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Targeting drugs to the enterohepatic circulation: A potential drug delivery system designed to enhance the bioavailability of indomethacin

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Summary

The effect of exogenously added bile acids upon the bioavailability of indomethacin was investigated in healthy human volunteers. In vitro dissolution studies were performed on formulations containing indomethacin and bile acids and the effects of enteric coating determined. Pharmacokinetic evaluation of the results of the human volunteer study suggests that bile acids increase the bioavailability of indomethacin by prolonging its enterohepatic circulation. The relevance of the findings to drug delivery systems for anionic drugs is discussed. A rapid and efficient means of determining indomethacin in human plasma, using solid-phase extraction, is described.

Introduction

Indomethacin is a non-steroidal anti-inflammatory drug (NSAID) which is commonly used in the treatment of both acute and chronic inflammatory states (Day et al., 1987). Indomethacin has a low plasma clearance, short plasma half-life, a low volume of distribution (Kwan et al., 1975) and is mainly excreted via the hepato-biliary route (Hucker et al., 1966). Extensive clearance of intact indomethacin into the bile has been shown to result in its enterohepatic recirculation (Duggan et al., 1975; Kwan et al., 1975). Interestingly, this phenomenon also occurs with other NSAIDs such as paracetamol (Siegers et al., 1983), ibuprofen (Dietzel et al., 1990), sulindac (Duggan and Kwan, 1979), tenoxicam and piroxicam (Guentert et al., 1988; Benveniste et al., 1990).

Bile acids, functioning as surfactants, are an important component of the human digestive

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process. Bile acids are recovered by a receptor-mediated process from the gastrointestinal tract (Lack et al., 1979) and then recycled by the liver where they are largely responsible for the production of bile (Coleman, 1987; Erlinger, 1988). The composition (primary and secondary bile acids, taurine and glycine conjugates) and overall size of the human bile acid pool depends upon hepatic metabolism, bacterial metabolism within the intestinal luman, diet and the efficiency of intestinal reabsorption (for reviews see Carey and Cahalane (1988) and Hofmann (1988)). It is therefore not surprising that the size and composition of the human bile acid pool are subject to wide inter-subject variation. One of the possible consequences of an inadequate bile acid pool in humans is the precipitation of cholesterol from bile, within the gall-bladder, to form cholesterol gallstones. Cholesterol gallstones can be dissolved by expanding the bile acid pool with bile acids, either chenodeoxycholate or ursodeoxycholate. Bile acid replacement therapy results in a decrease in inter-subject variation in both bile acid-pool size and composition (Hofmann, 1985, 1988).

Cholestyramine, a bile acid sequestrant, is widely used in the treatment of hypercholesterolemia. Reduction in plasma cholesterol results from its recruitment, by the liver, for the replacement of bile acids lost via the faecal route bound to the anion-exchange resin (Fallon and Woods, 1968). Previous studies have shown that the enterohepatic circulation of indomethacin can be interrupted by cholestyramine (Al-Meshal et al., 1990). Similar observations have been made for paracetamol (Dordoni et al., 1973; Siegers et al., 1983), piroxicam and tenoxicam (Guentert et al., 1988; Benveniste et al., 1990). Many of these studies suggested that the interruption of the enterohepatic circulation of NSAIDs results from their specific binding to cholestyramine. However, in view of the importance of endogenous bile acids in (i) solubilising indomethacin, and other NSAIDs, within the gastrointestinal tract (Miyazaki et al., 1979, 1980; Tripathi et al., 1991); and (ii) stimulating the production of bile by the liver (Coleman, 1987; Erlinger, 1988); their concomitant removal from the enterohepatic circulation, by cholestyramine, cannot be ruled out as a contributing factor in reducing the enterohepatic recycling of NSAIDs.

In an attempt to show the importance of bile acids in promoting the enterohepatic circulation of NSAIDs, the present study investigates the effects, in humans, of the simultaneous administration of bile acids upon the bioavailability of indomethacin. The relevance to the delivery of NSAIDs and other anionic drugs is discussed.

Materials and Methods

Chemicals

Indomethacin B.P. was obtained from Dinoval (U.K.) for formulation and bioavailability studies and from Sigma (U.K.) as an analytical standard. Bile acids and sucrose B.P. were obtained from Consolidated Chemicals Ltd (Wrexham, U.K.). Hydroxypropylmethylcellulose (HPMC), povidone and hydroxypropylmethylcellulose phthalate (HPMC phthalate), B.P. or USP.NF grade, were obtained from Stancourt, Sons & Muir Ltd and size 1 hard gelatin capsules from Capsugel Ltd. Indocid® 50 mg was supplied by Thomas-Morton Ltd. All other chemicals and solvents used were of an appropriate grade and obtained from Sigma or BDH.

