International Journal of Pharmaceutics, 64 (1990) 155–160
Elsevier

IJP 02148

Interruption of the enterohepatic circulation of indomethacin by cholestyramine in rabbits

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(Received 2 March 1990)
(Accepted 4 April 1990)

Key words: Indomethacin; Cholestyramine; Pharmacokinetic parameters; Accelerated clearance; Enteric binding

Summary

The purpose of this investigation was to determine whether the oral administration of cholestyramine would increase the systemic clearance of indomethacin following intravenous administration (2 mg/kg) to rabbits. In cholestyramine-treated rabbits a significant reduction in indomethacin serum concentration was observed compared to control animals. Cholestyramine treatment resulted in a significant decrease in the terminal elimination half-life (1.26 ± 0.13 and 0.85 ± 0.06 h for the control and treated groups, respectively) and the mean residence time (1.31 ± 0.13 and 0.78 ± 0.04 h for the control and treated rabbits, respectively). Furthermore, a 56% increase in the systemic clearance (1.91 ± 0.17 and 2.99 ± 0.2 ml min^{-1} kg^{-1} for the control and treated rabbits, respectively) and 36% decrease in the area under the serum concentration-time curve (17.57 ± 1.62 and 11.19 ± 0.7 μg h ml^{-1} for the control and treated rabbits, respectively) were also observed. Cholestyramine administration did not significantly alter the apparent volume of distribution parameters (V_c, V_{ss} and V_{area}). Regarding the microconstants of the two-compartment model which adequately described indomethacin kinetic in control and treated rabbits, cholestyramine administration produced a significant increase in the rate of transfer of indomethacin from the tissue compartment (K_{21}) and out of the central compartment (K_{10}). These findings indicate that cholestyramine administration accelerates the systemic elimination of indomethacin. This effect is thought to be due to augmentation of net biliary excretion through enteric binding.

Introduction

Cholestyramine, an anion-exchange resin, is widely used to lower the serum cholesterol levels in patients with hypercholesterolemia (Fallon and Woods, 1968). This agent binds cholesterol metabolites and bile acids in the intestinal lumen, prevents their reabsorption, and thus depletes total body cholesterol (Moore et al., 1968). Such binding is not limited to bile acids, so cholestyramine may markedly reduce the availability of coadministered drugs. Cholestyramine has been shown to reduce the oral bioavailability of cephalexin, sulphamethoxazole (Parsons and Paddock, 1975), thyroxine (Northcutt et al., 1969), warfarin (Robinson et al., 1971; Renowden et al., 1985), metronidazole (Molokhia and Al-Rahman, 1987), digoxin (Brown et al., 1977, 1985) and paracetamol (Dordoni et al., 1973) upon coadministration. It also reduced the absorption rate, but not the

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overall availability of trimethoprim (Parsons and Paddock, 1975), and does not appear to interfere with the absorption or bioavailability of propranolol (Schwartz et al., 1982). Of more interest, however, is the observation that for some drugs like digitoxin (Caldwell et al., 1971; Carruthers and Dujovne, 1980; Pieroni and Fisher, 1981), lorazepam (Herman et al., 1989), chlordecone (Cohn et al., 1978; Boylan et al., 1978; 1979), phenoprocoumon (Meinertz et al., 1977), warfarin (Jähnchen et al., 1978), paracetamol (Siegers et al., 1983) and tenoxicam and piroxicam (Guentert et al., 1988), oral administration of cholestyramine produced a significant increase in the rate of elimination from plasma, even when cholestyramine is administered during the post absorptive phase or when the drugs were administered parenterally. The main proposed mechanism by which cholestyramine enhances drug clearance is by interruption of enterohepatic circulation.

Indomethacin, a non-steroidal anti-inflammatory drug, is commonly used in clinical treatment of rheumatoid arthritis and other inflammatory states (Day et al., 1987; Szabo et al., 1989). Previous studies on indomethacin metabolism and excretion have shown that indomethacin and its conjugated metabolites undergo a significant enterohepatic cycle in most animal species and in man (Duggan et al., 1975; Duggan and Kwan, 1979; Kwan et al., 1975). Furthermore, it has been suggested that unchanged indomethacin and/or its conjugated metabolites, as secreted in the bile, are the primary causative factors for the observed intestinal lesions following indomethacin administration in many species (Duggan et al., 1975). Interruption of this pathway in the intestine may accelerate the elimination of indomethacin and decrease the side effects of this drug. We have previously demonstrated that oral administration of activated charcoal enhances the systemic elimination of intravenously administered indomethacin (El-Sayed et al., 1990). It has been postulated that enterohepatic recirculation of indomethacin may be interrupted and, to a lesser extent the charcoal in the gut could bind the drug fraction that diffuses back from the blood into the gut lumen (El-Sayed et al., 1990).

