Antiinflammatory Drug-Induced Small Intestinal Permeability: The Rat Is a Suitable Model

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Excretion of orally administrated ^{51}Cr-EDTA as a marker of small intestinal permeability (a proposed prerequisite for human enteropathy) is increased by corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs). We have investigated the suitability of the rat as an animal model of small intestinal permeability using orally administered ^{51}Cr-EDTA. We dosed Sprague-Dawley rats with NSAIDs and corticosterone followed by ^{51}Cr-EDTA under conditions reported for humans and measured urinary excretion of the marker. In control rats, the urinary excretion of ^{51}Cr-EDTA exhibited a skewed-to-the-left frequency distribution curve with a median of 2.13% of the dose. No sex-related differences were noticed in the baseline permeability. In male rats, single therapeutically equivalent doses of indomethacin, flurbiprofen, ibuprofen, naproxen, diclofenac, sulindac, nambumetone, and corticosterone, increased the intestinal permeability by different extents with indomethacin eliciting the maximum effect, and the last four drugs showing minimal potencies. Therapeutically relevant doses of aspirin did not have any significant effect. The increase in permeability was dependent upon the NSAIDs dose. Administration of glucose/citrate, misoprostol and sulfasalazine significantly reduced the effect of indomethacin. Misoprostol antagonized the effect of naproxen but H_2-antagonists and sucralfate did not. All the above observations made in the rat were similar to those previously reported for humans. Thus the rat is a suitable model for studies of small intestinal permeability.

KEY WORDS: intestinal permeability; ^{51}Cr-EDTA; NSAIDs; small intestine; misoprostol.

INTRODUCTION

The use of non-steroidal anti-inflammatory drugs (NSAIDs) is associated with various side-effects, the most frequent being gastrointestinal (GI) disturbances (1). Numerous articles examining the gastric and duodenal damage caused by NSAIDs have been published, however, only recently have the more distal intestinal disturbances caused by these drugs received closer attention (2). It has been reported that the incidence of small intestinal ulceration in patients prescribed NSAIDs may be less common than those in the stomach or duodenum, but may be more life-threatening (2).

An inherent problem in examining adverse effects in the small intestine is that diagnosis is difficult and is usually only demonstrated after one of the complications (obstruction, perforation, or hemorrhage) becomes clinically apparent (3). However, NSAIDs cause increased small intestinal permeability and this may lead to intestinal mucosal inflammation (4). ^{51}Cr-EDTA has been the most frequently employed probe in NSAID-induced permeability studies (5–6). The ^{51}Cr-EDTA test has been shown to be reproducible and relatively specific to the small intestine. The safety, simplicity, and accuracy of the procedure meet all the requirements for a permeability test (7,8).

A present trend in NSAID development is to improve therapeutic efficacy and reduce the severity of upper GI side-effects through modification of dosage forms of NSAIDs by enteric-coating or through sustained release (SR) formulations. Indeed, enteric coating and SR formulations of several NSAIDs have resulted in a reduction in endoscopic findings in the stomach and duodenal bulb as these formulations are intended to release NSAIDs in the intestine (9). However, the more distal intestinal toxicological manifestations of NSAIDs have been largely ignored.

The rat has become an accepted model for the study of NSAID-induced adverse effects in the upper GI tract (10). However, there does not appear to have been examination of the effects of NSAIDs on small intestinal permeability in this species. Several investigators have carried out preliminary studies of the assessment of intestinal permeability in the rat (11–12) but these studies have not been extended to the examination of NSAID-induced alterations.

Given the apparent parallels between the occurrence of upper GI adverse effects between rats and humans it is of interest to examine whether the permeability changes in the small intestine between these species also parallel one another. Integration of human literature data with appropriate animal models may increase our understanding of NSAID-induced intestinal permeability. In addition, a suitable animal model for studies of intestinal permeability may help to understand the relative safety profile of NSAIDs with different pharmacokinetic characteristics and formulations with different release patterns. In search of a suitable animal model we compared characteristics of the rat intestinal permeability with those reported for humans.

