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Report

Influence of Indomethacin Amphoteric Gel on Gastric Ulcerogenicity and Absorption of Indomethacin in Rats

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Indomethacin is a potent and efficacious antiinflammatory agent. However, a limiting side effect is its ability to cause gastric ulceration. This study was designed to investigate the effects of an amphoteric gel on the gastric ulcerogenicity and pharmacokinetics of indomethacin. Oral administration (5 mg/kg) in a suspension and a gel formulation were compared to an intravenous (iv) formulation of indomethacin in rats. The iv formulation administered to rats produced large severe ulcers in some rats but not in others. In contrast, the oral suspension produced small ulcers in all rats. The difference in toxicities is attributed to a centrally mediated action as a result of high plasma levels of indomethacin following iv administration, compared to locally mediated action with the suspension, resulting from local high concentrations of indomethacin on the apical epithelial surface because of the presence of indomethacin crystals. Oral administration of the gel formulation did not result in any gastric ulceration and improved the bioavailability of indomethacin to 115.5%, compared with 68.2% for the suspension. The reduced gastrointestinal toxicity of indomethacin in the gel was attributed to the gel's ability to dissolve indomethacin, preventing the localized high concentration observed with the suspension and possibly providing a gastric protectant phospholipid. The gel formulation doubled the oral bioavailability and the t_{max} of indomethacin compared to the suspension but did not affect the half-life. The results indicate that the local irritant effect of indomethacin, in rats, can be reduced by appropriate formulation design and suggest that the ulcerogenicity index for indomethacin can be improved by the use of an amphoteric gel formulation.

KEY WORDS: indomethacin; gastric ulcer; gastric irritancy; amphoteric gel; oral bioavailability.

INTRODUCTION

Indomethacin is a nonsteroidal, antiinflammatory agent that is used for the treatment of rheumatoid arthritis, ankylosing spondolytis, and osteoarthritis. The drug is administered by injection, tablet, capsule, or suppository (1). When administered by any route indomethacin can produce gastrointestinal side effects including irritancy and can cause ulceration of the stomach and intestine (2,3). These effects are most pronounced following oral administration (4). The mechanism(s) responsible for ulceration is unclear, however, several explanations have been proposed and include interference with arachidonate (cyclooxygenase) metabolism (5–7) and inhibition of ATP production (8) and inhibited production of mucous (7,9,10). Acute gastric ulceration following oral administration of indomethacin probably arises as a result of crystalline drug producing a local high concentration of indomethacin at the apical cell surface, resulting in local inhibition of cyclooxygenase activity. A dosage form that results in a molecular dispersion of indomethacin and that will prevent precipitation in the stomach might also prevent the local irritant effects observed with the suspension. To test this hypothesis, indomethacin was dissolved in an amphoteric gel and administered to rats. The gastric irritancy and plasma concentration of indomethacin were monitored and compared to oral administration of a suspension and intravenous (iv) administration of a solution.

MATERIALS AND METHODS

Materials

Indomethacin and trifluoroacetic acid obtained from Sigma (St. Louis, Mo.), egg phosphatide powder Type V from Asahi (Japan; Lot No. HVG01V), oleic acid from Mallinckrodt (St. Louis, Mo.), arginine from Allergan (Calif.), ethanol, HPLC grade, and 2-propanol, HPLC grade, from Baker (N.J.), and Methocel A4M from Dow (Midland, Mich.) were used as provided from the supplier.

Methods

Preparation of Dosages

The amphoteric gel was prepared by adding 7.16 g of

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oleic acid to 20.00 g of egg phosphatide powder type V. The mixture was cooled to 4°C and triturated to a fine powder. The powder was added to an aqueous solution of 4.41 g of arginine dissolved in 68.43 g of distilled water. The mixture was thoroughly mixed and heated to 35°C, forming a gel. Indomethacin, 10 mg, was then dissolved in 10 g of the gel, yielding a 0.1% (w/w) loading of indometnacin.

The oral suspension was prepared by adding 1 g Methocel (with stirring) to 30 ml of boiling HCl (0.01 *M*, pH 2.0), followed by indomethacin (100 mg) dispersed in 70 ml of 0.01 *M* HCl. The stirred mixture was then cooled and kept on ice for 30 min to allow thickening.

The iv solution was prepared by dissolving indomethacin (10 mg) in 1 ml of methanol and then adding 9 ml of Sorenson's phosphate buffer (pH 7.4).

Control vehicles were prepared as described above except that indomethacin was omitted.

