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Inhibitory Effect of Cholestyramine on the Absorption of Flufenamic and Mefenamic Acids in Rats[1] (37755)

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(Introduced by G. Levy)
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Cholestyramine has been used clinically to lower elevated plasma lipid and cholesterol levels (1–5). This hypocholesterolemic effect is due to the binding of bile salt anions in the fluids of the small intestine, which decreases their reabsorption. Thus, the absorption of exogenous cholesterol is significantly reduced, while the hepatic biotransformation of endogenous (plasma) cholesterol into additional bile salts is increased (6, 7).

The quaternary ammonium exchange sites on cholestyramine are strongly basic and remain ionized over the normal pH range of the gastrointestinal (GI) fluids. Therefore, a significant drug–cholestyramine interaction may occur within the GI tract upon concurrent oral administration of this nonabsorbable resin with certain anionic drugs. Few quantitative reports have appeared in the literature concerning such interactions *in vivo* (8–12). The possible occurrence of such an interaction should not be overlooked, as it may result in a significant decrease in the rate and/or extent of drug absorption and, hence, in the therapeutic effectiveness or toxicity of the drug.

The purpose of the present investigation was to quantitate the *in vitro* and *in vivo* binding tendencies of cholestyramine for the potent anti-inflammatory and analgesic agents, mefenamic acid [*N*-(2,3-xylyl) anthranilic acid, p*K*a 4.2] and flufenamic acid [*N*-(α,α,α-trifluoro-*m*-tolyl) anthranilic acid, p*K*a 3.9].

Materials and Methods. The cholestyramine[3] used in this investigation was of pharmaceutical grade, the particle size of which was 100% < 100 mesh, 80% < 200 mesh. Flufenamic acid,[4] mefenamic acid,[4] sodium taurocholate,[5] and sodium glycocholate[5] were dried *in vacuo* for at least 24 hr prior to use. All other chemicals were of reagent quality and were used as received.

In vitro *binding studies.* In all *in vitro* equilibrium binding experiments, 25 mg of cholestyramine was accurately weighed into 50-ml glass-stoppered flasks. Twenty-five-milliliter quantities of aqueous solution of each fenamate, over a concentration range of 0.70–3.5 m*M* in pH 7.4 phosphate buffer (0.15 *M*) were quantitatively transferred to the flasks, and the samples agitated on a water bath shaker[6] at 37° until equilibrium was established (*i.e.*, within 48 hr). The samples were then subjected to Millipore filtration (0.45 μm pore size), appropriately diluted with sodium hydroxide (0.1 *N*), and assayed spectrophotometrically[7] at 286 nm (mefenamic acid) and 290 nm (flufenamic

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[3] Generously supplied by Merck & Co., Inc., Rahway, NJ 07065.

[4] Generously supplied by Parke Davis and Co., Detroit, MI 48232.

[5] Grade A. Obtained from Calbiochem, Los Angeles, CA 90063.

[6] Precision constant-temperature water bath shaker, Precision Scientific Co., Chicago, IL 60647.

[7] Beckman DB-G Spectrophotometer, Beckman Instruments, Inc., Fullerton, CA 92634.

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acid). Taurocholate or glycocholate did not interfere with this assay.

Employing this experimental protocol, the influence of 4.0 m*M* initial concentrations of either taurocholate or glycocholate anions on the fenamate–cholestyramine binding reaction was examined. These conjugated bile salt anions are normal constituents of intestinal fluid and, therefore, may potentially function as competitive inhibitors of the drug–resin interaction, *in vivo*.

In vivo *absorption studies*. Adult male Sprague–Dawley rats,[8] weighing 250–300 g, were fasted for a period of 20–24 hr prior to drug administration but were allowed free access to water at all times.

A test group of animals was lightly anesthetized with ether and dosed orally with a 0.25% methylcellulose suspension of flufenamic acid (50 mg/kg) or mefenamic acid (100 mg/kg), and a separately prepared suspension of cholestyramine (500 mg/kg). A control group of rats received the same dose of drug suspension and the proper volume of resin-free methylcellulose suspension vehicle. The total dosing volume was maintained constant at 5.0 ml/kg. At predetermined time intervals after drug administration, blood samples were collected into heparinized centrifuge tubes by decapitation (flufenamic acid studies) or blood withdrawal from the tail vein (mefenamic acid studies). In the latter case, several 0.5-ml blood samples were taken from each rat as a function of time. The blood samples were centrifuged at 2000*g* for 10 min, and the plasma obtained therefrom frozen until assayed, in duplicate, for drug content.

