Evaluation of gastrointestinal toxicity of ibuprofen using surrogate markers in rats: Effect of formulation and route of administration

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Abstract

Objective

To elucidate the mechanism of gastrointestinal (GI) toxicity of ibuprofen and to examine the effect of altered site of drug release using gastroduodenal and intestinal permeability tests in the rat model.

Methods

Adult male Sprague-Dawley rats were administered (n = 6 per group) either: (1) 100 mg/kg immediate or sustained release ibuprofen; (2) 100 mg/kg immediate release and ibuprofen lysinate; or (3) 100 mg/kg or 200 mg/kg ibuprofen po *or* sc. *Upper and lower GI permeablity as a surrogate marker of toxicity were determined at pre-determined times using the urinary excretion of orally administered sucrose and ^{51}Cr-EDTA permeability probes, respectively.*

Results

Ibuprofen administration resulted in a dose-dependent increase in both upper and lower permeability of the GI tract. Both immediate and sustained release preparations of ibuprofen increased upper and lower GI permeability with no shift of toxicity to the site of drug release. Ibuprofen lysinate also induced significant increased upper and lower GI permeability comparable to immediate release ibuprofen. Oral doses were not more toxic than sc *doses.*

Conclusion

Ibuprofen-induced increased GI permeably appears to be independent of the type of formulation and route of administration. This indicates that, contrary to some other nonstereoidal anti-inflammatory drugs, ibuprofen's effect on GI permeability is mainly systemic and the direct local effect contributes minimally to its overall GI toxicity. Ibuprofen may be a suitable candidate for sustained release formulations since its effect may be prolonged without the danger of a shift of side effect from the upper to the lower GI tract.

Key words

NSAID, GI toxicity, permeability, ibuprofen.

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Received on April 30, 1999; accepted in revised form on August 26, 1999.

Introduction

Ibuprofen is a 2-phenylpropionic acid derivative with powerful analgesic and anti-inflammatory activities and is used extensively in the treatment of osteoarthritis and rheumatoid arthritis, and as an analgesic. Similar to other nonsteroidal anti-inflammatory drugs (NSAIDs), the most frequent and important adverse effect of ibuprofen is in the gastrointestinal (GI) tract (1). The GI toxicity of NSAIDs results from: (1) a direct local contact effect that can be attributed to a combination of local irritation of the NSAID and inhibition of prostaglandin synthesis at the GI site, and (2) a general systemic effect which is manifested after all routes of drug administration (2).

A number of different methods have been developed to assess GI toxicity (e.g., radiology, endoscopy, and permeability tests). The permeability tests using sucrose and ^{51}Cr-ethylenediaminotetraacetic acid (^{51}Cr-EDTA) as probes offer valid diagnostic methods that detect NSAID-induced injury in the upper (3) and lower (4) GI tract, respectively. The intact mucosa is normally poorly permeable to these probes. With the loss of integrity of the mucosal barrier caused by NSAID, these probes will pass through the surface epithelium, gain access to the blood, and ultimately appear in the urine. The amount of excreted sucrose and ^{51}Cr-EDTA in the urine is proportional to the degree of upper and lower GI damage, respectively. Sucrose is rapidly broken down in the small intestine by sucrase to its monosaccharid constituents, glucose and fructose. Therefore, sucrose excretion in the urine indicates permeation from the gastroduodenal tract. There is a significant correlation between GI endoscopic grades and the extent of sucrose permeability (3). On the other hand, ^{51}Cr-EDTA is a sensitive and reliable intestinal permeability probe. The ^{51}Cr-EDTA permeability changes which are probably due to NSAID-induced altered permeability of epithelial tight junctions (5) correlate well with ulceration and GI blood loss (4).

Ibuprofen is a frequently used anti-inflammatory, analgesic and antipyretic NSAID. Although safer NSAIDs, i.e., selective cyclooxygenase 2 inhibitor agents (6) have become available, ibuprofen is expected to continue to be a commonly used drug at least as an over-the-counter analgesic. A considerable number of different preparations of ibuprofen are being consumed every day. Attempts have been made to improve the efficacy and safety profile of ibuprofen by altering its absorption kinetics, such as preparation of sustained release formulations or ibuprofen lysinate (7, 8). As has been shown for flurbiprofen (9) and tiaprofenic acid (10), however, the modification of NSAID release may shift the toxicity to the site of drug release. Hence the rationale for the use of modified release formulations of NSAIDs has been questioned (11, 12). Altered release may influence the toxicity due to direct contact, but should have no effect on the toxicity that is contributed by the systemic inhibition of the prostaglandin synthesis. Therefore, it might be possible to delineate the mechanism of NSAID-induced GI toxicity by using different formulations and routes of administrations. The objective of this study was to elucidate the mechanism of induced GI toxicity of ibuprofen and to examine the effect of altered site of drug release. This was achieved by studying the pattern of increased GI permeability as a surrogate marker of GI toxicity (13, 14) caused by different preparations of ibuprofen, i.e., immediate release powder, sustained release granules, crushed ibuprofen lysinate tablets and also the effect of the route of administration.