Plasma Indomethacin Analysis

The plasma concentrations of indomethacin were analyzed by HPLC. Plasma samples (0.3 ml) were spiked with salacylic acid (250 μg in 50 μl) and then deproteinated by mixing with 600 μl of a 16.7% (v/v) solution of trichloroacetic acid for 5 min. Indomethacin and salicylic acid were extracted by adding 4 ml of dichloromethane to the acidified plasma. The mixture was then vortex mixed for 3 min and centrifuged at 3000 rpm for 10 min. The methylene chloride was removed and evaporated to dryness under a stream of nitrogen and then reconstituted with 0.5 ml of the mobile phase.

The analysis was conducted with a Waters WISP autoinjector (100-μl injection volume), an LDC HPLC pump Model ConstaMetric IIIG (flow, 0.6 ml/min), a Ranin column oven Model III (temp., 50°C), an LDC CCM system controller and data analysis station, and a Kratos UV detector Model Spectraflow 783 set at 235 nm. Indomethacin and the internal standard, salicylic acid, were resolved on a Dupont Zorbax ODS, 5-μm column (4.6 mm × 25 cm) and guard column (4.6 × 5 mm). The mobile phase consisted of 13% (w/v) *n*-butanol, 13% (w/v) ethanol, and 0.08 *M* perchloric acid/0.05 *M* phosphate, pH 3.45 (11).

In Vivo *Procedures*

Male Sprague–Dawley rats (Crl/CD BR) obtained from Charles River Laboratories, Wilmington, Mass., were kept in Bioclean rooms (Hazelton Systems, Aberdeen, Md.). Temperature was maintained at 22 ± 1°C, and relative humidity at 50 ± 10%, with a 12-hr light/dark cycle (0100–1900 hr). Rats were provided laboratory chow (certified Rodent Chow 5002, Ralston Purina Co., Mo.) and tap water *ad libitum*. Rats were delivered and maintained virus free as determined by monitoring of sentinel animals for rat corona virus/silalodacryoadenitis. Rats (450–550 g) were anesthetized with pentobarbitone, 1 g/kg, and the jugular vein was cannulated (12) to facilitate iv dosing and removal of blood samples. The rats were permitted to recover for 24 hr without food but with *ad libitum* access to water. They were then lightly anesthetized with ether to facilitate placement in Bollman cages and dosed by either iv injection or oral gavarge. Blood samples were withdrawn at appropriate time intervals. Six hours following dosing the rats were replaced in holding cages and removed at designated times for blood sampling. At the end of the experiment, 30 hr after treatment, the rats were anesthetized with ether and exsanguinated and the stomach was removed for examination.

Evaluation of Gastric Ulceration

The stomach was cut along the line of greater curvature from the duodenum to the pyloric sphincter. The stomachs were then spread flat and pinned out on a dissecting board, washed with 0.9% sodium chloride, and inspected under a low-power (×4) dissecting microscope for gastric irritancy. The scoring scheme for the degree of irritancy is given in Table I.

RESULTS

A typical HPLC chromatograph for a control plasma sample and an extracted plasma sample spiked with indomethacin and the internal standard, salicylic acid, is shown in Fig. 1. The retention time for indomethacin was 16.9 min and that for salicylic acid was 6.3 min. Recoveries following extraction were 86 and 67% for salicylic acid. The limit of detection for indomethacin was 100 ng on the column. The calibration curve based on peak area measurements was linear over the range 0.1–60 μg/ml, with a correlation coefficient of 0.999 and an equation for the line of $y = 0.1329x + 0.1241$. Between-day reproducibility was better than ±3%.

The mean plasma profiles of indomethacin following administration of an iv solution, oral suspension, and oral gel administered at 5 mg indomethacin/kg body weight to rats are shown in Fig. 2. A biphasic elimination of indomethacin occurs following iv bolus administration and the data were analyzed to obtain the pharmacokinetic parameters on the basis of a two-compartment open model using a two-exponent peeling method. The alpha-phase values are coefficient = 59.36 ± 29.9 μg/ml (±SD); exponent = 2.55 ± 1.76/hr; and $t_{1/2}$ = 24.7 min. The beta-phase values are coefficient = 24.09 ± 6.16 μg/ml; Exponent = 0.125/hr and $t_{1/2}$ = 346.8 min. The clearance was calculated to be 22.46 ± 3.10 ml × hr/kg, and the volume of distribution during the beta phase was 185.28 ± 34.97 ml/kg. The trapezoidal rule was used to calculate the AUC. The $AUC_{0-30\ hr}$ was 226 ± 35 μg/ml × hr and the $AUC_{0-\alpha}$ was 232 ± 37 μg/ml × hr. Fol-

Table I. Scoring Scheme for Quantitative Gastric Irritancy

Observation	Numerical score
Hyperemia of the mucosal surface	1
Increased surface mucus	1
Vascular leakage (surface erosion)	
1–2 areas, 1 mm (pinpoint) in diameter	1
3 areas or more	3–5
Ulcers	
Single, 3 mm or less	1
Multiple	
Mild	10
Moderate	20
Severe	30
Very severe	40