Manufacture of dosage forms

The bile acid/indomethacin formulations consisted of sugar spheres coated with a mixture of indomethacin and bile acids filled into size 1 hard gelatin capsules. The drug coating solution was made by dissolving indomethacin and povidone into an alcoholic solution containing bile acids. The weight ratio of bile acids to indomethacin was approx. 2:1. This solution was (bottom) sprayed onto sucrose utilising a Uni-Glatt® fluidised bed (air suspension system). The resulting spheres were then finally coated, in the same system, with a solution of HPMC in ethanol or enterically coated using a solution containing HPMC phthalate. In all cases the temperature and air flow in the Uni-Glatt® system were sufficient to evaporate efficiently the solvent used. The final formulations were assayed for in-

domethacin content by the high-performance liquid chromatography (HPLC) method described below and the capsule fill weight determined accordingly. Capsules were manufactured to obtain 50 mg of indomethacin.

HPLC of indomethacin formulations

A potency- and stability-indicating reverse-phase HPLC assay of indomethacin and its degradants, 4-chlorobenzoic acid and 5-methoxy-2-methyl-3-indoleacetic acid, was developed from methods previously described by Levine and Caplan (1985), Berninger and Darsh (1986) and Sauvaire et al. (1986). The system consisted of a Varian® 5500 liquid chromatography system incorporating a programmable UV/Vis wavelength detector, a 250 mm × 4.6 mm (i.d.) 5 μm C_{18} column Spherisorb®, ODS1 (Phase Separations Ltd, U.K.). The mobile phase was 0.1 M acetic acid : acetonitrile (55 : 45) at a flow rate of 2 ml min^{-1}. Detection was at 280 nm for 4.5 min, followed by 254 nm for 7 min. The detector sensitivity was controlled by a Varian® 4270 integrator set to an attenuation of 16 for 4.5 min and 512 for 7 min. The total sample volume injected was 20 μl. The approximate retention times for 5-methoxy-2-methyl-3-indoleacetic acid, 4-chlorobenzoic acid, diethyl phthalate and indomethacin were 2, 3, 5 and 9 min, respectively. The linear response range for indomethacin was 0.8–1.2 mg ml^{-1} with a correlation coefficient of 0.997, the corresponding data for 5-methoxy-2-methyl-3-indoleacetic acid being 8–12 μg ml^{-1} with a correlation coefficient of 0.991. System suitability tests, using six injections of standards, indicated that the performance of the system remained within a 2% confidence limit based on coefficient of variance. To analyse indomethacin in the bile acid/indomethacin formulation, spheres were weighed, dissolved in 50 ml of methanol and sonicated (Decon Ultrasonics Ltd, U.K.) to remove the coating material. The spheres were further sonicated after adding 50 ml of distilled water until the sphere contents were completely dissolved. The solution was then made up to 500 ml with methanol and filtered through a 0.45 μm cellulose acetate filter before HPLC analysis in duplicate or triplicate.

Dissolution testing

The method used was based upon the procedure outlined in the US Pharmacopoeia XXII monograph for indomethacin capsules.

Studies were carried out in a Hanson® 12 dissolution tester using the rotating basket method. Dissolution studies were performed at 37 °C using a range of buffers from pH 1.2 to pH 7.4 and samples taken at specified time intervals, usually 5, 10, 20, 30 and 60 min. A 20 ml sample was removed, 5 ml aliquots of which were then diluted to 25 ml with methanol and the indomethacin content determined using the HPLC technique described above. The linear response range was 0–32.4 μg ml^{-1} with a correlation coefficient of 0.999. The sample solutions were compared to a reference indomethacin standard solution equivalent to the indomethacin content in one capsule dissolved in 750 ml using a detection sensitivity controlled by the Varian® 4270 integrator at an attenuation of 16. Dissolution measurements were performed on six samples at each pH with a resulting coefficient of variance of the percent indomethacin released of within 2%.

Stability studies

The effects of storage temperature and time on the stability of the encapsulated formulations packaged into white, high-density polyethylene containers was assessed. The capsules were stored at all or some of the following temperatures: 4, 25, 30°C, and 30°C at 75% relative humidity; 37, 50°C, and 37°C at 75% relative humidity. At monthly intervals after manufacture, one container from each storage temperature was removed from the controlled temperature environment, and capsules were inspected visually and assayed by the HPLC method outlined above.

Determination of indomethacin in human plasma

Indomethacin in human plasma was determined by HPLC using naproxen as an internal standard. Indomethacin was extracted from 1 ml of human plasma after mixing with 0.5 ml of the internal standard solution, 80 mg ml^{-1} naproxen in methanol. Methanol was used to precipitate protein from plasma and was removed by centrifugation for 1.4×10^4 $g \cdot min$ in a microcen-

trifuge (MSE, U.K.). Solid-phase extraction was carried out on C_{18} Bond-Elut® columns (Technicol, U.K.), capacity 1 ml and containing 100 mg of solid-phase packing material, under a vacuum of 250–500 mmHg. The columns were initially activated by sequentially washing with 2 ml methanol, 4 ml water and 2 ml of 0.25 M acetic acid, pH 3.6. Extraction was carried out using 1 ml of the plasma supernatant. This was followed by a wash with 1 ml of 0.25 M acetic acid, pH 3.6, to remove remaining protein, before eluting the indomethacin with four 0.25 ml aliquots of methanol. Indomethacin was quantified by HPLC as described above, but with the detector sensitivity increased to an attenuation of 4 and the injection volume increased to 50 μl. The recovery of indomethacin and naproxen from standard human plasma extracts, concentration range 0.5–18 $\mu g\ ml^{-1}$, was found to be linear with a correlation coefficient of 0.994 and a recovery of 84.0%. Samples were determined in duplicate or triplicate.