Materials and methods

Chemicals

Racemic ibuprofen powder B.P/U.S.P. was obtained from Wintrop Laboratories (Newcastle, England). Sustained release granules of ibuprofen were from Apotex (Weston, Canada). Methyl cellulose, D-glucose were purchased from BDH Chemicals (Edmonton, Canada). Sucrose was purchased from Aldrich Chemical Company Inc (Milwaukee, WI, USA). ^{51}Cr-EDTA was purchased from Dupont NEN (Wilmington, DE, USA). Trinder's Reagent and lysine were obtained from Sigma (St. Louis, MO, USA). Ibuprofen lysine (dolormin) was purchased from Woelm Pharma GmbH & Co (Eschwege, Germany). Other reagents used in the study were of analytical grade.

Animals

Adult male Sprague-Dawley rats were housed at ambient temperature in individual metabolic cages (Fisher Scientific, Edmonton, Canada). Animals were fasted overnight and during the experiment with free access to water. The experiment was approved by the Animal Care Committee of the University of Alberta.

Formulations

Oral doses were administered to rats by oral gavage. An immediate release ibuprofen preparation was made by suspending ibuprofen powder in 1% methylcellulose. This formulation had an *in vitro* dissolution time for 50% ibuprofen (t50%) of 6 min. The time to reach the peak concentration (t_{max}) was 1 hr. For the dry sustained release ibuprofen granules t50% = 42 min and t_{max} = 2.5 hr (15). In humans, ibuprofen lysinate has a faster dissolution, and hence a more rapid absorption (t_{max} = 0.55 hr) than immediate release ibuprofen (t_{max} = 0.89 hr) (7, 8). Crushed ibuprofen lysinate was introduced into the stomach by a flexible plastic tube attached to the oral gavage, followed by 0.3 ml water. Water, methylcellulose, or lysine was used as placebo in control rats. Subcutaneous (*sc*) ibuprofen solution was prepared by dissolving ibuprofen powder in 1 ml ethanol, diluting, and adjusting its pH to 9 with sodium bicarbonate. The *sc* vehicle was used in control rats.

Effect of the formulation on GI permeability

To test immediate versus sustained released ibuprofen, 3 groups of 6 rats (300-350 g) were dosed orally with 100 mg/kg of immediate release ibuprofen, sustained release ibuprofen, or placebo. At 1, 3, and 8 hours post-dose, 1 ml of the permeability marker syrup containing 1 g sucrose and 10 µ Ci ^{51}Cr- EDTA was administered by oral gavage and urine was collected for 8 hrs.

To test ibuprofen lysinate versus immediate release, rats (6 per group) weighing 300-350 g were dosed orally with 100 mg/kg ibuprofen as either ibuprofen lysinate or immediate release ibuprofen. GI permeability changes were assessed after 1 hour. Control rats received either 1% methylcellulose or 75 mg/kg lysine.

To test the effect of the dose and route of administration on GI permeability, rats (6 per group) with a weight of 250-300 g were dosed with ibuprofen 100 and 200 mg/kg either orally or *sc*. GI permeability changes were assessed at the maximum effect time (15), i.e. after 1 hour. Control rats received either 1% methylcellulose or an *sc* vehicle.

^{51}Cr-EDTA and sucrose permeability assessment

Relative permeability was assessed by calculating the percent of administered dose of sucrose and the activity of ^{51}Cr-EDTA excreted in urine over 8 hrs. An 8 hour collection period was shown to be adequate (4, 14). The time post-dose administration of permeability probes was selected according to the release profile of the examined formulations. Sucrose and ^{51}Cr-EDTA were measured using a spectrophotometer (13) and gamma counting (14), respectively.

Statistical analysis

Differences between two means were determined by the Student's unpaired t-test. One way ANOVA followed by Duncan's New Multiple Range test was used to determine the difference between more than two means. A $p < 0.05$ was considered significant. Data are presented as means ± the standard error of the mean.

