Influence of Dosage Form on the Gastroenteropathy of Flurbiprofen in the Rat: Evidence of Shift in the Toxicity Site

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Purpose. Gastroduodenal and intestinal permeability were compared after single doses of sustained release and regular release flurbiprofen in the rat to assess possible site-specific formulation-dependent toxicity.
Methods. Pharmacokinetics was assessed and gastrointestinal permeability was evaluated using sucrose and ^{51}Cr-EDTA as gastroduodenal and intestinal permeability probes, respectively.
Results. The two formulations demonstrated equal areas under the flurbiprofen concentration-time curve. The sustained release formulation peaked 2–3 h slower with 57–74% lower concentrations than regular release formulation. In comparison, the regular release powder induced greater gastroduodenal permeability while sustained release granules induced greater intestinal permeability. When S-flurbiprofen concentrations were plotted versus intestinal permeability, a linear relationship and an anti-clockwise hysteresis were obtained for regular and sustained release formulations, respectively.
Conclusions. Sustained release formulations of flurbiprofen demonstrate reduced gastroduodenal permeability but shift the site of this side-effect to the more distal intestine.

KEY WORDS: non-steroidal antiinflammatory drugs; gastroduodenal; intestinal permeability; flurbiprofen.

Non-steroidal antiinflammatory drugs (NSAIDs) which are commonly associated with upper gastrointestinal tract side-effects, have recently been shown to cause significant and life-threatening mucosal damage within the small and large intestine (1).

NSAID-induced toxicity in the small intestine was initially observed in animal studies conducted 28 years ago on inflammation and ulcers (2). It has been subsequently suggested that NSAID-induced small intestinal damage in rats resembles NSAID-induced enteropathy of the human intestine (3). It has also been demonstrated that NSAID induced increased gastroduodenal and intestinal epithelial permeability is a pre-requisite to the development of inflammation and ulceration (4,5). Gastrointestinal permeability tests, therefore, are considered as facile and sensitive non-invasive markers of NSAID-induced gastrointestinal damage (4–6). These tests are non-invasive and involve determination of the urinary excretion of orally administered non-absorbable probes such as polysugars. We have demonstrated that the rat is a suitable animal model for evaluating NSAID-induced gastroduodenal (7) and intestinal (8) permeability changes as measured by urinary excretion of sucrose and ^{51}Cr-EDTA, respectively.

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To improve therapeutic efficacy and reduce the severity of upper gastrointestinal side-effects, modified dosage forms of NSAIDs such as enteric-coated and sustained release formulations have been developed. The therapeutic rationale behind these new formulations has not been unequivocally proven, instead it has been suggested that these formulations may increase the exposure of active drug to the mucosa distally to the duodenal bulb, and thereby increase toxicity to this region where the effects are difficult to monitor (9).

The objectives of this work included 1) an examination of the possibility of formulation-dependent toxicity of flurbiprofen, a member of the 2-arylpropionic acid NSAIDs, throughout the gastrointestinal tract as assessed by the urinary excretion of sucrose and ^{51}Cr-EDTA, 2) delineate the possibility of a relationship between the pharmacokinetics of a sustained release and regular release formulation and increased intestinal permeability.

METHODS

Animals

Male Sprague Dawley rats (250–300 g) were fed with commercial diet (Purina Rat Chow, Ralston Purina, St. Louis, MO). Rats were allowed free access to both food and water for the duration of the experiments and housed at ambient temperature and humidity with a 12 h light-dark cycle. All experimental procedures were approved by the Animal Care Committee of the University of Alberta.

Chemicals

Racemic flurbiprofen and sucrose were purchased from Sigma Chemical Company (St. Louis, MO). Flurbiprofen sustained release 200 mg capsules (Organon Canada Ltd., Westhill, Canada) were purchased from the University of Alberta Health Services Pharmacy. ^{51}Cr-EDTA (specific activity 570 MCi/mg) was from NEN Dupont (Wilmington, DE).