Clinical study

Ten healthy male and female subjects aged between 18 and 40 years, and within $\pm 10\%$ of ideal body weight, participated in the study. Subjects were shown to be in good health by a physical examination and a series of hospital laboratory tests. The protocol for the study was approved by independent researchers at The Academy of Medicine, Bialystok, Poland, and the subjects gave their informed consent.

The subjects were asked to abstain from taking any medication for the 2 weeks before the start of the study and until after the collection of the last blood sample. Alcohol, tea, coffee and other xanthine-containing beverages were prohibited from 24 h prior to the beginning of the study until its completion. Food was withdrawn for 12 h over the night preceding each part of the study. A light breakfast was allowed 3 h post-dose after which time the subjects were allowed to follow their normal daily diets.

The study was of a randomised two-way cross-over design with the subjects receiving either Indocid or bile acid/indomethacin formulation with an equivalent dose of 50 mg of indomethacin. The medication was taken with approx. 250 ml of boiled tap water. The zero time blood samples were taken within a 5 min period preceding the administration of the medication. Subsequent samples were taken at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 10.0 and 12.0 h. Blood samples were collected into lithium heparin tubes, gently mixed and centrifuged at $2.0 \times 10^3\ g \cdot min$ within 15 min of collection. Separated plasma was transferred into a clean tube and stored at $-20\,^{\circ}C$. After a 1 week interval, the subjects received the alternative medication and the blood sampling protocol was repeated. Plasma samples were analysed by the HPLC method described above. No degradation of indomethacin was detected during the period of storage at $-20\,^{\circ}C$.

The results of the clinical study were evaluated using the observed values of maximum plasma concentration of indomethacin (C_{max}) and time to C_{max} (t_{max}). The areas under the plasma concentration curves were calculated using the trapezoidal rule. The results were further assessed using a modification of the method described by Gupta and Hung (1989) for the evaluation of drug delivery systems. The statistical significance of the results was assessed by confidence intervals and p values calculated using a paired t-test (SPSS v3.0).

Results

Stability studies

Two batches of indomethacin and bile acid coated sugar spheres were manufactured and coated with either HPMC film coating (to prevent the spheres from aggregating) or an enteric coating containing HPMC phthalate. The results of the stability studies are listed in Table 1. After 3 months the indomethacin content of the two bile acid/indomethacin formulations, determined by HPLC, was similar to that at zero time except at $37\,^{\circ}C$ and 75% humidity. The contents of these capsules were found to have fused together. Storage of the formulations at 37°C also led to the formation of unidentified degradants with no significant increase in 4-chlorobenzoic acid and 5-methoxy-2-methyl-3-indoleacetic acid. For clini-

TABLE 1

Effects of storage conditions on the indomethacin content of bile acid / indomethacin formulations coated with HPMC or HPMC phthalate

Temperature (°C)	% Initial indomethacin content (± 2%)					
	1 month		2 months		3 months	
	HPMC	HPMCP	HPMC	HPMCP	HPMC	HPMCP
4	100.8	98.4	100.8	103.2	100.8	101.6
25	100.4	101.6	101.6	100.0	100.0	101.6
30	100.0	101.6	100.8	101.1	98.4	97.6
37°C and 75% R.H.	98.4	94.4	97.6	97.6	107.0	88.1

The values represent the mean indomethacin content of 10 capsules, determined by HPLC, as a percentage of their zero time content. The values were all within an error margin of 2%, coefficient of variance. R.H., relative humidity.

cal trial and dissolution study purposes, the samples were stored below 25 ° C and used within 3 months of manufacture.

Dissolution studies

Dissolution profiles of encapsulated bile acid/indomethacin spheres coated with HPMC in various pH buffers are shown in Fig. 1a. The results show that above pH 6.0 indomethacin is rapidly released and solubilised by bile acids. However, it is interesting to note that fairly rapid solubilisation of indomethacin also occurred at pH 5.0 where indomethacin is not usually readily soluble. A comparison between the bile acid/indomethacin formulation and Indocid® at pH 5.0 (Fig. 1a and b) shows that 58% of the indomethacin has been released at 30 min for the bile acid/indomethacin formulation, compared to 30% release from standard Indocid® capsules.