This study was carried out to evaluate the effect of oral administration of cholestyramine on the systemic clearance and other pharmacokinetic parameters of indomethacin following intravenous administration to rabbits. Adsorption studies in vitro were also performed.

Materials and Methods

Chemicals

Indomethacin vials (5 mg/ml) were obtained from Dumex (Copenhagen, Denmark). Cholestyramine (Questran) was obtained from Mead Johnson Laboratories (Evansville, IN, U.S.A.). All chemicals, reagents and solvents used in this study were of analytical and HPLC grade.

Adsorption studies

Adsorption studies were carried out as previously described (El-Sayed et al., 1990). Indomethacin solutions (5–50 mg/50 ml, buffered at pH 7.5) were added to 250 mg cholestyramine (Sigma, St. Louis, U.S.A.) in separate bottles. The bottles were shaken at $37 \pm 0.5\,^{\circ}C$ in a constant temperature water bath. After attaining equilibrium (1 h), indomethacin concentration was determined spectrophotometrically at 265 nm. Desorption was determined by three successive washings with 20 ml buffer at $37\,^{\circ}C$, following desorption from 10 mg/50 ml indomethacin solution (El-Sayed et al., 1990).

Animal studies

New Zealand white male rabbits (3–5 kg) were used. The animals were fasted for 48 h prior to and during the experiment and water was allowed ad libitum. All animals, in a random fashion, received the drug intravenously and either Questran (cholestyramine equivalent, 0.17 g/kg) suspended in water (1 g/10 ml) for the treated group ($n = 8$), or water for the control group ($n = 8$) by gastric intubation. The marginal vein of one ear was cannulated with a polyethylene tube (Terumo 22 G) for blood sampling. 30 min after cholestyramine administration, indomethacin (2 mg/kg) was injected over a period of 1 min into the marginal vein of the opposite ear where that tube was introduced. Blood samples (1.5 ml each)

were collected into glass tubes just prior to drug administration and at 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0 and 6.0 h post-drug administration and allowed to clot. Serum samples were taken after centrifugation and frozen pending analysis.

Analysis of indomethacin

Indomethacin serum concentrations were assayed using a specific and sensitive high-performance liquid chromatographic procedure (Al-Angary et al., 1990).

Pharmacokinetic analysis

The data on serum indomethacin concentrations after intravenous administration were analyzed by a linear two-compartment open model with elimination from the central compartment. The concentration of indomethacin in serum (C_p) is described by the following equation:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t}$$

where, A, B, α and β are hybrid constants and t is the time. The relevant pharmacokinetic parameters such as the terminal elimination half-life ($t_{1/2}\beta$), the apparent volume of distribution at steady state (V_{ss}), volume of the central compartment (V_c), the V_{area}, the area under the serum concentration-time curve (AUC), the total systemic clearance (Cl) and the mean residence time (MRT) of the drug were calculated using compartmental and non-compartmental equations (Gibaldi and Perrier, 1982).

Statistical analysis

The data are presented as means ± S.D. The t-test for unpaired data (two-tailed) was employed to assess the effects of cholestyramine treatment on the pharmacokinetic parameters. Differences between two related parameters were considered statistically significant for p values equal to or less than 0.05.

Results

The decline in serum concentrations of indomethacin after intravenous injection of a single dose (2 mg/kg) to rabbits with or without treatment with cholestyramine is shown in Fig. 1. Orally administered cholestyramine produced a significant reduction in indomethacin concentrations from 0.25 h onwards, but did not affect the general shape of the serum concentration-time curve (Fig. 1).

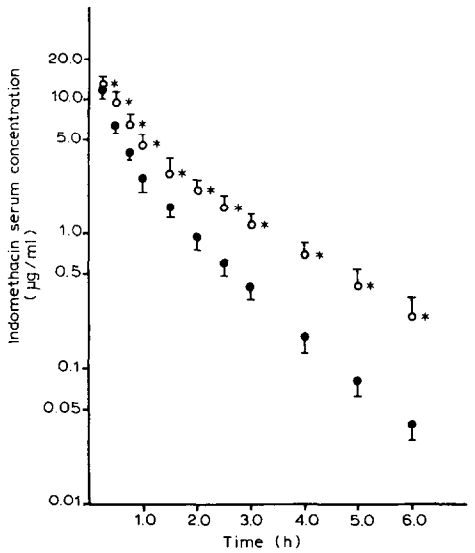

Fig. 1. Serum indomethacin concentrations following intravenous administration (2 mg/kg) to rabbits with (●) and without (○) treatment with cholestyramine. Each point represents the mean ± S.D. of eight rabbits. * p 0.001.