Results

Both sustained and immediate release preparations significantly increased upper GI permeability at 1 hr post-dose as compared with control rats (Fig. 1). At 3 hrs post-dose only the sustained release formulation induced a significant increase in upper GI permeability. The immediate release preparation, on the other hand, significantly increased lower GI permeability at 1 hr, while the sustained release preparation did so at 3 hrs. Following both preparations, both upper and lower GI toxicity returned to baseline

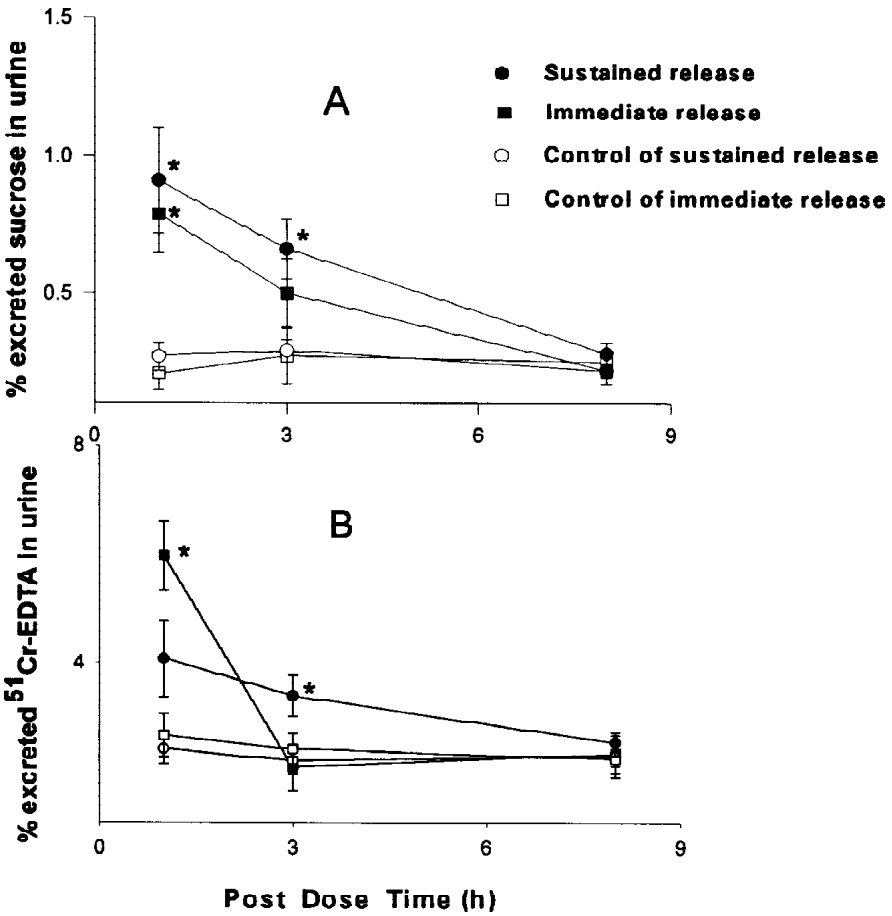

Fig. 1. Sucrose (gastroduodenal) and ^{51}Cr-EDTA (lower GI tract) permeability at 1, 3, and 8 hours after administration of 100 mg/kg ibuprofen in the form of immediate release or sustained release preparations. Control rats received placebo in either immediate release or sustained release form. * Significantly different from control. Error bars represent standard error of the mean.