Assay of fenamates in plasma. The concentration of flufenamic acid in plasma samples was determined spectrophotometrically after a two-step extraction procedure. The method essentially involved acidifying 0.5 ml of plasma with an equal volume of sulfuric acid (1 *N*) and extracting the drug into 4.0 ml of carbon tetrachloride. The drug was then reextracted from a 2.0-ml aliquot of the organic phase into 3.0 ml of a 0.1 *N* sodium hydroxide solution, and the absorbance of the alkaline phase was read on a spectrophotometer[7] at 290 nm. The absorbance value for each plasma sample was converted to concentration units with the aid of a Beer's law plot prepared by subjecting plasma containing known concentrations of drug to the extraction procedure.

Plasma samples containing mefanamic acid were assayed by a modification of the fluorometric procedure described by Glazko (13). Accordingly, 0.2 ml of plasma was acidified with 0.1 ml of sulfuric acid (1 *N*) and the drug extracted into 5.0 ml of *n*-heptane (Spectroanalyzed grade). After separation of the phases by centrifugation, a 3.0-ml aliquot of the heptane phase was mixed with 0.1 ml of a 50% solution of trichloracetic acid in carbon tetrachloride (Spectroanalyzed grade), and the fluorescence of the resultant solution read on spectrofluorometer[9] using excitation and emission wavelengths of 368 nm and 470 nm (uncorr.), respectively. On each assay day, standard samples containing 0.5–4.0 μg of mefenamic acid were simultaneously assayed with unknown plasma samples, and a linear calibration plot constructed. The concentrations of mefenamic acid in the plasma samples were determined from such a plot.

Recovery studies showed that the extraction procedures quantitatively removed both drugs from plasma. Thin-layer chromatographic analyses, performed on extracted plasma samples, indicated that over the entire experimental time period, only intact flufenamic and mefenamic acids were detectable. The solvent system utilized in the chromatographic analyses consisted of isopropanol, ammonium hydroxide, and distilled water (20:1:2, v/v). The average R_f values obtained for pure and plasma-extracted samples, respectively, were: flufenamic acid, 0.640 and 0.633; mefenamic acid, 0.565 and 0.560.

All drug plasma concentration data were corrected for blank values. The Student's *t* test was used to statistically evaluate the *in vivo* results.

[8] Obtained from Blue Spruce Farms, Altamont, NY.

[9] Aminco-Bowman Spectrofluorometer, American Instrument Co., Inc., Silver Spring, MD 20910.

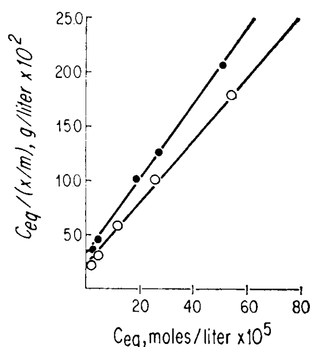

FIG. 1. Langmuir adsorption isotherm for the binding of flufenamic acid (○) and mefenamic acid (●) to cholestyramine at 37°.

Results and Discussion. In vitro *binding studies.* The results of all *in vitro* equilibrium fenamate–cholestyramine binding studies were fitted to the linear form of the Langmuir adsorption isotherm (14), $Ceq/(x/m) = 1/k_1k_2 + Ceq/k_2$; where Ceq is the concentration (moles/liter) of unbound drug present in solution at equilibrium, x is the amount (moles) of drug bound to the resin, m is the mass (g) of resin employed, k_1 is the association constant (liters/mole) governing the drug–resin interaction, and k_2 is the maximum capacity constant (*i.e.*, the maximum amount (moles) of drug bound monomolecularly per mole-equivalent of resin). Representative Langmuir plots are shown in Fig. 1. The interaction constants, k_1 and k_2, determined from the least-squares intercept and slope values of such plots, are summarized in Table I. The flufenamate anion binds more strongly and extensively to cholestyramine than does the mefenamate anion. These results are consistent with the organic anion–resin binding mechanism previously suggested by Johns and Bates (15). Accordingly, such reactions involve both a primary electrostatic interaction (*i.e.*, between the negatively charged carboxyl group on the organic anion and the cationic quaternary ammonium group on the resin) and a secondary, nonelectrostatic interaction between the two reactants. Apparently, the electron withdrawing capabilities of the trifluoromethyl group on the flufenamate anion provide for a greater interaction with the resin as compared with the methyl group-containing mefenamate anion.