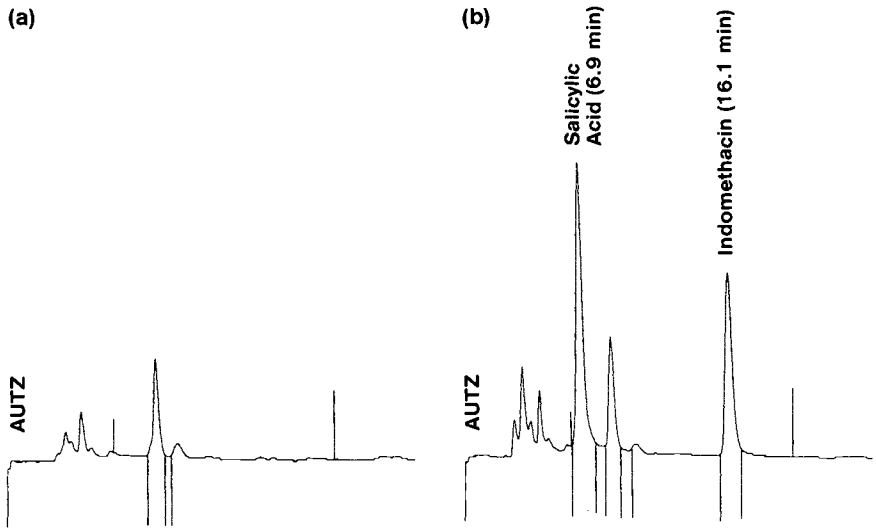

Fig. 1. HPLC chromatograms for (a) an extracted control plasma sample and (b) an extracted plasma sample spiked with indomethacin and the internal standard, salicylic acid.

lowing oral administration of an indomethacin suspension the mean of the individual t_{max} values was calculated to be 71.5 ± 15.3 min, the mean of individual C_{max} values was 11.20 ± 1.89 μg/ml, the $AUC_{0-30\ hr}$ was 126 ± 36 μg/ml × hr, and the $AUC_{0-\alpha}$ was 154 ± 68 μg/ml × hr. Following oral administration of the gel the mean t_{max} was 187.3 ± 116.4 min, the C_{max} was 16.06 ± 4.30 μg/ml, the $AUC_{0-30\ hr}$ was 252 ± 79 μg/ml × hr, and the $AUC_{0-\alpha}$ was 280 ± 64 μg/ml × hr. Based on the blood profiles, the rate of absorption from the oral suspension and gel formulations was similar for approximately the first 70 min. However, the duration of the absorption phase following gel administration was prolonged and continued for approximately 187 min, compared to 72 min for the suspension.

The gastric irritancy following administration of an iv solution, oral suspension, and oral gel of indomethacin (5 mg/kg) to rats is summarized in Table II. No gastric ulcers were detected following administration of the oral gel formulation, in contrast to the suspension, which caused an irritant response in all rats. Intravenous administration resulted in very severe ulceration in two of the rats, minor irritation in a third rat, and no observed ulceration in three rats. Rats treated with vehicle alone by any of the routes did not show any evidence of a gastric irritant response.

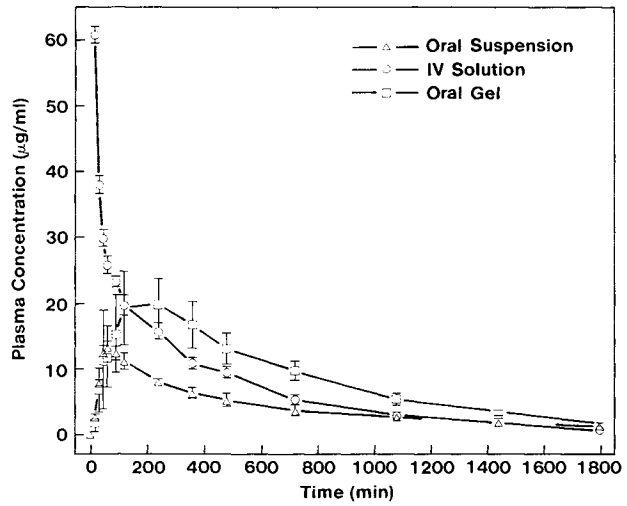

Fig. 2. Plasma profiles (±SE; n = 6) of indomethacin (5 mg/kg) following iv administration in a solution formulation and oral administration in either a gel or a suspension formulation to rats.

