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Report

The Biliary Elimination and Enterohepatic Circulation of Ibuprofen in Rats

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The biliary and urinary excretion of ibuprofen and its metabolites were determined in rats after intravenous and peroral administration of 25 and 100 mg/kg of the drug. Within 24 hours 48% of the low i.v. dose and 59% of the high i.v. dose were eliminated via bile as ibuprofen and its metabolites. Following oral administration 40 to 41% of the dose were recovered in bile, whereas 16 to 32% of the dose were eliminated in urine, resulting in an overall drug recovery of 66 to 79% within 24 hours. Upon infusion of bile containing ibuprofen and its metabolites into the duodenum substantial enterohepatic cycling of the drug occurred in the rat.

KEY WORDS: nonsteroidal antiinflammatory drugs (NSAID); ibuprofen; pharmacokinetics in rats; biliary elimination; enterohepatic cycling.

INTRODUCTION

Ibuprofen, 2-(4-isobutylphenyl)propionic acid, is an arylpropionic acid with a molecular weight of 206 and a pK_a value of 4.6 (1). It belongs to the nonsteroidal antiinflammatory drugs (NSAID). The analgesic, antipyretic and antiinflammatory properties made it a useful compound for the treatment of pain and rheumatic diseases during the last 20 years (2,3).

The metabolism of ibuprofen in man and several animal species is well documented (4). The major metabolites are 2-[4-(2-hydroxy-2-methylpropyl)phenyl)]-propionic acid (metabolite A) and 2-[4-(2-carboxypropyl)phenyl]-propionic acid (metabolite B).

Some of the NSAIDs, e.g., indomethacin, have been shown to undergo enterohepatic cycling to a variable degree depending on the species investigated (5). The major side effect of these drugs is irritation of the gastrointestinal mucosa, which may result in bleeding, ulceration, and perforation (6–9). As shown for indomethacin there is a direct correlation between the minimum intestinal toxic dose and the amount of unchanged drug recovered in bile in different species (5). It can be concluded that the incidence of lesions of small intestine may be related to the degree of biliary elimination and enterohepatic circulation of NSAIDs. For this reason the investigation of biliary elimination is of importance in order to define a possible risk factor of gastrointestinal toxicity. Preliminary results of Mills *et al.* (4) indicate that ibuprofen and metabolites are eliminated via bile in rats and dogs. We therefore investigated the time course of ibuprofen and metabolites in the bile of rats following different intravenous as well as intragastral doses of ibuprofen. In addition, we determined the amount of drug which could undergo enterohepatic cycling by infusing bile containing ibuprofen and its metabolites into the small intestine of rats.

MATERIALS AND METHODS

Chemicals

Ibuprofen and the two main metabolites were obtained from Kanoldt (Höchstadt/Donau, FRG). All reagents used for sample preparation and HPLC analysis were of analytical grade.

Animal Protocol

Male Sprague-Dawley rats weighing from 250 to 300 g were obtained from Savo Ivanovas (Kisslegg, FRG). They had free access to food and water until two hours before surgery. One hour before drug administration the bile duct was isolated through abdominal midline incision and cannulated with polyethylene tubing (PE-25) under ether anesthesia. The animals chosen for intravenous drug application had an additional polyethylene catheter (PE-50) implanted in the femoralis vein which was removed immediately after injection.

To determine the amount of drug undergoing enterohepatic cycling a second polyethylene tube was inserted into the duodenum via the papilla major. By means of an infusor (Braun Melsungen, FRG) a 5 ml bile sample obtained from another rat after i.v. administration of ibuprofen was infused

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continuously into the duodenum for 5 hours (i.e. with an infusion rate of 1 ml/h) to imitate the physiological bile flow. The infused bile contained 11.2 mg of ibuprofen, 3.2 mg of metabolite A and 0.2 mg of metabolite B as determined by HPLC analysis prior to application.

Immediately after drug administration the animals were placed into restraint devices prior to full recovery. Bile was collected from 0 to 4, 4 to 8, and 8 to 24 hr. Urine was obtained up to 24 hr using a petri dish which was placed below the animal. The volume and pH of bile and urine samples were measured and an aliquot was frozen and stored at −20°C until analysis.

The animals received 25 and 100 mg/kg ibuprofen both intragastrically and intravenously. The i.v. dosing solution was prepared in 0.03 *M* phosphate buffer, pH 7.4. For intragastral administration ibuprofen was suspended in 1% methylcellulose solution in 0.9% sodium chloride. The control animals received the vehicle only intragastrically. In each case the applicated volume was 5 ml/kg body weight.