Fig. 2 illustrates the effect of enteric coating on the release of indomethacin from bile acid/indomethacin formulations. Formulations, with and without enteric coating, were initially incubated, at 37°C, in a dissolution bath at pH 1.2 for 60 min. This procedure resulted in the release of approx. 10% of the indomethacin from the non-enteric coated formulation. Indomethacin release from the enteric-coated formulation at pH 1.2 was below the levels detectable by the analytical method used. Replacing the pH 1.2 buffer with pH 7.2 buffer resulted in a rapid release and solubilisation of indomethacin from the enteric-coated spheres, 90% release being achieved within 10 min. Release of indomethacin from the non-

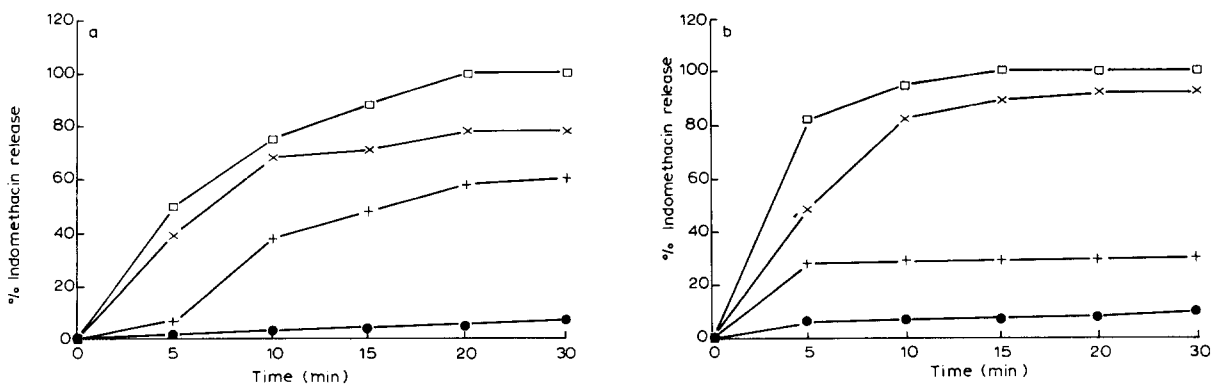

Fig. 1. Dissolution profiles of Indocid and bile acid/indomethacin formulation: effect of pH. Dissolution profiles at 37°C of (a) HPMC coated-bile acid/indomethacin formulations and (b) Indocid® at pH values of 1.2 (●), 5.0 (+), 6.2 (×) and 7.2 (□).

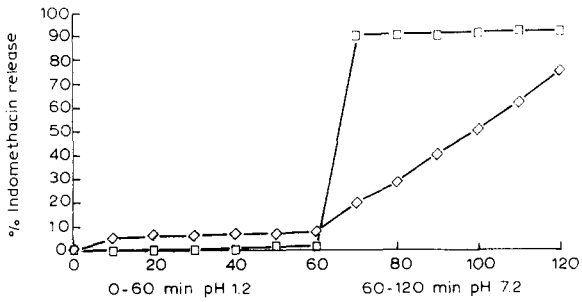

Fig. 2. Comparison of the dissolution profiles of enteric HPMC phthalate (□) and non-enteric HPMC (◇) coated bile acid/indomethacin formulations at pH 1.2 followed by pH 7.2.

enteric coated formulation was slower, reaching 40% after 30 min and 75% after 60 min.

The effect of pH on the dissolution of the enteric-coated bile acid/indomethacin formulation is shown in Fig. 3. The release profiles at pH 2.5, 5.0 and 6.2 show a slow, almost linear release of indomethacin which is probably indicative of the leaching of indomethacin through the enteric coating at pH 2.5, with the slow breakdown of the enteric coat occurring as the pH was increased from 5.0 to 6.2. The release profile at pH 7.2 exhibits a more rapid release of indomethacin, indicative of the rapid removal of the enteric coat and subsequent rapid solubilisation of the indomethacin by bile acids.

Clinical studies using indomethacin

The pharmacokinetic data for indomethacin in plasma for each of the formulations are shown in Table 2. Concentration-time curves for each subject receiving the separate treatments are depicted in Fig. 4 (a–j), in which data points are the mean of duplicate or triplicate determinations. Mean plasma indomethacin concentrations are listed as a function of time in Table 3.

The confidence intervals for the mean of the differences between treatments was calculated for C_{max}, t_{max} and AUC. A paired t-test was used to determine the significance (Table 2). Interestingly, the inter-individual variation with respect to AUC was considerably reduced with the bile acid/indomethacin formulation in comparison to Indocid. This decrease in inter-subject variability was combined with a 50% increase in AUC, from 10.95 to 16.26 $\mu g\ ml^{-1}$. Plasma half-lives for indomethacin with the two formulations were not calculated because of the nature of the terminal plasma indomethacin values (see Fig. 4a–j) showing the appearance of secondary peaks. This phenomenon was particularly noticeable in subjects 3, 4, 5 and 8 receiving Indocid® (Fig. 4c–e, h) and subjects 6 and 8 receiving the bile acid containing formulation (Fig. 4f, h). In the case of Indocid, these secondary peaks appear after the first meal at 3 h post-dose. This feature of the data is also apparent for Indocid in the mean plasma indomethacin concentrations, shown in Table 3, at 3 and 8 h post-dose. Meals were taken at 3 and 7 h post-dose. These secondary peaks probably result from the emptying of indomethacin, stored in the gall-bladder, into the gastrointestinal tract from where it is recycled into the systemic circulation. The addition of bile acids with indomethacin reduces the incidence of secondary peaks. This is probably due to secondary peaks being masked by the generally enhanced levels of indomethacin in the circulation (see Tables 2 and 3).