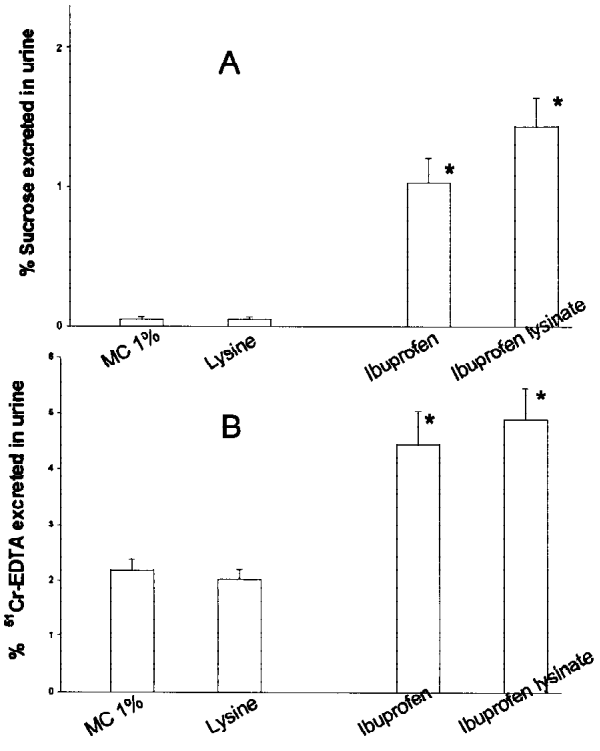

Fig. 2. Sucrose (gastroduodenal), and ^{51}Cr-EDTA (lower GI tract) permeability at 1 hour after the administration of 100 mg/kg ibuprofen in the form of either immediate release or ibuprofen lysinate preparations. Control rats received 1% methylcellulose or lysine. The results for ibuprofen and ibuprofen lysinate were not significantly different.
* Significantly different from control. Error bars represent the standard error of the mean.

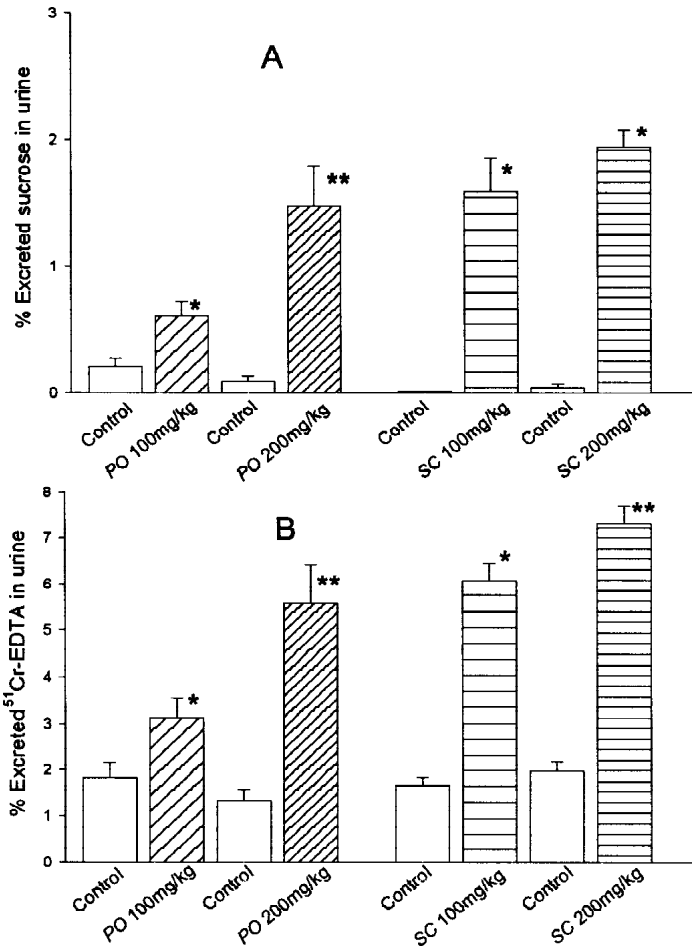

Fig. 3. Effect of the route of administration (oral and subcutaneous [*sc*]) and the dose (100 and 200 mg/kg) on the gastroduodenal (sucrose) and ^{51}Cr-EDTA (lower GI tract) permeability of ibuprofen.
*Significantly different from the control; **significantly different from the control and patients receiving the 100 mg/kg dose. Error bars represent the standard error of the mean.

levels in 8 hr (Fig. 1).

Ibuprofen lysinate induced similar significant increased upper and lower (Fig. 2) GI tract permeability as compared with control rats, which received either 1% methylcellulose or lysine (Fig. 2).

Increased permeability was dose-dependent after both *po* and *sc* administration. Administration of 100 and 200 mg/kg of ibuprofen via *sc* induced a significant increase in upper and lower GI tract permeability (Fig. 3) as compared to control rats. The increased upper and lower GI permeability following 100 mg/kg *sc*, but not 200 mg/kg, was significantly greater than the permeability after oral administration (Fig. 3).

Discussion

The GI side effects of NSAIDs are often perceived to be due to their local effect. Hence, considerable attempts have been directed to reduce GI toxicity caused by direct contact of the upper GI tract with NSAIDs. They include preparation of pro-drugs to temporarily mask the acidic group of NSAIDs (16-17) or modification of the site of release (e.g., sustained release or enteric coated preparations). However, the effectiveness of these approaches depends on the importance of the local versus systemic effects of a drug. Nabumetone is a non-acidic prodrug NSAID (18). Nonetheless, the incidence of peptic ulcer in patients using nabumetone is comparable to that observed with other examined NSAIDs (17, 19). Thus, it appears that, even though the prodrug nabumetone may offer reduced direct GI toxicity, its systemic effect still presents a significant risk of serious GI complications.