Analytical Methods

HPLC Equipment

The concentrations of ibuprofen and the two main phase I metabolites were determined employing a modified high performance liquid chromatographic method (10,11). The HPLC equipment consisted of a Model 114 M pump (Beckman), a Promis (Spark) auto sampler, and a CR 3 A Shimadzu integrator. Separation was achieved by means of a prepacked column (Bischoff, Leonberg, FRG; 250 × 4.5-mm i.d., Nucleosile 5 μm, RP 8). Detection was performed at 220 nm for all samples. Injection volume was 50 μl. Under these conditions the detection limit for ibuprofen and both metabolites was 0.5 μg/ml bile and urine, respectively.

The mobile phase used for the separation of ibuprofen consisted of methanol–water (65:35). One milliliter of phosphoric acid conc. was added to each liter of eluent. The delivery rate was 1.6 ml/min. Ibuprofen was eluted after 12.2 min.

The two main metabolites were separated using a mobile phase of acetonitrile–water (27:73), with 1 ml of phosphoric acid added to each liter of eluent. The flow rate was 1.5 ml/min, and retention times were 10.1 min (metabolite A) and 12.2 min (metabolite B), respectively.

Sample Preparation

For the analysis of the total amount of the compounds (free + conjugated) in bile and urine an alkaline hydrolysis was performed according to Ref. 11. After acidifying 0.1 ml of bile or urine with 0.15 ml of 1 *N* HCl, ibuprofen and metabolites were extracted into 1.2 ml of a hexane–ether (8:2) mixture. One milliliter of the upper organic layer was removed, evaporated to dryness under a stream of dry nitrogen, reconstituted with 0.5 ml of the acetonitrile–water mixture (27:73), and used for HPLC analysis.

Bile Acids

The concentration of total bile acids in each bile sample was determined by UV-spectrophotometry at 340 nm according to Paumgartner *et al.* (12).

Data Analysis

The biliary flow, the bile acid concentrations, and the eliminated amounts of ibuprofen and metabolites were tested for significant differences using Student's *t* test. A *P* value of less than 0.05 was considered to be statistically significant. All results in the tables are expressed as mean ± standard deviation (SD).

Table I. Bile Flow and Bile Acid Concentration of Bile Samples (G_1 = 0–4 hr, G_2 = 4–8 hr, G_3 = 8–24 hr)

Dose, appl. (mg/kg)	Weight (g)	Bile flow (ml/kg/hr)			Total bile acids (μmol/ml)		
		G_1	G_2	G_3	G_1	G_2	G_3
Control[a]	268[b]	2.8	2.8	2.0	24.8	12.6	3.8
	(7.6)	(0.8)	(0.6)	(0.1)	(5.1)	(4.9)	(0.3)
25 i.v.	292	3.4	2.5	1.9	20.3	3.6[c]	1.1[d]
	(7.2)	(0.7)	(0.4)	(0.3)	(5.0)	(1.2)	(1.9)
25 i.g.	298	2.9	2.4	1.7[d]	19.7	2.6[c]	2.1[d]
	(5.9)	(0.3)	(0.4)	(0.1)	(1.5)	(0.7)	(0.8)
100 i.v.	281	4.9[c]	3.7[d]	2.0	13.3[c]	5.9[c]	2.3[d]
	(4.5)	(0.6)	(0.4)	(0.4)	(1.4)	(0.9)	(1.0)
100 i.g.	274	3.7	3.2	2.3	21.1	5.9[c]	3.8
	(6.3)	(0.4)	(0.4)	(0.3)	(11.6)	(1.5)	(1.5)
Recycling	271	4.8[d]	3.9[d]	2.3[d]	41.9[a]	16.4	5.3
	(19.0)	(0.5)	(0.1)	(0.1)	(8.7)	(4.8)	(2.4)

[a] The control animals received the vehicle only i.g.
[b] Means (n = 6); standard deviation in parentheses.
[c] $P < 0.01$ relative to control group.
[d] $P < 0.05$ relative to control group.

RESULTS

The bile flow and the bile acid concentration of each sample are listed in Table I. A significant increase in bile volume resulted after i.v. administration of 100 mg/kg ibuprofen and in the recyling experiment, possibly due to osmotic effects and the absorption of bile acids and other bile constituents in the latter case. The pH of bile shifted in all cases from 7.8–8.1 during the first and second sample to approximately 8.7, except for the recycling experiment, where no significant elevation of pH was recorded. The pH of bile during the first 8 hr was lower after drug administration as compared to the control animals. In all cases bile acid concentrations decreased during the experiment (see Table I), so that the total net amount of bile acids was comparable to that in drug-treated and control animals. In the recycling experiment the bile acid concentration was greater during the first 8 hr because of the absorption of the infused bile.