Discussion

The results of the present investigation have demonstrated that the concomitant administra-

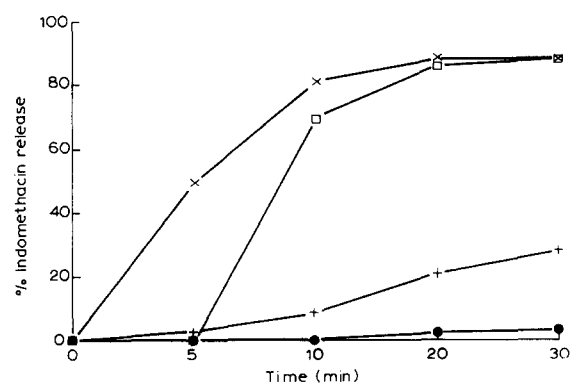

Fig. 3. Dissolution profiles of HPMC phthalate coated, bile acid/indomethacin formulations at pH 2.5 (●), 5.0 (+), 6.2 (×) and 7.2 (□).

TABLE 2

Pharmacokinetic parameters for indomethacin using a bile acid / indomethacin formulation and Indocid

	Subjects	C_{max} ($\mu g\ ml^{-1}$)	t_{max} (h)	AUC ($\mu g\ h\ ml^{-1}$)	
Indocid	1	3.11	2.1	6.43	
(*A*)	2	4.04	1.0	7.79	
	3	3.34	4.0	12.29	
	4	4.75	0.5	9.21	
	5	5.35	0.5	9.18	
	6	6.14	1.5	8.41	
	7	6.43	1.5	10.16	
	8	4.13	4.0	14.60	
	9	7.51	3.0	16.58	
	10	6.68	2.5	14.87	
	Mean	5.15	2.1	10.95	
	SD	1.50	1.3	3.43	
	CV%	29.0%	63.0%	31.1%	
					AUC ratio (*B/A*)
Bile acid/indomethacin	1	9.35	1.0	16.45	2.6
formulation (*B*)	2	5.70	2.0	21.33	2.7
	3	9.30	1.0	8.70	0.7
	4	11.16	2.0	18.94	2.1
	5	5.61	1.0	11.02	1.2
	6	8.36	1.5	16.74	2.0
	7	4.66	2.0	16.91	1.7
	8	3.38	4.0	17.05	1.2
	9	5.52	4.0	18.98	1.2
	10	6.00	2.5	16.48	1.1
	Mean	6.90	2.1	16.26	1.5
	SD	2.50	1.1	3.75	
	CV%	36.0%	53.5%	23.0%	
95% Confidence intervals and *p* values for the mean of the difference between treatments					
95% CI	−0.63 to 4.15		−0.87 to 0.97	1.71 to 8.45	
p	0.131		0.904	0.010	

tion of bile acids with indomethacin in healthy subjects increases the bioavailability, in terms of total AUC, by about 50% compared to a conventional formulation of the drug. Part of the explanation for this increased bioavailability may be the rapid dissolution of indomethacin, by bile acids, in the rising pH of the duodenum, as predicted on the basis of the dissolution studies (see Figs 1 and 2). Previous studies have also shown that bile acids enhance the dissolution of indomethacin (Miyazaki et al., 1979), probably by the formation of mixed micelles (Miyazaki et al., 1981). The absence of bile acid mixed micelles was suggested to be the explanation for the reduced indomethacin levels observed in the plasma of rats undergoing biliary drainage (Miyazaki et al., 1980). Interestingly, however, the results of the present study do not provide any statistically significant evidence of a more rapid absorption of indomethacin from the bile acid-containing for-