Table 1 summarizes the calculated pharmacokinetic parameters from the serum concentration-time data in control and cholestyramine-treated rabbits. Significant decrease was observed between control and cholestyramine-treated rabbits in the terminal elimination half-life ($t_{1/2}\beta$) (1.26 ± 0.13 and 0.85 ± 0.06 h for the control and treated groups, respectively) and the mean residence time (MRT) (1.31 ± 0.13 and 0.78 ± 0.04 h for the control and treated groups, respectively). Treatment with cholestyramine significantly increased the total clearance (1.91 ± 0.17 and 2.99 ± 0.2 ml min^{-1} kg^{-1} for the control and treated rabbits, respectively) and showed pronounced reduction in the area under the serum concentration-time curve (AUC) (17.57 ± 1.62 and 11.19 ± 0.7 μg h ml^{-1} for the control and treated rabbits, respectively). The calculated apparent gastroin-

TABLE 1

Pharmacokinetic parameters of indomethacin administered intravenously (2 mg/kg) to rabbits with or without treatment with cholestyramine administered orally [a]

Pharmacokinetic parameters	Control	Treated	Significance (P) [b]
AUC (μg h ml^{-1})	17.57 ± 1.62	11.19 ± 0.7	< 0.001
Cl (ml min^{-1} kg^{-1})	1.91 ± 0.17	2.99 ± 0.2	< 0.001
V_c (ml kg^{-1})	85.16 ± 5.7	86.63 ± 3.53	NS
V_{ss} (ml kg^{-1})	148.94 ± 17.25	139.43 ± 11.3	NS
V_{area} (ml kg^{-1})	209.30 ± 32.43	219.9 ± 28.22	NS
MRT (h)	1.31 ± 0.13	0.78 ± 0.04	< 0.001
$t_{1/2}\beta$ (h)	1.26 ± 0.13	0.85 ± 0.06	< 0.001
K_{12} (h^{-1})	0.85 ± 0.11	0.83 ± 0.13	NS
K_{21} (h^{-1})	1.16 ± 0.13	1.38 ± 0.13	< 0.001
K_{10} (h^{-1})	1.35 ± 0.13	2.07 ± 0.1	< 0.001

[a] Each value represents the mean ± S.D. of 8 rabbits.
[b] Student's t-test.
NS, not significant.

testinal clearance (Cl with cholestyramine–Cl without cholestyramine) of indomethacin was found to be 1.08 ml min^{-1} kg^{-1}. There were no significant differences in the apparent volume of distribution V_c, V_{ss} and V_{area} between the control and cholestyramine-treated rabbits (Table 1).

The microconstants of the two-compartment model that adequately described indomethacin kinetic in control and treated rabbits were calculated. Cholestyramine administration produced a marked increase in the tissue compartment rate constant (K_{21}) (1.16 ± 0.13 and 1.38 ± 0.13 h^{-1} for the control and treated rabbits, respectively) and the rate of elimination out of the central compartment (K_{10}) (1.35 ± 0.13 and 2.07 ± 0.1 h^{-1} for the control and treated groups, respectively). Nevertheless, no significant difference was observed in the transfer rate of indomethacin from the central to the tissue compartment (K_{12}) (0.85 ± 0.11 and 0.83 ± 0.13 h^{-1} for the control and treated groups, respectively).

The percent change in the pharmacokinetic parameters produced by the oral administration of cholestyramine relative to the control is depicted in Fig. 2. There were significant decreases in $t_{1/2}\beta$

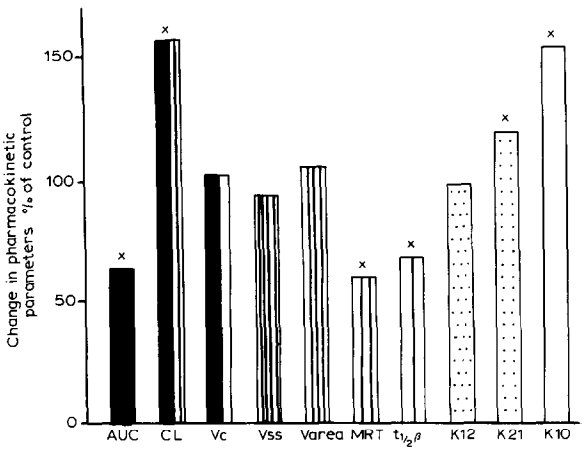

Fig. 2. Changes in the pharmacokinetic parameters of indomethacin expressed as percent of control values. * p 0.001.

(32.5%), MRT (40.5%) and AUC (36.3%) and increases in Cl (56.5%), K_{21} (19%) and K_{10} (53.3%).

These changes are compatible with an acceleration of indomethacin elimination induced by oral administration of cholestyramine. The changes in the parameters K_{12}, V_c, V_{ss} and V_{area} were not statistically significant (Fig. 2).