Dosage Forms and Administration

Rats were dosed orally with 10 mg/kg flurbiprofen via gastric intubation. The formulations were either flurbiprofen powder suspended in 0.5 mL of 2% (w/v) methylcellulose or sustained release granules contained inside a commercially available capsule (Organon, Westhill, Canada) followed by 0.5 mL of 2% (w/v) aqueous methylcellulose solution. A single dose of 10 mg/kg racemic flurbiprofen was chosen since it had previously been shown to induce significant and measurable intestinal permeability at approximately 50% of the maximum effect (8).

Pharmacokinetic Studies

As described elsewhere (11) male Sprague-Dawley rats were cannulated in the left jugular vein. Two groups of rats (n = 4 for each group) were dosed orally with flurbiprofen powder or sustained release granules. Whole blood samples were withdrawn from the cannula at 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12, 24, 36, and 48 h after drug administration, plasma separated and stored at −15°C until analysis. A stereospecific

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assay was used for quantification of flurbiprofen enantiomers (10) and pharmacokinetic indices were calculated (11).

Pharmacodynamic Study

To establish baseline permeability values, control rats received either sucrose or ^{51}Cr-EDTA in the absence of flurbiprofen. The effect of the NSAID on the permeability was examined using rats, which received flurbiprofen followed by either sucrose or ^{51}Cr-EDTA.

Assessment of Gastroduodenal Permeability

Gastroduodenal permeability was assessed using a previously described method using the urinary excretion of orally administered sucrose as a marker (7). Flurbiprofen dosage forms were administered orally to each group of rats (n = 4) at the same time of day (9 a.m.) 1 h prior to the sucrose solution (predetermined time of maximum effect (7). Urine was collected 0 to 8 h following the administration of the sucrose solution and individual volumes measured. Permeability was determined by calculating the sucrose present in each urine sample as a percent of the administered dose after correcting for baseline levels of glucose and sucrose present in urine for each individual rat (7).

Assessment of Intestinal Permeability

Intestinal permeability was assessed using ^{51}Cr-EDTA as a surrogate marker (8). Urine was collected from 0 to 8 h following oral administration of ^{51}Cr-EDTA and counted directly in a Gamma counter. The relative permeability was calculated as a percentage by dividing the count/min present in 0 to 8 h samples by that of the dosing solution after correcting for background radiation. To study the time course of the permeability changes, ^{51}Cr-EDTA was administered 0, 1, 2, 3, 4, 6, 12, 24, 36, 48, or 72 h (n = 4 at each time point) after flurbiprofen administration to individual rats and the 0–8 h urinary excretion of the marker was measured.

Statistical Analysis

Differences between two means were determined by Student's unpaired t-test. Differences between more than two means were determined by one-way ANOVA followed by Duncan's multiple range test at $\alpha = 0.05$. Data are presented as mean ± standard deviation.

RESULTS

Pharmacokinetics

Following administration of the regular release suspension, the plasma concentration of both enantiomers of flurbiprofen peaked rapidly in 0.25 to 0.5 h (Fig. 1a). Following administration of the sustained release formulation, on the other hand, both enantiomers of flurbiprofen exhibited slow absorption rates as reflected in significantly longer T_{MAX} values (2.25–3.5 h) and lower C_{MAX} values as compared with the regular release form (Fig. 1b). There was no significant difference between the $AUC_{0-\infty}$ values between the powder and the sustained release granules (R, 76 ± 20; S, 215 ± 55 and R, 84 ± 12; 225 ± 46 mg.h.L^{-1}, respectively). The plasma concentration of the S enantiomers was significantly higher than that of the R enantiomer. The S:R plasma concentration ratio was not significantly different between the two formulations. The terminal t1/2 of R and S were approximately 5.0 and 7.5 h, respectively, with no significant differences between the products. The observed pharmacokinetic indices were very close to those reported earlier (11).