MATERIALS AND METHODS

Chemicals

Indomethacin, sulindac, nambumetone, diclofenac, naproxen and corticosterone were purchased from Sigma (St. Louis, MO, USA). Flurbiprofen and ibuprofen were kindly supplied by Boots Pharmaceuticals, (Nottingham, UK), and Sanofi-Winthrop of Canada, (Ontario, Canada), respectively. Misoprostol (Cytotec, Searle, Canada), sulfasalazine (Salazopyrin, Pharmacia), sucralfate (Sulcrate, Nordic, Canada), cimetidine (Tagamet, Smith-Kline-Beecham, Canada), and famotidine (Pepcid, Merk-Sharp-Dome, Canada) were purchased from a local pharmacy. Aspirin was purchased from Mallinckrodt Chemical Works (St. Louis, MO, U.S.A.). Methyl cellulose, citric acid, and d-glucose were purchased from BDH Chemicals Canada Ltd.

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(Edmonton, Canada). ^{51}Cr-EDTA (specific activity 570 MC/mg) was purchased from Dupont NEN (Wilmington DE, USA).

Animals

Male and female Sprague-Dawley rats (wt = 300–500 g) were housed at ambient temperature and humidity in individual metabolic cages with wire mesh floors allowing for separate quantitative collection of urine and feces. Animals were fed a standard rat chow and allowed free access to food and water for the duration of the experiment.

Drugs, ^{51}Cr-EDTA Dosing, Sampling, and Assay

To test intestinal permeability, 0.5 ml of a solution containing 10 μCi/ml of ^{51}Cr complexed with EDTA was administered orally following the dose of placebo or NSAIDs. A 50 mg/kg ibuprofen dose induced measurable and significant permeability changes in the rat. Other NSAIDs therefore, were administered in doses therapeutically equivalent to that of ibuprofen from: Dose (mg/kg) = D(50/2500), where D is the recommended daily human dose of the NSAID in mg, and the recommended dose of racemic ibuprofen is 2500 mg/day/70 kg.

Each drug was suspended in 2% methylcellulose and 0.5 ml was administered to each group of rats (n = 4) at the same time of day (9 a.m.) administered orally at predetermined maximum effect time of NSAIDs (12 h prior to indomethacin and 3 h prior for others). Other drugs (misoprostol, sucralfate, sulfasalazine, cimetidine, and famotidine) were suspended in 2% methylcellulose and 0.5 ml administered thirty minutes prior to each NSAID tested. Glucose/citrate solution was administered concomitantly with indomethacin. The suspensions were delivered through an 18 gauge 5 cm curved feeding needle (Harvard Apparatus) attached to a 1 ml syringe. Urine was collected 0 to 4, 4 to 8, and 8 to 24 h following the administration of the ^{51}Cr-EDTA solution. The urine was collected in cups containing 1 ml of 1 M H_2SO_4 to inhibit microbial growth. After each collection period 10 ml of tap water was used to rinse the urine collection trays and was transferred to scintillation vials. Each experiment contained 3 to 4 untreated animals as baseline controls.

Urine samples were counted by a gamma counter Beckman Gamma 8000 (Irvine, California) for 1 minute in a counting window scanning within a range of 0–2 Mev. At least two standards of 100 μl of the administered ^{51}Cr-EDTA solution were counted with every set of urine samples. Relative permeability was determined by calculating the activity present in each urine sample as a percent of the administered dose after correcting for background radiation.

Twenty-four h fecal collections in 5 untreated male rats were obtained after oral ^{51}Cr-EDTA administration. Fecal samples were counted in a similar manner to the urine samples.

Statistical Analysis

Differences between two means were determined by Student's unpaired t-test at $\alpha = 0.05$.

RESULTS

The urinary excretion of the ^{51}Cr-EDTA was approximately evenly divided between the 0 to 4 and 4 to 8 h collection intervals although some rats produced little urine in one or the other collection interval. An excellent correlation between the urinary excretion from 0 to 8 h and that of 0 to 24 h was found for both male ($r^2 = 0.95$) and female ($r^2 = 0.98$) control rats (Fig. 1). Therefore, unless otherwise stated, the combined 0 to 8 h interval was used as a measure of ^{51}Cr-EDTA permeability. The mean baseline permeability in untreated male rats (n = 100) after oral administration was determined to be 1.78 ± 0.44% of the ^{51}Cr-EDTA dose administered. There were no differences found between the two sexes (n = 7) in baseline permeability values (Fig. 1).