DISCUSSION

The use-limiting side effect with indomethacin is its ability to cause gastric irritancy. An approach that could prevent or ablate this side effect would result in a substantial decrease in the ulcerogenicity index for indomethacin. Administration of the gel formulation of indomethacin to rats resulted in the total ablation of the gastric irritant responses and an increase in bioavailability. The absorption rate from the gel was similar to that from the suspension, however, the

Table II. Evaluation of Gastric Effects 30 hr After Dosing with Indomethacin (5 mg/kg) to Rats

Rat No.	Administration	Number of ulcers	Score
1	iv	3	90
2	iv	5	123
3	iv	1	5
4–6	iv	0	0
7–9	iv controls	0	0
10	Oral suspension	2	40
11	Oral suspension	3	15
12	Oral suspension	6	45
13	Oral suspension	1	3
14	Oral suspension	3	40
15	Oral suspension	5	40
16–18	Control suspension	0	0
19–24	Oral gel	0	0
25–28	Control gel	0	0

gel formulation prolonged the time taken for t_{max} to be reached to approximately 187 min and resulted in a higher C_{max} of approximately 16 μg/ml; the bioavailability was improved to 111.5%, compared to the suspension bioavailability of 68.2%. The data were compared using the paired *t*-test method at $P > 0.1$. In contrast to these findings, Hilton and Summers (17) report a relationship between peak plasma levels following oral administration and ulcerogenicity with dispersions of indomethacin in polyvinylpyrrolidone. However, the dose of indomethacin used in their studies was higher (20 mg/kg) than we used in this study and the resulting blood concentration of indomethacin was correspondingly higher. Thus, it would be difficult to determine the separate influences of locally and centrally mediated ulcerogenicity in their study. To obtain a blood level vs ulcerogenicity relationship, the same formulation at varying doses must be employed; in the studies described by Hilton and Summer (17) various different formulations were compared at the same dose and ulcerogenicity related to serum levels of indomethacin.

Large, very severe ulcers occurred following iv administration of indomethacin to two of six rats and mild ulcers in one rat; however, in three of the iv-treated rats there was no evidence of ulceration. The ulceration is attributed to the initial high peak plasma concentrations of indomethacin, inducing a centrally mediated mechanism. Modeling of the iv plasma concentration data indicates that the plasma concentration at T_0 is 117 μg/ml. Such high peak plasma concentrations of indomethacin have been associated with an increase in the frequency and severity of side effects (16,17). The biphasic elimination behavior following bolus iv administration is consistent with that reported in human, dog, and rabbit (13–15).

Ulceration of the stomach was evident in all the animals receiving the suspension formulation. The ulcers were smaller, more benign, and more numerous than those observed following iv administration. The low peak plasma concentration of indomethacin (11 μg/ml) compared to that with iv dosing and the pattern of ulceration suggest that a locally opposed to a centrally, mediated mechanism may be responsible for the ulceration associated with oral administration of the suspension. This observation is similar to the findings of Cioli *et al.* (4), who reported that oral administration of a suspension results in more gastric ulcerogenicity than does iv administration of comparable doses. The severity of gastric lesions induced by indomethacin has been correlated with the particle size of the large crystals; a high local concentration of indomethacin is maintained at the mucosal surface for longer periods of time than with smaller crystals, resulting in more severe ulcerogenicity. In our study, ulceration following oral administration of the suspension probably arises as a result of indomethacin crystals adhering to the apical surface of the gastric epithelium, producing a local high concentration.

The gastric ulceration observed following oral administration is ameliorated when indomethacin is administered in a gel formulation. The amphoteric gel used in this study can accommodate high quantities of indomethacin (>200 mg of indomethacin/g of gel), and upon dilution the gel spontaneously forms liposomes. In the gel formulation, indomethacin should not produce local high concentrations at the apical epithelial cell surface since it is present as a dilute molecular dispersion. Further, the continuous phase in which indomethacin is dissolved spontaneously forms a dispersion when placed in the gastric pouch, reducing the potential for orally associated gastric lesions. The phospholipids used in the gel may provide additional protection to the gastric mucosa by incorporating into the epithelial lipid membrane bilayer (19). Exogenously administered phospholipids have been reported to prevent rat stomach necrosis and bleeding induced by 0.6 *M* hydrochloric acid (20).

Our studies have shown the profound influence different formulations of indomethacin can have on ulcerogenicity and do not support a relationship between peak plasma levels and ulcerogenicity following oral administration of various formulations of indomethacin. This report does not distinguish the relative importance of the phospholipid vs molecular dispersion of indomethacin in inhibiting orally induced gastric ulcerogenicity.

The improved bioavailability and concomitant ablation of gastric ulcerogenicity make the gel formulation approach to the oral administration of nonsteroidal antiinflammatories particulary attractive. However, further studies to elucidate the toxic implications of such an approach on the small intestine must be conducted.

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