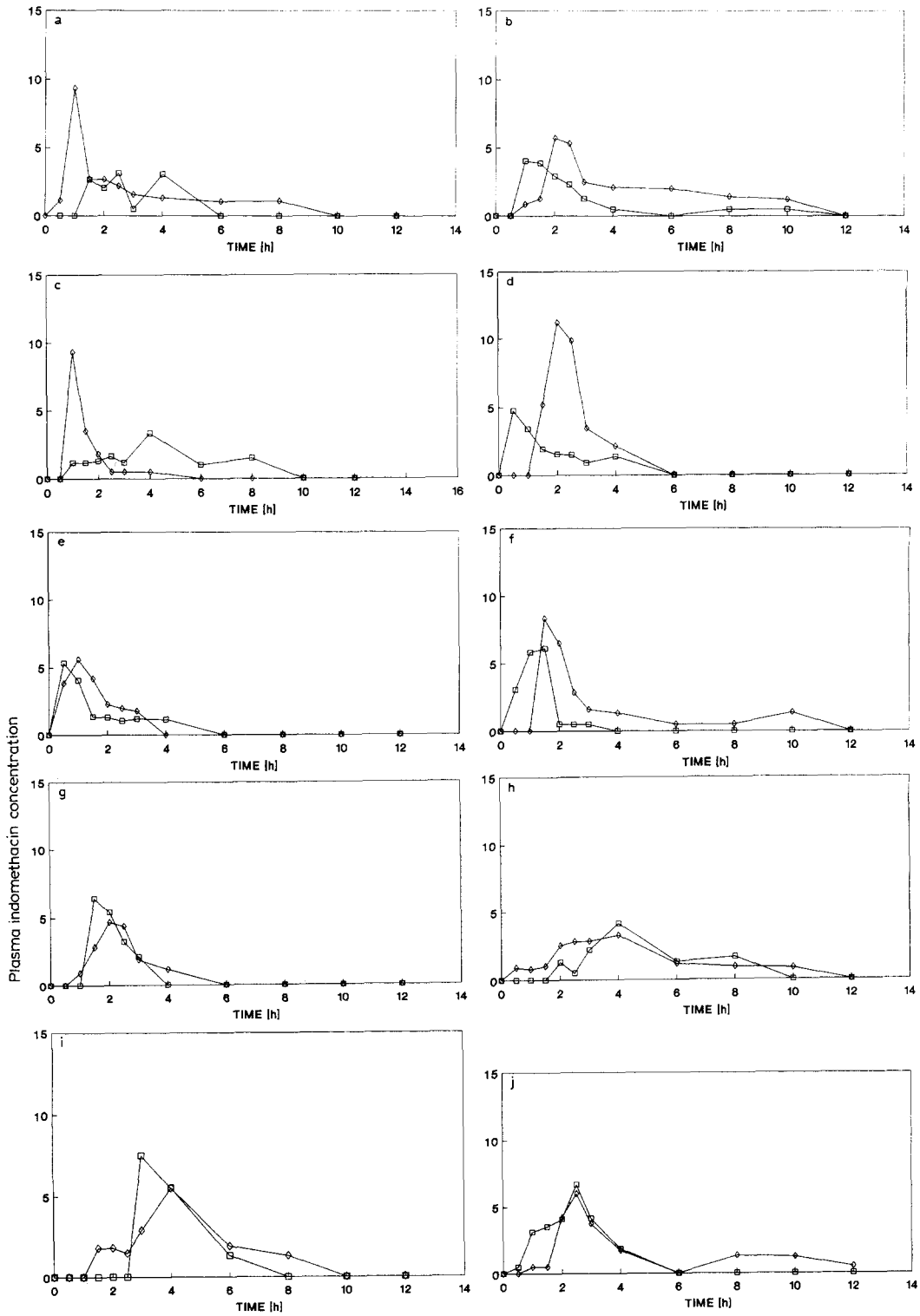

Fig. 4. (a–j) Individual plasma indomethacin concentrations (μg ml^{-1}) in subjects receiving Indocid® (□) and bile acid/in domethacin formulations (◇).

TABLE 3

Summary of plasma indomethacin concentrations

Time (h)	Bile acid/indomethacin formulation		Indocid	
	Mean	95% CI	Mean	95% CI
0	N.D.	-	N.D.	-
0.5	0.59	0 -1.47	1.37	0 -3.57
1.0	2.74	0 -5.50	2.43	0.96-3.90
1.5	3.14	1.47-4.81	2.66	1.03-4.29
2.0	4.33	2.25-6.41	2.18	0.98-3.38
2.5	3.73	1.76-5.70	1.65	0.63-2.67
3.0	2.25	1.55-2.95	2.43	0.94-3.92
4.0	1.82	1.30-2.34	1.79	0.41-3.17
6.0	0.66	0.07-1.25	0.37	0 -0.78
8.0	0.66	0.21-1.11	0.47	0 -0.97
10.0	0.46	0.03-0.89	0.05	0 -0.16
12.0	0.05	0 -0.16	N.D.	-

Values are means with 95% confidence intervals (CI) for 10 subjects receiving each formulation. Plasma indomethacin levels (in $\mu g\ ml^{-1}$) were determined using the solid-phase extraction procedure and HPLC assay outlined in Materials and Methods. N.D. represents indomethacin levels in plasma not detectable using the analytical method used.

mulation, compared to Indocid, in terms of t_{max} (Table 2) or in the plasma concentration summary shown in Table 3. There is also little evidence of a substantially increased C_{max} to explain the increased AUC with the bile acid/indomethacin formulation (Table 2).

An alternative explanation for the enhanced bioavailability of indomethacin with bile acids is that they increase the biliary excretion and/or the enterohepatic recycling of the drug. The extent to which indomethacin undergoes enterohepatic recycling (21–41%) in man and animals is well established (Duggan et al., 1972, 1975; Kwan et al., 1975; Duggan and Kwan, 1979). Similarly well documented is the effect of bile acids in increasing the biliary excretion of organic anions (Mandiola et al., 1972; Berk et al., 1974; Vonk et al., 1974; Delage et al., 1975; Esteller et al., 1984; Kanai and Kitani, 1986). Therefore, it is likely that indomethacin, an organic anion, will be excreted in bile to an extent determined by the size of the circulating bile acid pool in a given individual. This hypothesis, if correct, would explain the extensive inter-individual variation in dose response to indomethacin since this dose response would relate to the corresponding inter-individual variation in human bile acid pool size. Furthermore, it could be predicted that expanding the human bile acid pool with exogenous bile acids would increase the enterohepatic recycling of indomethacin and therefore, via a 'spill-over effect', the systemic bioavailability of the drug. It is likely that individuals with a small circulating bile acid pool, and therefore poor indomethacin bioavailability, would respond with the greatest increase in indomethacin bioavailability to the addition of exogenous bile acids. Interestingly, analysis of the AUC data, based on a method described by Gupta and Hung (1989), shows the relationship between increased bioavailability of indomethacin with bile acids, compared to Indocid, to have a strong correlation ($r = 0.769$) which would support this idea (Fig. 5).