The baseline permeability values in male rats from 0–24 h are positively skewed with a mean of 2.36 ± 1.13, a median of 2.13% (95% CI = 2.11–2.86), and a range of 0.70–7.68 (Fig. 2). Uniform fecal distribution of radioactivity was seen in rat feces (n = 5) and greater than 99% of the administered dose of ^{51}Cr-EDTA was recovered during the first 24 h post-test.

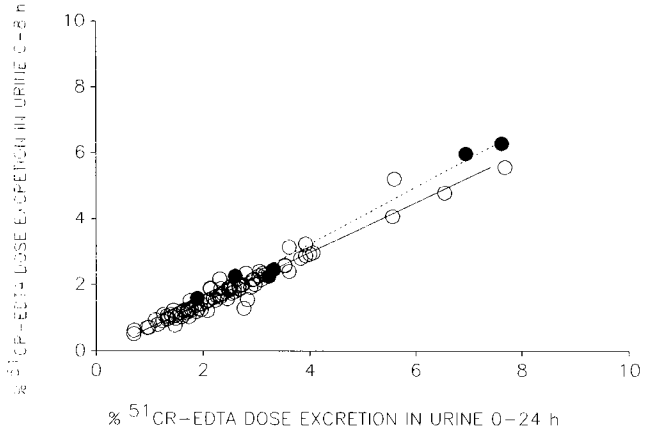

FIG 1. Correlation between 0–8 and 0–24 h urinary excretion of ^{51}Cr-EDTA in individual rats after oral administration of 0.5 ml of a solution containing 10 μCi/mL of the probe in male (○) and female (●) Sprague-Dawley rats.

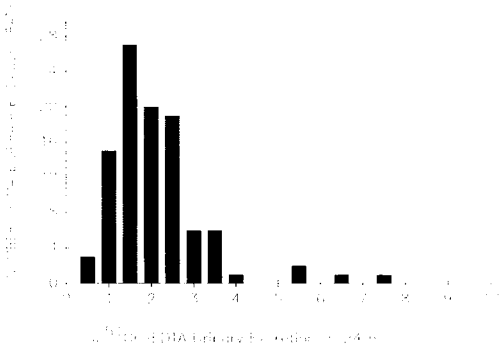

FIG 2. Frequency distribution histogram of baseline ^{51}Cr-EDTA urinary excretion in control male Sprague-Dawley rats after oral administration of 0.5 ml of a solution containing 10 μCi/ml of the probe.

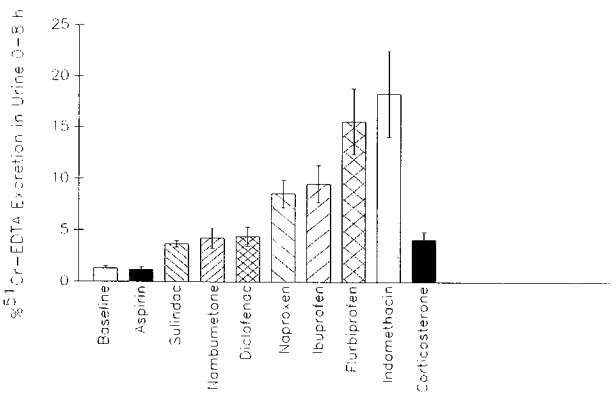

FIG 3. Mean (± s.e.m., n = 4) intestinal permeability measured as % urinary excretion of ^{51}Cr-EDTA following oral administration of single doses of various antiinflammatory drugs. All treatments are significantly different from baseline except aspirin. Doses in (mg/kg): Aspirin (42), Sulindac (8.3), Nambumetone (42), Diclofenac (4), Naproxin (20), Ibuprofen (50), Flurbiprofen (5), Indomethacin (10), and Corticosterone (10).