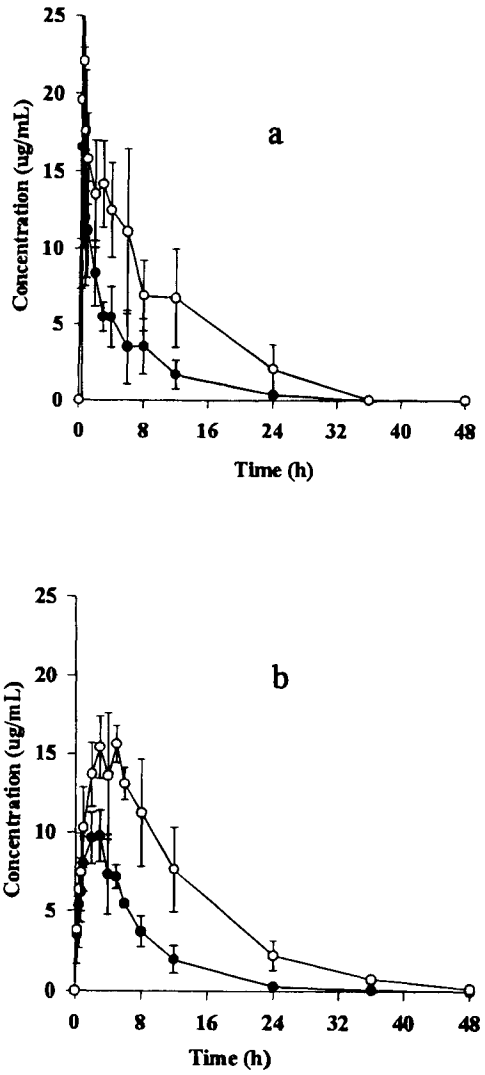


Fig. 1. Mean (±SD, n = 4) plasma concentration vs time curves of S- (open circles) and R-flurbiprofen (closed circles) in rats dosed orally with 10 mg/kg racemic flurbiprofen as regular release powder (a) or sustained release granules (b).

Gastroduodenal and Intestinal Permeability

Both the regular release and sustained release flurbiprofen treatments significantly increased gastroduodenal permeability above baseline values 1 h post-dose (Fig. 2). This effect was, however, significantly greater for regular release as compared with the sustained release formulation.

The urinary excretion of ^{51}Cr-EDTA as a measure of intestinal permeability was significantly increased following administration of both formulations (Fig. 3). Both treatments resulted

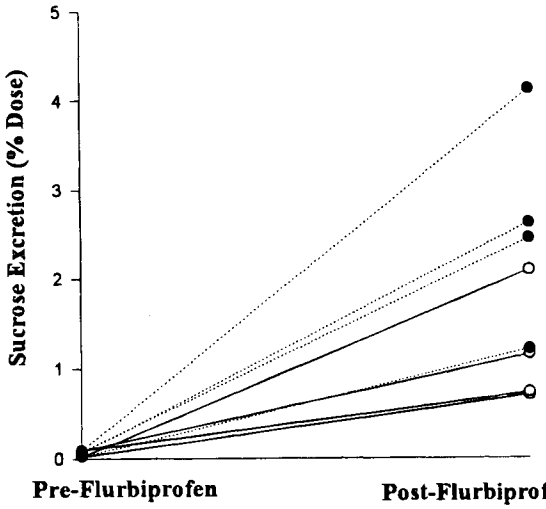


Fig. 2. Increased gastroduodenal (sucrose) permeability induced by regular (closed circles) and sustained release (open circles) flurbiprofen formulations one h after the NSAID administration. Each circle represents one individual rat. The two observations were significantly different.

in a rapid and significant increase in intestinal permeability beginning 1 h post-dose and reaching maximal values 1 h and 5 h post-dose for the regular and sustained release formulation, respectively. Intestinal permeability returned to control values 48 h following the regular release powder but remained above base-line values even after 72 h following sustained release granules. The time course of permeability of the two groups did not overlap one another. In particular the effect was significantly higher for the sustained release granules at 10, 48 and 72 h post-dose as compared with the powder.