Previous studies have shown that the incidence of gastrointestinal lesions correlates with the increased enterohepatic recycling of indomethacin (Duggan et al., 1975). In view of the present findings, it is believed that the extent of enterohepatic recycling of indomethacin correlates with the size of the endogenous bile acid pool of the individual. Thus, the subjects with the largest endogenous bile acid pool would receive a higher

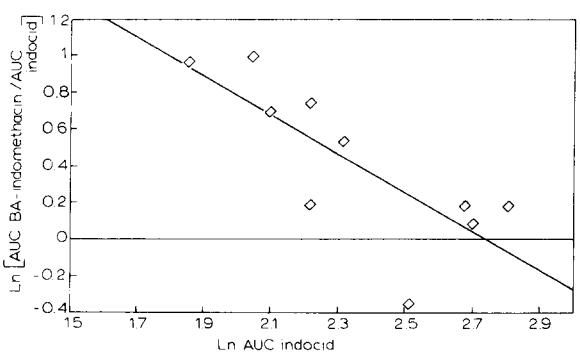

Fig. 5. Relationship between the ln ratio of indomethacin AUC with the bile acid/indomethacin formulation to the AUC for Indocid® and ln of AUC indomethacin from Indocid® for 10 subjects.

than average dose of indomethacin from a standard formulation such as Indocid®. Stabilizing the bile acid pool within a population, by administering bile acids, would have the effect of increasing the bioavailability of indomethacin in subjects with an initially small endogenous bile acid pool and would therefore allow for a substantial reduction of administered dose while maintaining consistent therapeutic efficacy. It is believed that a dose reduction of up to 50%, compared to standard formulations, would result in a decreased incidence of gastrointestinal side-effects while also reducing the overall chemical load of indomethacin. Development of a reduced dose delivery system targeting the enterohepatic circulation could, in addition to indomethacin, be applicable for use with other pharmaceutical organic anions excreted in bile.

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References

Al-Meshal, M.A., El-Sayed, Y.M., Al-Angary, A.A., Lufti, K.M. and Gouda, M.W., Interruption of the enterohepatic circulation of indomethacin by cholestyramine in rabbits. *Int. J. Pharm.*, 64 (1990) 155–160.

Benveniste, C., Striberni, R. and Dayer, P., Indirect assessment of the enterohepatic recirculation of piroxicam and tenoxicam. *Eur. J. Clin. Pharmacol.*, 38 (1990) 547–549.

Berk, R.N., Golberger, L.E. and Lueb, P.M., The role of bile salts in the hepatic excretion of iopanoic acid. *Invest. Radiol.*, 9 (1974) 7–15.

Berninger, R.W. and Darsh, D.A., The use of reverse phase cartridges (C_{18}) in processing plasma for analysis of indomethacin by high performance liquid chromatography. *J. Clin. Chem. Clin. Biochem.*, 24 (1986) 227–232.

Carey, M.C. and Cahalane, M.J., Enterohepatic circulation. In Arias, I.M., Jakoby, W.B., Popper, H., Schachter, D. and Shafritz, D.A. (Eds), *The Liver: Biology and Pathobiology*, 2nd Edn, Raven, New York, 1988, pp. 573–615.

Coleman, R., The biochemistry of bile formation. *Biochem J.*, 244 (1987) 249–261.

Day, R.O., Graham, G.G., Williams, K.M., Champion, G.D. and Jager, D.J., Clinical pharmacology of non-steroidal anti-inflammatory drugs. *Pharmacol. Ther.*, 33 (1987) 383–433.

Delage, Y., Erlinger, S., Duval, M. and Benhamou, J.P., Influence of dehydrocholate and taurocholate on bromsulphthalein uptake, storage and excretion in the dog. *Gut*, 16 (1975) 105–108.

Dietzel, K., Beck, W.S., Schneider, H.T., Geisslinger, G. and Brune, K., The biliary elimination and enterohepatic circulation of ibuprofen in rats. *Pharm. Res.*, 7 (1990) 87–90.

Dordoni, B., Willson, R.A., Thompson, R.P.H. and Williams, R., Reduction of absorption of paracetamol by activated charcoal and cholestyramine. A possible therapeutic measure. *Br. Med. J.*, 14 (1973) 86–87.

Duggan, D.E. and Kwan, K.C., Enterohepatic recirculation of drugs as a determinant of therapeutic ratio. *Drug Metab. Rev.*, 9 (1979) 21–41.