The overall recovery in urine and bile ranged from 66 to 79% of the dose as ibuprofen and metabolites in 24 hr (Table II). In the recycling experiment, 74% of the administered ibuprofen and metabolites was recovered. If the recovery was calculated as the percentage of the amount of ibuprofen given, 95% was excreted. In both bile and urine samples collected after 24 hr, only negligible amounts of ibuprofen and metabolites could be detected.

In bile 40 to 59% of the dose was eliminated, mainly as ibuprofen and metabolite A. Little metabolite B was formed, especially following the 100-mg/kg doses. In urine, ibuprofen was barely detectable.

The concentration–time courses of ibuprofen and metabolites in bile are given in Fig. 1. After i.v. application the concentrations of ibuprofen and metabolite A in the first bile sample (i.e., 0–4 hr) were higher than after the i.g. dosage, which resulted in maximal bile concentrations in the second fraction after 4 hr. Overall, the biliary recoveries were similar. After a 100-mg/kg i.v. dose, however, a higher biliary excretion (59% of dose) and decreased renal elimination (16%) were observed (Table II). Further, the amount of metabolite A in bile was also increased (31%), whereas the amount in urine was decreased (9%).

DISCUSSION

The overall recovery of ibuprofen and metabolites in bile and urine amounted to 75 and 79% after i.v. administration and to 71 and 66% after i.g. application, indicating a slightly reduced bioavailability of ibuprofen after i.g. administration in the dosage range investigated. The recycling experiment showed that most of the amount excreted in bile was reabsorbed. If the fraction recovered in bile and urine is calculated as the percentage of ibuprofen given with bile, one finds significantly higher recovery data than after i.g. administration of ibuprofen without metabolites suspended in methylcellulose solution (95 vs 66 and 71%). This observation can be interpreted as follows.

First, the absolute bioavailability of ibuprofen, when given together with bile, might be somewhat higher as compared to the bioavailability of ibuprofen given i.g. without bile. Second, the parent drug ibuprofen and its major metabolites, A, are absorbed from the GI tract and undergo enterohepatic circulation, i.e., not all of the recovered metabolite A is processed from ibuprofen. This interpretation gains support from the fact that the amount of metabolite A is relatively high in urine in this experiment (41 vs 9–22% in all other experiments). If the sum of administered ibuprofen and metabolites is used for the calculation of the percentage elimination, 74% of dose could be recovered in accordance with the i.g. and i.v. experiments.

The fraction being excreted via bile is almost the same if the drug is given i.v. or i.g. However, the time course of biliary elimination is different. This difference does not re-

Table II. Biliary and Renally Excreted Amounts of Ibuprofen and Metabolites After Intragastral and Intravenous Application of Ibuprofen and After the Administration of Ibuprofen and Metabolites with Bile During 24 hr (n = 6; Means, Standard Deviations in Parentheses)

Dose, appl. (mg/kg)	Bile (% of dose)				Urine (% of dose)				Urine + bile (% of dose), total
	Ibuprofen	Met. A	Met. B	Total	Ibuprofen	Met. A	Met. B	Total	
25 i.v.	$12.8^{A,I,*}$	$25.7^{a,B}$	$9.3^{a,A,I}$	$47.9^{b,A}$	0	$20.5^{B,II}$	11.1	31.6^{B}	$79.4^{b,II}$
	(2.6)	(2.9)	(1.4)	(6.0)	(—)	(7.3)	(4.8)	(11.9)	(7.9)
25 i.g.	15.4^{I}	18.6^{I}	$5.4^{A,I}$	39.7^{II}	0	21.5^{I}	7.3	28.8^{I}	66.3^{I}
	(2.4)	(1.7)	(1.9)	(5.5)	(—)	(6.0)	(2.6)	(8.5)	(11.2)
100 i.v.	26.1^{B}	31.4^{II}	1.5^{II}	$59.0^{a,II}$	0	$9.2^{a,I}$	7.2	$16.4^{a,I}$	75.4^{I}
	(5.0)	(3.9)	(0.3)	(5.7)	(—)	(2.5)	(1.1)	(1.9)	(5.1)
100 i.g.	16.9^{II}	23.9	0.6	41.3	0.1	20.1^{I}	9.8	29.9^{I}	71.2^{I}
	(2.9)	(5.6)	(0.4)	(5.9)	(0.1)	(4.4)	(1.3)	(4.2)	(5.6)
Recycling 1[a]	21.6	26.4	0.6	48.6	0.6	40.6	5.0	46.2	94.8
	(2.3)	(1.6)	(0.4)	(3.6)	(0.7)	(2.0)	(4.8)	(2.7)	(1.1)
Recycling 2[b]	16.8	20.6	0.5	38.0	0.5	31.7	3.9	36.1	74.1
	(1.8)	(1.2)	(0.3)	(2.8)	(0.5)	(1.6)	(3.9)	(2.1)	(0.8)

[a] Relative to the administered amount of ibuprofen.