When the mean time courses of the change in intestinal permeability (Fig. 4) for the regular release suspension were plotted against the mean S-flurbiprofen plasma concentration

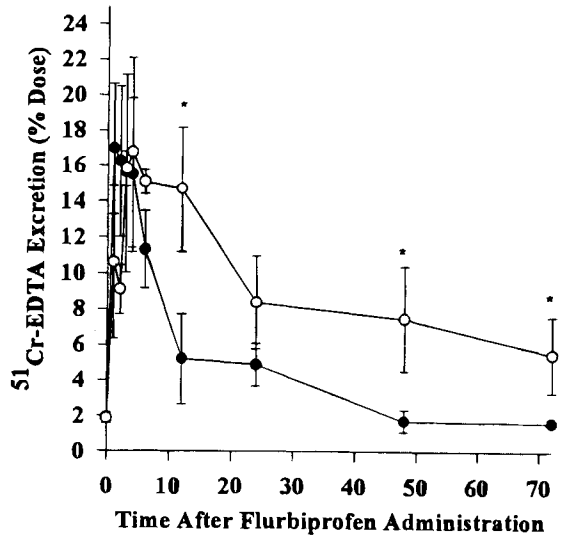
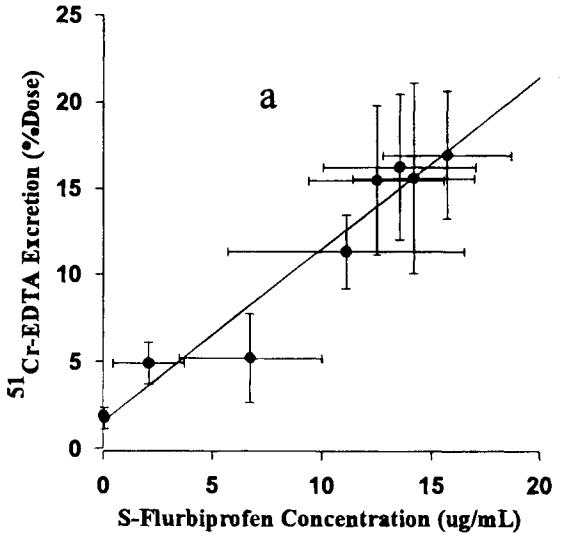


Fig. 3. Mean (±SD, n = 4) time courses of intestinal (^{51}Cr-EDTA) permeability changes resulting from the administration of 10 mg/kg flurbiprofen as either regular release powder (closed circles) or sustained release granules (open circles). * denotes a significant difference.



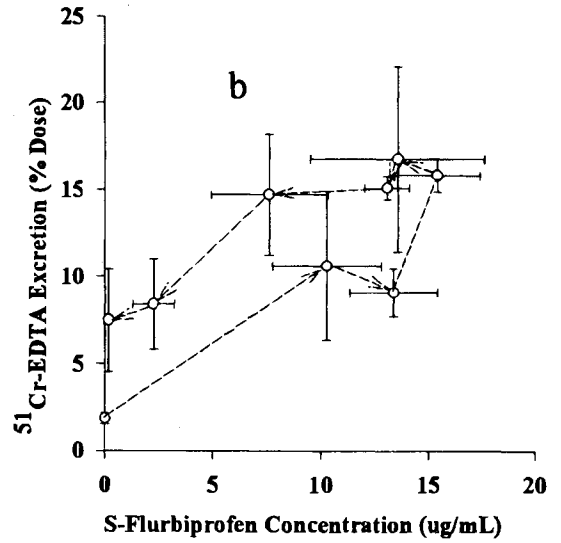


Fig. 4. Change in intestinal permeability vs S-flurbiprofen plasma concentration following oral doses of 10 mg/kg racemic flurbiprofen either as regular release powder (a) or sustained release granules (b). In b the points are joined in temporal order.

a linear relationship (r^2 = 0.95) was found (Fig. 4a). This relationship, on the other hand, was of a counterclockwise hysteresis type for the sustained release formulation (Fig.4b).