Duggan, D.E., Hogans, A.F., Kwan, K.C. and McMahon, F.G., The metabolism of indomethacin in man. *Pharmacol. Exp. Ther.*, 181 (1972) 563–575.

Duggan, D.E., Hooke, K.F., Noll, R.M. and Kwan, K.C., Enterohepatic circulation of indomethacin and its role in intestinal irritation. *Biochem. Pharmacol.*, 25 (1975) 1749–1754.

Erlinger, S., Bile flow. In Arias, I.M., Jakoby, W.B., Popper, H., Schachter, D. and Shafritz, D.A. (Eds), *The Liver: Biology and Pathobiology*, 2nd Edn, Raven, New York, 1988, pp. 643–661.

Esteller, A., Gonzalez, J., Hidalgo, F. and Lopez, M.A., Enhancement of maximal bilirubin excretion by bile salts in the anaesthetized rabbit. *Q.J. Exp. Physiol.*, 69 (1984) 217–225.

Fallon, H.J. and Woods, J.W., Response of hyperlipoproteinemia to cholestyramine resin. *J. Am. Med. Assoc.*, 204 (1968) 1161–1164.

Guentert, T.W., Defoin, R. and Mosberg, H., Accelerated elimination of tenoxicam and piroxicam by cholestyramine. *Clin. Pharmacol. Ther.*, 43 (1988) 179 Abstr.

Gupta, P.K. and Hung, C.T., Quantitative evaluation of targeted drug delivery systems. *Int. J. Pharm.*, 56 (1989) 217–226.

Hofmann, A.F., Targeting drugs to the enterohepatic circulation: Lessons from bile acids and other endobiotics. *J. Controlled Release*, 2 (1985) 3–11.

Hofmann, A.F., Bile acids. In Arias, I.M., Jakoby, W.B., Popper, H., Schachter, D. and Shafritz, D.A. (Eds), *The Liver: Biology and Pathobiology*, 2nd Edn, Raven, New York, 1988, pp. 553–572.

Hucker, H.B., Zacchei, A.G., Cox, S.V., Brodie, D.A. and Cantwell, N.H.R., Studies on the absorption, distribution and excretion of indomethacin in various species. *Pharmacol. Exp. Ther.*, 153 (1966) 237–249.

Kanai, S. and Kitani, K., Effect of tauroursodeoxycholate on the biliary transport maximum of sulfobromophthalein in the rat. *J. Lab. Clin. Med.*, 108 (1986) 601–607.

Kwan, K.C., Breault, G.O., Umbenhauer, E.R., McMahon,

F.G. and Duggan, D.E., Kinetics of indomethacin absorption, elimination and enterohepatic circulation in man. *J. Pharmacokinet. Biopharm.*, 4 (1975) 255–280.

Lack, L., Properties and biological significance of the ileal bile salt transport system. *Environ. Health Perspect.*, 33 (1979) 79–90.

Levine, B. and Caplan, Y.H., Simultaneous liquid chromatographic determination of five non-steroidal anti-inflammatory drugs in plasma or blood. *Clin. Chem.*, 31 (1985) 346–347.

Mandiola, S., Johnson, B.L., Winters, R.E. and Longmire, W.P., Biliary excretion of ampicillin in the anaesthetized dog. I. Effect of serum ampicillin concentration, taurocholate infusion rate, biliary secretion pressure and secretin infusion. *Surgery*, 71 (1972) 664–674.

Miyazaki, S., Inoue, H., Yamahira, T. and Nadai, T., Interaction of drugs with bile components. I. Effects of bile salts on the dissolution behaviour of indomethacin and phenylbutazone. *Chem. Pharm. Bull.*, 27 (1979) 2468–2472.

Miyazaki, S., Yamahira, T., Inoue, H. and Nadai, T., Interaction of drugs with bile components. II. Effect of bile on the absorption of indomethacin and phenylbutazone in rats. *Chem. Pharm. Bull.*, 28 (1980) 323–326.

Miyazaki, S., Yamahira, T., Morimoto, Y. and Nadai, T., Micellar interaction of indomethacin and phenylbutazone with bile salts. *Int. J. Pharm.*, 8 (1981) 303–310.

Sauvaire, D., Cociglio, M. and Alric, R., Liquid chromatographic measurement for plasma indomethacin and its prodrug apyramide; oral rat and intravenous dog pharmacokinetics. *J. Chromatogr.*, 375 (1986) 101–110.

Siegers, C.P., Rozman, K. and Klassen, C.D., Biliary excretion and enterohepatic circulation of paracetamol in the rat. *Xenobiotica*, 13 (1983) 591–596.

Tripathi, M., Kohli, D.V. and Uppadhyay, R.K., Effect of cholic and deoxycholic acid conjugates on solubility and dissolution of indomethacin and phenylbutazone, *Int. J. Pharm.*, 67 (1991) 207–209.

Vonk, R., Van der Veen, H., Prop, G. and Meijer, D.K., The influence of taurocholate and dehydrocholate choleresis on plasma disappearance and biliary excretion of indocyamine green in the rat. *Naunyn Schmiedeberg's Arch. Pharmacol.*, 282 (1974) 401–410.