Conjugated bile salt anions, which are present in the luminal fluids of the small intestine, have been found to rapidly and significantly interact with cholestyramine (15–17). As a result, they may function as competitive inhibitors of fenamate–resin interactions, *in vivo*. To explore this possibility, the *in vitro* binding of both fenamates to cholestryramine was assessed in the presence of 4.0 mM initial concentrations of these physiologic surfactants. The sodium salts of taurocholic and glycocholic acids were employed for these studies, since they comprise approximately 80% of the total bile salts present in rat bile (18). The taurocholate anion is significantly more effective as an inhibitor of the drug–resin interactions than is the more polar glycocholate anion (Table I). Under the experimental conditions, taurocholate decreases the inherent affinity constant of flufenamic and mefenamic

TABLE I. Effect of Added Conjugated Bile Salt Anions on the Binding of Flufenamic and Mefenamic Acids to Cholestyramine at 37°, pH 7.4.[a]

Added conjugated bile salt anion	Association constant, k_1 (liters/mole)		Maximum capacity constant, k_2 (moles of drug bound per mole-equivalent of resin[b]	
	Flufenamate	Mefenamate	Flufenamate	Mefenamate
None	1.56×10^4	1.13×10^4	0.763	0.659
Taurocholate (4.0 mM)[c]	1.19×10^4	0.680×10^4	0.678	0.661
Glycocholate (4.0 mM)[c]	1.47×10^4	0.960×10^4	0.720	0.667

[a] Total electrolyte concentration maintained constant at 150.0 mM.
[b] Based on a monomer equivalent weight for cholestyramine of 230.
[c] Initial concentration added to the fenamate–resin binding system.

TABLE II. Effect of Concomitant Oral Administration of Cholestyramine (500 mg/kg) on the Absorption of Flufenamic Acid (50 mg/kg).

Time (hr)	Dosage regimen	Mean plasma concentration[a] (μg/ml)	SE[b]	Level of significance[c]	Percent reduction
0.25	Flufenamic acid	51.2	8.05	$P < 0.02$	47.7
	Flufenamic acid + resin	26.8	1.68		
0.5	Flufenamic acid	79.8	12.5	$P < 0.001$	72.1
	Flufenamic acid + resin	22.3	3.26		
1.0	Flufenamic acid	89.2	16.3	$P < 0.01$	63.9
	Flufenamic acid + resin	32.3	6.10		
1.5	Flufenamic acid	58.4	7.17	$P < 0.001$	63.3
	Flufenamic acid + resin	21.4	2.98		
2.5	Flufenamic acid	45.8	4.41	$P < 0.001$	61.9
	Flufenamic acid + resin	17.4	1.55		
3.5	Flufenamic acid	41.2	6.51	$P < 0.005$	60.6
	Flufenamic acid + resin	16.2	1.64		
4.5	Flufenamic acid	29.6	2.39	$P < 0.02$	34.9
	Flufenamic acid + resin	19.3	2.87		

[a] Mean of seven animals.
[b] SE = standard error of the mean.
[c] Level of significance as determined by Student's t test.

acids by 23.7% and 39.8%, respectively, while the glycocholate anion only produces 5.8% and 15.0% reductions. The greater inhibitory effect of taurocholate anions on the binding process for both fenamates parallels its greater inherent strength of interaction with cholestyramine as compared to the glycocholate anions (16). The fact that the magnitude of the maximum capacity constants (k_2) was insignificantly affected by the presence of either conjugated bile salt anion suggests that their inhibitory effect is competitive in nature.

In vivo *absorption studies.* If a significant fenamate–resin interaction is to take place in the fluids of the GI tract, it must compete kinetically with the process of drug absorption. Consequently, it was of considerable importance to quantify this interaction *in vivo*. Therefore, the absorption profiles of flufenamic (50 mg/kg) and mefenamic (100 mg/kg) acids alone and upon concurrent oral administration of cholestyramine (500 mg/kg) were studied in the intact rat. Cholestyramine in the GI tract of the test animals reduced significantly the mean plasma concentrations of both fenamates over the entire experimental time period (Table II, Fig. 2). The lower plasma levels at 0.5 and 1 hr indicate that the resin is capable of rapidly and strongly interacting with both fenamates within the GI tract. The fact that coadministration of the resin produces statistically significant decreases in the magnitude of peak plasma fenamate concentrations (Table II, Fig. 2) but not in their time of occurrence, suggests strongly that the amount of unbound fenamate available for absorption, rather than the rate constant associated with the process, is being affected by the ion-exchange resin.

