Effect of Competing Anions on Binding of Bile Salts by Cholestyramine

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The binding of bile salts by cholestyramine may be influenced by other anions, as the Langmuir adsorption coefficients for three bile salts tested were similar to the model anion, citrate. However, the selectivity coefficient indicated preferential binding of cholate anion in comparison to citrate anion. Binding experiments confirmed cholestyramine's preference for bile salts as the presence of other anions reduced but did not prevent the binding of cholate anion. Binding of cholate anion was reduced in direct relationship to the citrate anion concentration. Prior exposure of cholestyramine to citrate anion caused the binding of cholate anion to decrease slightly. Sequential exposure of cholestyramine to simulated gastric fluid and simulated intestinal fluid containing cholate anion resulted in a decrease in cholate binding which was attributed to competition with anions present in the gastrointestinal fluids. Components of tomato juice and orange juice, fluids commonly used to enhance ingestion of cholestyramine, also reduced the binding of cholate anion.

KEY WORDS: cholestyramine; bile salt binding; competitive binding.

INTRODUCTION

Cholestyramine is a therapeutically important anion exchange resin which consists of a styrene divinylbenzene copolymer containing quaternary ammonium groups (1). Its bile salt sequestering properties make it a useful agent for the management of hypercholesterolemia. Bile salts are sorbed from the intestinal fluid by cholestyramine and thereby excreted in the feces (2). Cholesterol is metabolized in response to the decreased return of bile acids to liver cells.

Because of the anion-exchange nature of cholestyramine, its binding capabilities are not restricted to bile salts and may include other anions. Orally administered cholestyramine encounters numerous anions while in the gastrointestinal tract. Possible sources of anions capable of competing with bile salts include dietary constituents, excipients in the cholestyramine dosage form, and anions naturally found in gastrointestinal fluids.

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The purpose of this study was to examine the effect of competing anions on the binding properties of cholestyramine for bile salt anions.

EXPERIMENTAL

The cholestyramine, sodium cholate, sodium glycocholate, sodium taurocholate, pepsin, and pancreatin were obtained commercially (Sigma).

Bile salts and citrate anion were quantified by an established HPLC method (3) using methyl paraben as an internal standard. The amount of anion bound by cholestyramine was calculated from the difference between the initial concentration of anion introduced into the system and the concentration present in solution upon sampling.

The effect of pH on binding was studied by placing 100 mg of cholestyramine in 100 ml of 3.75 m*M* sodium cholate at 37°C. The suspension was titrated to the desired pH by use of a pH-stat titrator (PHM 62, TTT 60, ABU 12, REA 160, Radiometer) with either 0.05 *N* HCl or 0.05 *N* NaOH. The volume of titrant added to control the pH was less than 4.0 ml, with adjustments made to account for the dilution of the samples. Following a 2-hr exposure period, the suspension was centrifuged at 6000 rpm for 20 min. The clear supernatant was used for analysis of cholate anion.

The Langmuir adsorption parameters (4) of cholestyramine for various anions were determined at pH 7.5, 37°C. The anions examined and their concentration ranges were as follows: cholate, 2.0–12.5 m*M*; glycocholate, 1.0–7.5 m*M*; taurocholate, 1.0–7.5 m*M*; and citrate, 0.75–3.0 m*M*. One hundred milliliters of the appropriate anion solution and 100 mg of cholestyramine were equilibrated using the above procedure.

The same general procedure was used for a series of competitive binding experiments. A 1-g sample of cholestyramine was suspended in 100 ml of water containing from 0 to 5 mmol of cholate and/or citrate anions.

Sequential competitive binding of cholate and citrate anions was studied by exposing cholestyramine to a solution containing one anion. After 1 hr, the cholestyramine was separated from the suspension by centrifuging and a sample of the clear supernatant was removed for analysis. Next, the cholestyramine was washed with double-distilled, deionized water and then exposed to the appropriate second solution. Following a 1-hr exposure period, the mixture was centrifuged and the supernatant collected for analysis.

Competitive binding of cholate and citrate was investigated further by determining the selectivity coefficient (5) of the resin for the two anions. One gram of cholestyramine was converted to the required ionic form by dispersing the resin in 100 ml of the appropriate solution (22 m*M* citrate or 45 m*M* cholate) at pH 7.5, 37°C. After 1 hr, a sample of the supernatant was collected by centrifugation. The resin was then placed in 100 ml of the appropriate second solution at pH 7.5, 37°C. Following a 1-hr exposure period, the mixture was centrifuged to give a clear supernatant, which was analyzed for the amount of citrate and cholate remaining in solution.