The in vitro adsorption of indomethacin onto cholestyramine followed the Langmuir adsorption isotherm (Fig. 3). The limiting adsorptive capacity as calculated via linear regression was 140 mg/g.

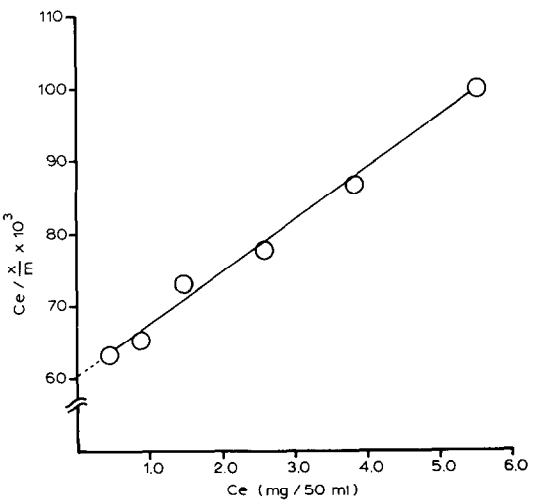

Fig. 3. Langmuir adsorption isotherms of indomethacin on cholestyramine.

Three successive washings of the drug-adsorbent mixture, with 20 ml buffer solution, resulted in 9% desorption.

Discussion

Oral administration of cholestyramine showed a pronounced increase in the systemic elimination of intravenously administered indomethacin as demonstrated by the observed increase in the systemic clearance (about 56%) and decrease in AUC (about 36%). A significant decrease (about 32%) in the elimination half-life was also noted.

The proposed mechanism underlying the cholestyramine-induced enhancement of indomethacin clearance involves the following events: cholestyramine interrupts the enterohepatic circulation of indomethacin that is excreted into the bile, either unchanged or as metabolites which are converted to indomethacin in the gastrointestinal tract, eventually causing their excretion in the feces. The question as to whether indomethacin itself is secreted (exsorbed) into the gut lumen with subsequent trapping by cholestyramine cannot be resolved as yet and remains to be established.

Previous studies on the metabolism and clinical pharmacology of indomethacin have suggested that enterohepatic circulation of indomethacin may influence its duration of action and the sensitivity to intestinal lesions (Duggan et al., 1975). Further, it was reported by Kwan et al. (1975) that between 24 and 115% of an intravenous dose of indomethacin undergoes biliary secretions and reabsorption. Therefore, the intestinal lumen may be considered as one of the routes of drug distribution. The sequestering of indomethacin in the gastrointestinal tract by cholestyamine interrupts the enterohepatic cycle and thereby enhances indomethacin clearance from the body.

The apparent lack of effect of cholestyramine on the volume of distribution parameters (Table 1, Fig. 2) could, at least in part, be explained by assuming that the adsorption of indomethacin onto cholestyramine in the gut is practically an irreversible process or that the desorption of the drug from cholestyramine is very slow in comparison to the rate of adsorption. The in vitro adsorption and desorption studies support this explanation. Cholestyramine has a high adsorptive capacity for indomethacin, and a small amount (9%) of the drug is desorbed following repetitive washings. The unaltered volume of distribution of indomethacin observed in vivo is consistent with nearly irreversible adsorption observed in vitro. The indomethacin adsorbed onto cholestyramine in the gastrointestinal lumen is distant from the central pool of the drug in the body, therefore, the gastrointestinal tract in the presence of cholestyramine could represent an elimination compartment.

In previous studies, a highly significant correlation between total biliary secretion of indomethacin and sensitivity to intestinal lesions was achieved in rabbits and in man (Duggan et al., 1975). Further, the ratio of biliary clearance to total plasma clearance of indomethacin has been reported to be similar in rabbits and in man (Duggan et al., 1975). Therefore, the rabbit can be considered as a useful animal model to study the effect of cholestyramine on the interruption of enterohepatic cycling of indomethacin.

In conclusion, it was found that oral administration of cholestyramine can enhance the clearance of intravenously administered indomethacin. This effect is postulated to be due to augmentation of net biliary excretion through enteric binding. Because of the reported similarities between man and rabbits with regard to the extent of biliary secretion of indomethacin, it may be expected that cholestyramine administration can increase the systemic elimination of the drug in cases of overdosage or intoxication. On the other hand, this effect may result in lowering indomethacin blood levels in patients treated concurrently with cholestyramine for hypercholesterolemia.

Acknowledgements

The authors gratefully acknowledge the support of the Research Center, College of Pharmacy, King Saud University, for this research through Grant No: CPRC-39. Efficient typing by Mr K. Abbas is also gratefully acknowledged.

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