In the mefenamic acid study, the area under the plasma concentration versus time curve from time zero to infinity, $(AUC)_{0-\infty}$ was calculated for each control and resin-treated animal. This pharmacokinetic parameter is directly proportional to the fraction of the dose of drug absorbed and can, therefore, be used as a measure of relative bioavailability (19). The results of the area measurements are summarized in Table III. It was found by this method that only 29.9% of the dose of mefenamic acid is available for

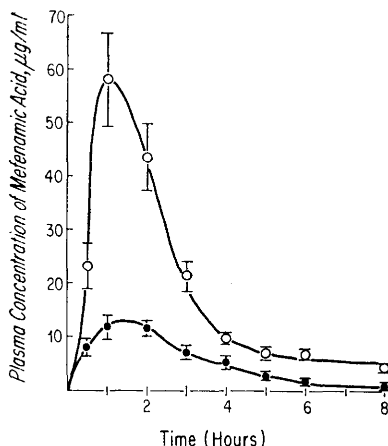

FIG. 2. Mean plasma concentrations of mefenamic acid after its oral administration at a dosage level of 100 mg/kg, alone (○) and concurrently with a 500 mg/kg dose of cholestyramine (●). Each point represents the mean of six animals. Bars denote standard errors of the mean.

absorption in the presence as compared to the absence of cholestyramine. This marked, 70.1% reduction in the relative extent of absorption or bioavailability of mefenamic acid suggests that cholestyramine rapidly and strongly binds the drug within the GI tract of the test animals. A similar relative bioavailability determination was not feasible with the flufenamic acid data, since a complete time course of drug levels in the plasma could not be established in the same animals and because post-absorptive conditions were not reached in the resin-treated animals. The available data show that the resin produces a statistically significant, 34.9 to 72.1% reduction in the plasma concentrations of flufenamic acid in the experimental time period examined.

The results of the present investigation have provided evidence that cholestyramine markedly decreases both the rate and extent of absorption of flufenamic and mefenamic acids at resin to drug, single-dose ratios considerably lower than those expected in their clinical use. The ratios employed in this study were 10:1 and 5:1, whereas, clinically they would be on the order of 50:1 and 20:1 for flufenamic and mefenamic acids, respectively. As a result, studies in man are warranted to establish the influence of the resin on the therapeutic efficacy and to explore the potential usefulness of cholestyramine for the treatment of acute fenamate intoxication.

Summary. The binding characteristics of the hypocholesterolemic agent cholestyramine for flufenamic and mefenamic acids were examined *in vitro* and *in vivo*. Both drugs strongly interact *in vitro* with the resin, and the presence of physiologic concentrations of conjugated bile salt anions competitively inhibits, but does not totally prevent, this interaction. When flufenamic acid or mefenamic acid and the resin were coadministered orally to fasted rats, a 60–70% reduction in both the rate and extent of absorption of these acids was observed. These results suggest that the therapeutic response elicited by these drugs may be seriously affected by concurrent administration of the resin. Also, the resin may prove to be useful in the treatment of acute fenamate intoxication.

TABLE III. Effect of Concomitant Oral Administration of Cholestyramine on the Extent of Absorption of Mefenamic Acid.

	Area under the plasma concentration vs time curve from time zero to infinity $(AUC)_{0-\infty}$ (μg-hr/ml)[a]	
	Mefenamic acid, 100 mg/kg	Mefenamic acid, 100 mg/kg plus cholestyramine, 500 mg/kg
	145.6	48.3
	115.2	39.9
	163.0	45.5
	135.2	46.7
	183.7	48.8
	127.1	31.2
Mean (SE)[b]	145.0 (10.2)	43.4 (2.77)
	$P < 0.001$[c]	

[a] Area from time zero to 8 hr determined by the trapazoidal rule. Area from 8 hr to infinity determined from the relationship, $C_8 \times 1.44 \times t_{1/2}$; where C_8 is the experimentally determined plasma concentration at 8 hr and $t_{1/2}$ is the biological half-life of the drug.

[b] Standard error of the mean in parentheses.

[c] Determined by Student t test.

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