Fig. 3 summarizes the NSAID-induced permeability data in male Sprague-Dawley rats using ^{51}Cr-EDTA. The studies in rats demonstrate an increased intestinal permeability after oral NSAID administration except for aspirin. The ^{51}Cr-EDTA excretion in urine also appears to be dose-dependent for ibuprofen, naproxen, and indomethacin (Fig. 4). Indomethacin induces the highest permeability changes (Fig. 4).

Further, the oral administration of 10 mg/kg corticosterone, a corticosteroid, also appeared to induce significant permeability changes in the rat with 4.13 ± 0.7% (n = 4) excreted into urine (Fig. 3).

The indomethacin-induced permeability changes in the rat model appear to be reduced by co-administration of a stable PGE_1 analog (misoprostol), glucose/citrate, and sulfasalazine but not by famotidine (Fig. 5).

Naproxen-induced changes in intestinal permeability are not antagonized by H_2-antagonists or sucralfate but are significantly reduced by misoprostol administration (Fig. 5).

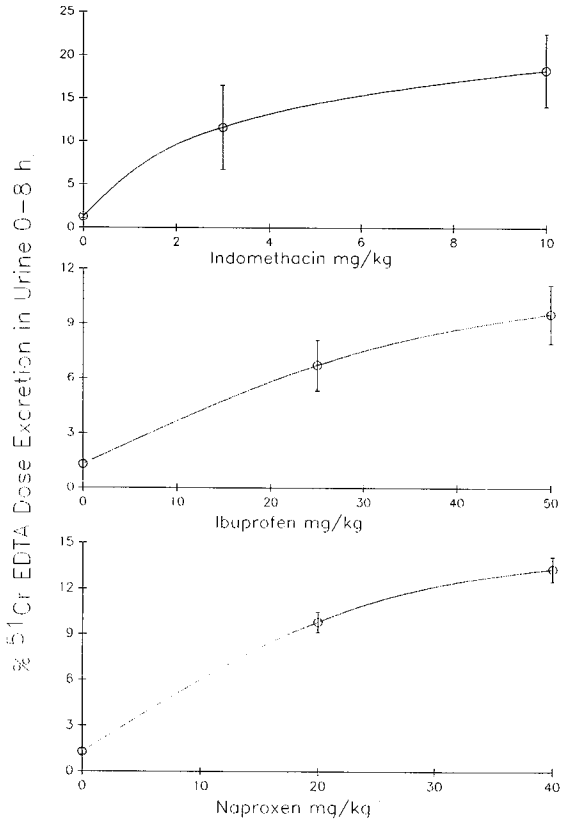

FIG 4. Dose dependency of the mean (± s.e.m., n = 4) intestinal permeability after single oral doses of indomethacin, ibuprofen and naproxen. All treatments are significantly different from baseline.