[b] Relative to the ibuprofen equivalents, i.e., ibuprofen and metabolites A and B.

* Between identical doses, i.v. vs i.g.: (a) $P < 0.01$; (b) $P < 0.05$. Between identical applications, 25 vs 100 mg/kg: (A) $P < 0.01$; (B) $P < 0.05$. Relative to recycling 1: (I) $P < 0.01$; (II) $P < 0.05$.

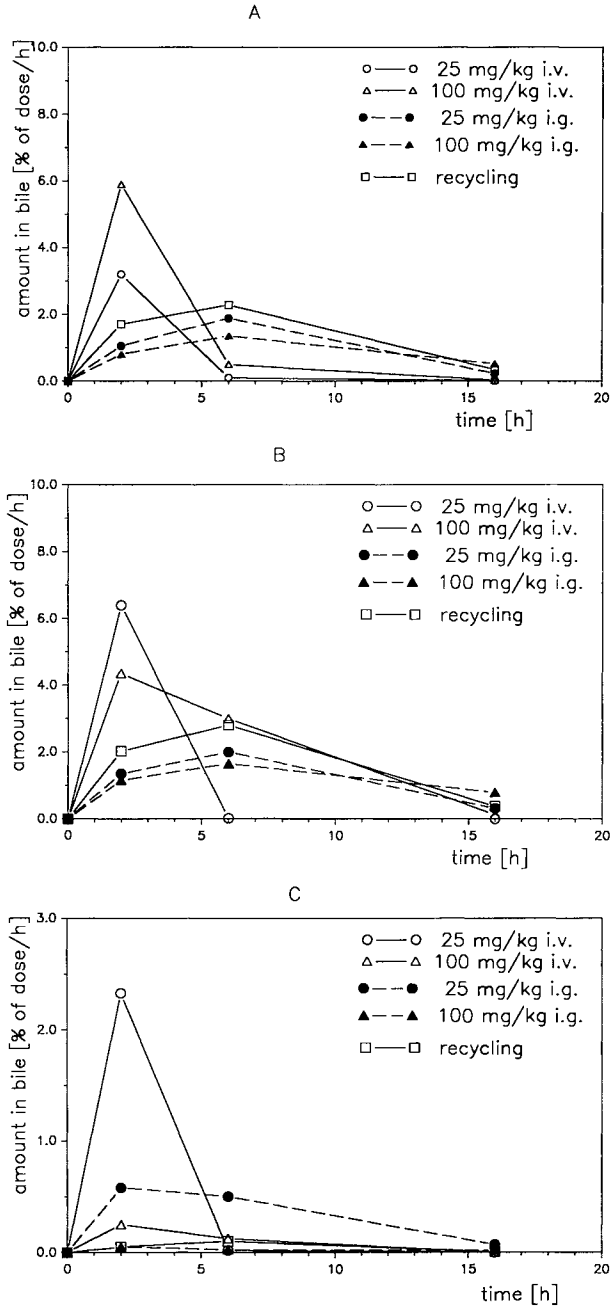

Fig. 1. Amount of ibuprofen (A), metabolite A (B), and metabolite B (C) eliminated in bile expressed as percentage of dose per hour. Data points represent means of six animals.

sult from the saturation of the biliary excretion process, as shown by the rapid appearance of drug in bile after the high dose of 100 mg/kg of ibuprofen, given intravenously. The amount excreted in bile of ibuprofen and its metabolite A increased, while renal elimination of metabolite A decreased.

In conclusion, the data presented here show that a substantial fraction of ibuprofen is encountering enterohepatic circulation in the rat. This enterohepatic circulation also appears to involve the main metabolite of ibuprofen (metabolite A).

Ibuprofen, like other NSAIDs, produces gastrointestinal side effects after i.g. as well as after parenteral administration (5,13,14). Further, the toxic effects of indomethacin on the small intestine were shown not to be caused by systemic action. Rather, the direct contact of the drug with the mucosa seems to be necessary for the development of intestinal erosions and ulcerations (15). The amount of unchanged ibuprofen excreted into bile after i.v. application of 100 mg/kg was comparable to the 25-mg/kg oral dose. Since most of the drug excreted in bile was reabsorbed in the recycling experiment, the intestinal mucosa is exposed to high levels of active drug even after parenteral drug application. These results support the hypothesis that enterohepatic cycling of NSAIDs may be a major factor for the development of intestinal side effects (5,16).

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