different levels assessed. Firstly, upper gastrointestinal permeability was differentially affected. While naproxen produced significantly increased urinary excretion of sucrose, this effect was not observed with slow release indomethacin or the COX-2 selective and preferential inhibitors celecoxib and meloxicam. However, even with naproxen, the degree of gastric mucosal compromise was low. We believe that these findings are related to intrinsic factors of the present protocol such as the low dosages used. In this regard, it is important to note that NSAID induced gastric mucosal lesions are time and dose dependent.[31] Furthermore, it has been demonstrated that sucrose permeability correlates with the severity of upper gastroduodenal damage.[35] In the interpretation of our results, it must be remembered that we designed our protocol to evaluate acute administration of equipotent doses as they are currently recommended for treatment in moderately severe conditions.[36] Our results support the concept that inhibition of "cytoprotective" prostaglandin synthesis by NSAIDs is a major factor in the development of gastric damage.[20] Furthermore, the study also provides support for the use of alternative formulations, such as those assessed in this study, to protect the gastric mucosa from aggressive agents. This effect was previously reported by Davies *et al* who also used sucrose as a marker of gastric permeability after administration of a modified release formulation (sustained release) of flurbiprofen in rats.[37]

Small intestinal permeability has been used to quantify mucosal alterations induced by acetylsalicylic acid and other NSAIDs. These permeability changes have been detected by oral administration of probes such as ^{51}Cr-EDTA,[38] lactulose,[39] cellobiose,[31] and polyethylene glycol.[40] In general, most of these studies showed increased permeability that was dose dependent. It has been demonstrated that this

effect can be antagonised by concomitant administration of misoprostol,[41] glucose/citrate,[42] glutamine,[43] or metronidazole.[44] Furthermore, permeability also increases to a similar extent after either oral or rectal NSAID administration, suggesting a systemically mediated mechanism and/or that biliary excretion is important.[22 45] Data from our study suggest that intestinal permeability is significantly increased by most NSAIDs used. However, a differential effect was observed depending on the NSAID used. Meloxicam administration was associated with the lowest number of abnormal permeability determinations (42%) compared with indomethacin or naproxen (61% and 75%, respectively). Our results for indomethacin and naproxen induced small intestinal mucosal injury are in agreement with those previously reported by others.[38] When enterogranulate capsules of indomethacin were used, our findings supported previous observations suggesting that new formulations do not solve the problem of NSAID induced gastrointestinal damage but shift the problem to a more distal site within the gastrointestinal tract.[45] In contrast, the observation that celecoxib induced no change in small intestinal permeability compared with baseline data suggests that this may be a safer drug in terms of small intestinal damage. Whether the observed small intestinal damage is secondary to a reduction in prostaglandin synthesis or a topical effect cannot be answered by this study.

There is little published information on gastrointestinal permeability following ingestion of these new selective COX inhibitors. Using an animal model, Sigthorsson and colleagues[46] assessed the characteristics of intestinal permeability in response to nimesulide, a very weakly acidic (pKa 6.5) preferential COX-2 inhibitor. The study provided evidence of no significant changes in intestinal permeability in rats at conventional doses (15 mg/kg nimesulide). Bjarnason and colleagues[47] also demonstrated normal small bowel permeability (lactulose/L-rhamnose) in 22 healthy volunteers receiving nimesulide 200 mg/day for 10 days. Finally, the highly selective COX-2 inhibitor rofecoxib at therapeutic doses for a week had no significant effect on intestinal integrity tested by ^{51}Cr-EDTA/L-rhamnose.[48] Therefore, our results using celecoxib are in agreement with those obtained in the experimental rat by Tibble *et al* who reported no significant increase in intestinal permeability (^{51}Cr-EDTA) after this new NSAID compared with the effect of a conventional NSAID such as indomethacin.[49] With respect to meloxicam, this is the first demonstration that one of the newest preferential COX-2 inhibitors can affect small intestinal permeability.

Injury to the lower gastrointestinal tract by NSAIDs is less frequently reported, is often associated with certain type of NSAIDs (fenemate group),[50] and seems to be dose and time dependent. Sucralose, a non-digestible and poorly absorbed sweetener that passes through the digestive tract unaltered, was recently described as a marker of colonic damage.[32] Previous experience in animal models demonstrated that a significant increase in sucralose permeability was only reached after high dose indomethacin.[32] Thus considering the low doses administered in our study and the low frequency and severity of colonic lesions in response to NSAIDs, it is not surprising that we were not able to observe any differences in colonic permeability after NSAID ingestion in humans devoid of any underlying colonic disease.

In conclusion, we observed that gastric mucosal integrity was preserved by the new NSAIDs regardless of whether the protective strategy applied was pharmaceutical or pharmacological formulations. However, the acute doses used in this study demonstrated that small intestinal damage still remains a potential problem. The only exception appears to be celecoxib which, in our opinion, deserves further evaluation.

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