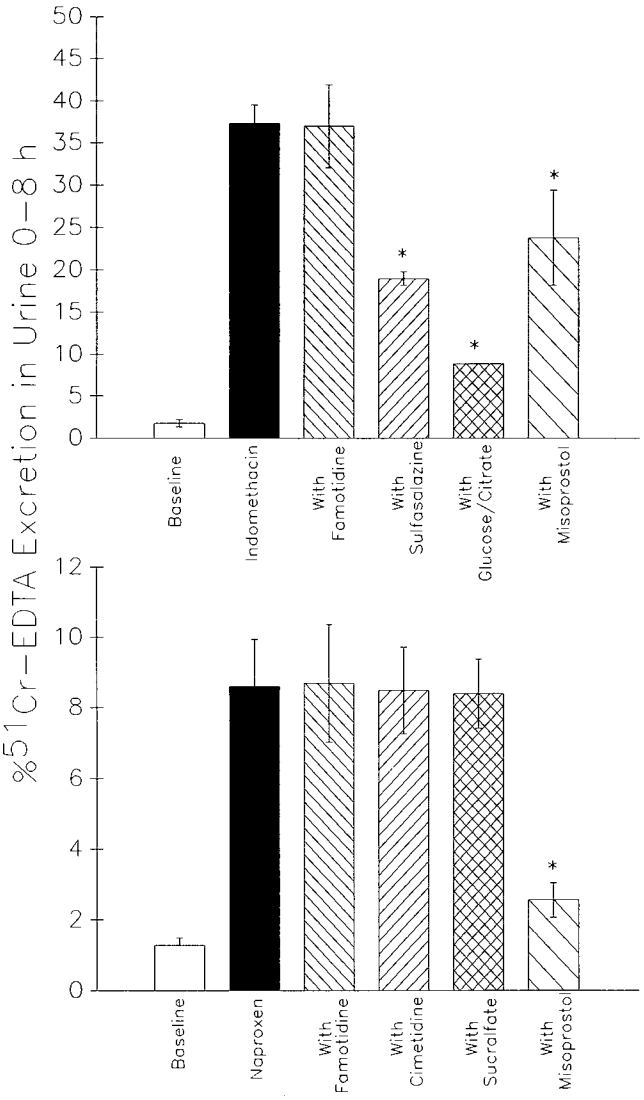

FIG 5. Top: Mean (± s.e.m., n = 4) intestinal permeability after 20 mg/kg single oral doses of indomethacin in rats dosed concomitantly with 170 μg/kg misoprostol, 90 mg of both glucose and citrate, 1 mg/kg famotidine and 84 mg/kg sulfasalazine. Bottom: 20 mg/kg oral naproxen in rats dosed with 170 μg/kg misoprostol, 40 mg/kg sucralfate, 10 mg/kg cimetidine, and 1 mg/kg famotidine. All treatments are significantly different from baseline. * Denotes significantly different from indomethacin or naproxen alone. All treatments are significantly different from baseline.

DISCUSSION

The intestinal permeability of ^{51}Cr-EDTA in 12 normal rats was previously determined to be 2.06 ± 0.44 over 5 h following intragastric administration (11). Additionally, the mean % of a ^{51}Cr-EDTA dose by gavage from 0–24 has previously been reported to be 1.6% (12). Thus our findings (Figs. 1 and 2) are consistent with previously reported results.

Similar to our observations in the rat (Fig. 2), the frequency distribution of baseline permeability values in 65 healthy male volunteers humans from 0–24 h are also positively skewed with a median of 2.45% (95% CI = 2.11–2.86) (8). In humans there is a good linear relationship between the 0–6 and 6–24 h cumulative excretion of ^{51}Cr-EDTA (5,13) which parallels the results in the rat model (Fig. 1). Further, there does not appear to any sex related differences in baseline permeability values in human studies (13) which parallels the results we have seen in male and female Sprague-Dawley rats (Fig. 1).

It is believed that ant-inflammatory drugs may modulate the small intestinal tight junction thereby inducing increases in paracellular permeability (5). Increased intestinal permeability is suggested to be a prerequisite to NSAID-induced ulceration (2). Similar to our observations in the rat (Fig. 3), NSAIDs enhance human intestinal permeability as has been reported for indomethacin (4–5, 14–19), naproxen (7–8,20–23), flurbiprofen (6), ibuprofen (4–5), and diclofenac (6). After therapeutically equivalent doses, the highest potency was observed with indomethacin and flurbiprofen. Interestingly, aspirin, a very potent inhibitor of cyclooxygenase has little or no effect on the intestinal permeability in both humans (5–6) and rats (Fig. 3). This may be due to the rapid absorption of aspirin from the upper part of the GI tract and its efficient hydrolysis to salicylic acid (24) which limit both direct exposure of the more distal intestine to the drug and the availability of aspirin for systemic distribution into the intestinal mucosa. Salicylic acid is a very weak inhibitor of cyclooxygenase. The concentrations required for 50% inhibition of cyclooxygenase 1, have been determined to be 0.3 and 35 mg/L for aspirin and salicylic acid, respectively (25). Nevertheless, the minimal effect of aspirin on the intestinal permeability, despite the well documented ulcerogenic effect of the drug on the upper GI is a common observation in humans (4) and rats.

The prodrugs, sulindac and nambumetone increased intestinal permeability significantly but to a limited extent (Fig. 3). In human studies, the increasing effect of these two drugs did not reach statistical significance (16,19). This may suggest a higher sensitivity for the rat model to detect increased intestinal permeability as compared with humans. Indeed, a comparison of human and rat data indicates less variability in response for the latter species.