It has been assumed that sustained release and enteric coated NSAIDs have less toxicity due to lowered peak plasma concentrations and decreased local exposure of the upper GI tract to the preparations (20, 21). However, these formulations may increase exposure to the active substance in the lower GI tract, where the adverse reactions are usually more risky, life-threatening, and difficult to diagnose (11, 22). In the rat, the gastroduodenal permeability of sustained release flurbiprofen (9) and tiaprofenic acid (10) is significantly less than that of regular release formulations, due to

exposure of the gastroduodenum to a relatively lower concentration of the drug. On the other hand, sustained release formulations of the latter drugs induce increased permeability in the distal intestine. This suggests a shift in the site of toxicity of these NSAIDs from the upper to the lower GI tract. Similarly a retrospective study of NSAID users has revealed that the GI toxicity of sustained release NSAIDs may indeed be more frequent than that of regular release NSAID (12). For ibuprofen and naproxan, comparative studies focusing on the formulation dependency of GI toxicity have been carried out (23, 24). However, the methods used (i.e., endoscopy and ^{51}Cr-labled erythrocytes) were not capable of detecting a side shift in GI toxicity.

In this study, 3 different ibuprofen formulations with different release profiles were used to assess the contribution of the local effect to the overall induced toxicity. The pattern of ibuprofen release and the subsequent local effect on the GI tract following administration of the lysinate (7, 8), a powder, and the sustained release formulation (15) were different. The extent of sucrose permeability (i.e., gastroduodenal toxicity) after administration of all the latter ibuprofen products was generally similar, suggestive of a lesser importance of the local toxicity caused in the upper GI tract. The only difference between the immediate and sustained release formulations was that the effect of the latter on sucrose permeability persisted longer than the former. At 3 hours post-dose, the permeability following the immediate release, but not after the sustained release formulation, returned to the baseline (Fig. 1). The difference between the two formulations at 3 hours was, however, insignificant. This minor difference between formulations could be a reflection of the more sustained systemic presence of the sustained release formulation.

The lack of local effect was further confirmed by the administration of ibuprofen via the *sc* route. The increased permeability in the upper GI tract following 100 mg/kg *sc* administration of ibuprofen was even greater than that after oral administration. Similarly, in the lower GI tract *sc* ibuprofen (100 or 200 mg/kg) induced higher levels of permeability than equal oral doses. The significantly increased permeability following sc administration might be due to a more complete absorption of the drug. These findings suggest that there is not a substantial local effect in the events leading to increased GI permeability caused by ibuprofen. There was no shift of increased permeability from the gastroduodenum to the lower intestine following the administration of sustained release ibuprofen. Indeed, similar to the observation made following sucrose administration, ibuprofen lysinate, ibuprofen powder, and sustained release ibuprofen caused a comparable increased permeability in the lower GI tract. The only minor difference between the immediate and sustained release formulations was a longer onset of activity for the latter (Fig. 1B) which may be explained by the delayed absorption of the sustained release formulation. Despite significant inter-product differences in the plasma concentration-time curves, no major difference in the pattern of increased permeability was noticed. Hence, one does not expect a formulation-dependent relationship between plasma concentrations and the GI effect.

In contrast to ibuprofen, it has been reported that ^{51}Cr-EDTA permeability in humans is significantly enhanced following the one-week administration of a sustained release formulation of diclofenac, but not after a regular release formulation (25). Moreover, administration to the rat of modified release formulations of flurbiprofen (9) or tiaprofenic acid (10) induced a significant increase in lower GI tract permeability and indeed there was a shift of toxicity from the upper to the lower GI tract. These reports indicate involvement of a local effect for diclofenac, flurbiprofen, and tiaprofenic acid. The mechanism of ibuprofen-induced GI toxicity, however, appears to mainly involve a systemic effect.

In conclusion, the induced GI toxicity by ibuprofen was significant regardless of the route of administration. Oral doses were not more toxic than *sc* doses, indicating the lack of a local effect. Rapidly absorbed, immediate release and sustained release preparations demonstrated similar permeability patterns, indicating no shift of toxicity to the site of drug release and further suggesting the lack of local toxicity. Therefore, ibuprofen's effect on GI permeability is mainly systemic. Hence, sustained release formulations of ibuprofen may offer the advantages of prolonged efficacy without increasing GI toxicity. This conclusion should not, however, be extrapolated to other NSAIDs, e.g., flurbiprofen, tiaprofenic acid, and diclofenac.

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