DISCUSSION

Both sucrose and ^{51}Cr-EDTA cross the gastrointestinal tract of healthy subjects only minimally. Upon administration of NSAIDs, on the other hand, the gastrointestinal permeability of these markers increases. An increased urinary excretion of sucrose indicates increased permeability of the gastroduodenal site due to the fact that, in the distal intestine, sucrose is immediately hydrolyzed (5,7). Enhanced urinary excretion of ^{51}Cr-EDTA, however, reflects mainly increases in the permeability of the more distal intestine due to the small surface area of the

gastroduodenal segment and short drug residence time therein (4). The gastroduodenal permeability of sucrose after administration of the regular release flurbiprofen powder was significantly and substantially higher than after the sustained release formulation (Fig. 2). This is postulated to be a reflection of the difference between the release rate of the products. Upon administration of a regular release, but not the sustained release, the gastroduodenum is exposed to a high concentration of the NSAID. Following the sustained release granules, the site of increased permeability was shifted from gastroduodenum to the lower intestine as demonstrated by greater urinary excretion of ^{51}Cr-EDTA as compared with the regular release powder (Fig. 3). Regular release flurbiprofen is rapidly absorbed so that the only flurbiprofen that reaches the small intestine is through minor biliary excretion and systemic circulation. The observed shift in the site of flurbiprofen effect may be a reflection of limited release of flurbiprofen from the sustained release product in the gastroduodenum but gradual release in the intestine. The sustained release granules, indeed, demonstrated a slower but more continuous pattern of absorption than the regular powder (Fig. 1). The sustained presence of flurbiprofen in the intestine coupled with the circulating concentration of the drug is likely responsible for the observed substantially greater overall excretion of ^{51}Cr-EDTA following administration of the granules as compared with the powder (Fig. 3). These results indicate that the gastrointestinal damage of flurbiprofen is, indeed, formulation dependent.

Similar observations have been made for diclofenac in humans (12). Choi *et al.* have recently shown that permeability of ^{51}Cr-EDTA is significantly increased following administration of a sustained release formulation of diclofenac but not after a regular release capsule (12). It is realized that permeability tests are only surrogate markers of NSAID-induced gastrointestinal damage such as ulceration (6). Nevertheless, our observations appear to agree with clinical observations of distal intestinal damage induced by sustained release NSAIDs (9,13). In addition, in a retrospective study of 1609 patients with reported NSAID-induced adverse effects, Figueras *et al.* observed a significantly greater risk associated with sustained release as compared with regular release formulations of NSAIDs (14).

Flurbiprofen is a racemic NSAID for which both the antiinflammatory and cyclooxygenase inhibitory effects are ascribed mainly to the S enantiomer (15). Following administration of the regular release powder, a direct and significant relationship was observed between the plasma concentration of the main pharmacologically active enantiomer of flurbiprofen and the changes in intestinal permeability (Fig. 4a). This is not surprising since the effect of a rapidly absorbed formulation of flurbiprofen is likely through its systemic distribution into the gut epithelial cells rather than local exposure. After the sustained release formulations, however, the S-flurbiprofen concentration-effect plot demonstrated an anticlockwise hysteresis when the data points were connected in temporal order (Fig. 4b). This may suggest that the intestinal permeability changes by the sustained release formulation is not only due to the systemic availability of the NSAID since it may be also resulted from continuous exposure of the intestinal tract to the drug.

Although the more distal intestinal toxicological manifestations of sustained release NSAID formulations have been largely ignored, the likelihood of its increased occurrence with more frequent use of these medications exists (13,14). In addition, since cost containment of pharmaceuticals is of current interest to health-care systems, the use of generally more expensive sustained release formulations needs justification. These data suggest that sustained release NSAIDs do not solve the problem of NSAID-induced gastrointestinal toxicity but merely shift the problem to a more distal site within the gastrointestinal tract. In order to properly assess the contribution of the drug and formulation on the overall toxicity of NSAIDs, the entire gastrointestinal tract should be examined. In addition, the issue of therapeutic relevance should be a major consideration in development of modified release formulations.

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