The effect of indomethacin (5), naproxen (5,8), and ibuprofen (5) on human intestinal permeability has been shown to be dose dependent. Similarly, in the rat, elevation of the dose of these drugs resulted in further increases in the urinary excretion of ^{51}Cr-EDTA (Fig. 4).

The regulation of intestinal permeability may be, at least in part, regulated by prostaglandins (26). However, Bjarnason et al (4) who examined the influence of concomitant administration of prostin E_2, a naturally occurring prostaglandin, did not notice a reduction in the NSAIDs-induced permeability changes perhaps due to the instability of the preparation. However, it did seem that prostaglandin E_2 itself significantly decreased baseline absorption of ^{51}Cr-EDTA (4). On the other hand, misoprostol which had no effect on permeation of ^{51}Cr-EDTA when given alone, partially protected the small bowel mucosa from the effects of indomethacin (14). The protective effect of misoprostol seems to be dose-dependent as 800 μg misoprostol doses did not alter the effect on either indomethacin (16) or naproxen (21) while 1200 μg doses partially blocked the effect of indomethacin (14). The observations in the rat model (Fig. 5) are consistent with the findings in humans.

Similar to the observations made in humans, indomethacin (5) and naproxen (22–23) effects on permeability were not reduced in the rat by H_2-antagonists (Fig. 5). H_2-antagonists may reduce the incidence of gastric ulceration by elevating the gastric pH whereas intestinal damage appears to be pH independent. Similar to the results found in humans with naproxen (7), effects on permeability were not reduced in the rat by sucralfate (Fig. 5). Sucralfate may reduce the incidence of gastric ulceration by cytoprotection in the stomach whereas it appears to afford no protection in the intestine.

In parallel with the results found in humans, indomethacin (18) effects on permeability were reduced in the rate by sulfasalazine (Fig. 5). It has been speculated that sulfasalazine may limit neutrophil recruitment to sites of inflammation and thereby ameliorate the inflammatory process (2).

In addition to inhibiting prostaglandin synthesis, NSAIDs may inhibit glycolysis and the tricarboxylic acid cycle resulting in inhibition of oxidative phosphorylation thereby reducing adenosine triphosphate (ATP) production damaging the enterocyte leading to cell death (2). It has been suggested that the presence of these sugars in the intestinal lumen may modify the reaction to indomethacin or that citrate may be cytoprotective against free radical damage caused by NSAIDs (17). In humans, a formulation containing 15 mg glucose and 15 mg citrate to each mg of indomethacin prevented the expected effect of the NSAID on the intestinal permeability (17). A similar observation was made in the rat (Fig. 5). In addition, the protective effects of glucose/citrate have also been previously demonstrated in NSAID-induced intestinal ulceration in the rat (27).

Mielants et al. (13) report that there were no significant difference in altered gut permeability between patients taking NSAIDs and patients taking corticosteroids. This further suggests that alteration of gut permeability may not only be accounted for by an inhibition of mucosal cyclooxygenase activity but that other pathways in the arachidonic cascade might be implicated. We observed increased intestinal permeability after administration of corticosteroids to the rat (Fig. 3).

Tests of intestinal permeability have been employed in a wide array of applications in the investigation of intestinal disease (28). These tests are safe, well tolerated, reproducible, and easy to perform and because of their non-invasive nature can be easily applied to diagnostic screening and research, and could possibly replace the need for invasive investigations of small intestinal disease such as radiology, bi-

opsy and enteroscopy. An implicit advantage of permeability tests is that they reflect the functional integrity over a major area of the small-intestinal mucosa, whereas biopsy may suffer from sampling error if damage is distributed randomly to inaccessible areas.

The use of ^{51}Cr-EDTA urinary excretion test has previously been questioned in the claim that NSAIDs may increase glomerular filtration and that this could account for the increased urinary excretion of the probe (29). However, subsequent studies have proven this concern to be unwarranted (30).

Given the well documented changes in small intestinal permeability parallel with the increasing body of NSAID related GI side-effects in the literature (2); the rat model may help understand the mechanisms of action of NSAIDs in producing GI abnormalities. The rat appears to be a suitable animal model to study NSAID-associated changes in intestinal permeability since it seems to respond in a similar fashion to the changes described in humans.

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