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Table I. Effect of pH on Binding of Cholate Anion by Cholestyramine[a] at 37°C

pH	Cholate anion bound (mmol)
2.0	0.36
3.0	0.36
4.0	0.36
5.0	0.34
6.0	0.31
7.0	0.30
7.5	0.30
8.0	0.30

[a] 0.375 mmol sodium cholate exposed to 100 mg cholestyramine in 100 ml.

Sequential exposure to gastrointestinal fluids was studied by dispersing cholestyramine in simulated gastric fluid (6). After 1 hr, the cholestyramine was separated by centrifugation. The resin was then placed in simulated intestinal fluid (6) containing 2.0 mmol of sodium cholate. Following a 2-hr exposure period, the sample was centrifuged and the supernatant analyzed.

The effects of suspending the cholestyramine in different liquids on the binding of cholate anion was investigated by placing 1 g of cholestyramine in 50 ml of the beverage to be investigated. The suspension was magnetically stirred for 15 min. Fifty milliliters of 0.04 *M* sodium cholate was added to the beverage–cholestyramine suspension and the mixture was magnetically stirred in a glass-jacketed vessel at 37°C for 30 min. The pH was maintained at 7.5 by the use of a pH-stat titrator with 0.05 *N* NaOH. Following the exposure period, the liquid was removed and centrifuged to obtain a sample of the clear supernatant.

The rate of cholate anion binding by cholestyramine at pH 7.5, 37°C, was investigated by placing 100 mg of cholestyramine in 100 ml of 5.0 or 7.1 m*M* sodium cholate. Samples taken at 15, 30, 60, and 180 min were centrifuged to produce a clear supernatant.

RESULTS AND DISCUSSION

Cholate binding to cholestyramine was only weakly affected by pH (Table I), indicating that both cholic acid (pK = 6.4) and cholate anion bind by hydrophobic sorption, in addition to charge interaction for cholate only (see Ref. 7). This result is consistent with the hydrophobic nature of the styrene divinylbenzene copolymer.

Table II. Adsorption Constants[a] for Adsorption of Bile Salts by Cholestyramine at pH 7.5, 37°C

Adsorbate	Adsorption coefficient (L/mol)	Adsorption capacity (mmol/g)
Cholate anion[b]	1.23×10^4	3.49
Glycocholate anion[c]	0.95×10^4	3.79
Taurocholate anion[d]	1.67×10^4	3.59
Citrate anion[d]	1.03×10^4	1.48

[a] All isotherms had $R^2 > 0.99$.
[b] Seven points.
[c] Six points.
[d] Four points.

Table III. Binding of Cholate (1 mmol Sodium Cholate/100 ml) by Cholestyramine (1 g/100 ml) in the Presence of Citrate at pH 7.5, 37°C

mmol cholate:mmol citrate	Cholate anion bound (mmol)
1:0	0.95
1:0.5	0.87
1:1	0.76
1:2	0.69
1:5	0.60

The Langmuir adsorption characteristics of cholate, glycocholate, and taurocholate anions by cholestyramine at pH 7.5, 37°C, were investigated to determine the affinity of the resin for bile salts. pH 7.5 was selected since final adsorption of bile salts by anion-exchange resins takes place in the ileum (8). Citrate anion was also examined as a model anion. Linear plots of the isotherms were used to calculate the adsorption coefficients and the adsorption capacities (Table II). The adsorption coefficients are similar for the three bile salts and citrate anion. Thus, cholestyramine has a broad affinity for bile salts but its adsorptive capabilities are not restricted to bile salts and may include other anions.

The adsorption capacity obtained for citrate anion was approximately one-half that observed for the bile salts, possibly as a result of charge repulsion. Citrate anion is trivalent at pH 7.5 (pK_3 = 5.41), and thus, initial binding may interfere with the binding of additional citrate anions by adjacent sites.

Based on the adsorption characteristics presented in Table II, citrate was selected to investigate the effect of anions on the binding of cholate by cholestyramine. In competitive binding studies, citrate reduced the amount of bound cholate from 0.95 to 0.6 mmol at a ratio of 1 mmol cholate to 5 mmol citrate (Table III).

Table IV contains the results of binding experiments in which the ratio of sodium cholate to sodium citrate was held constant at 1:1 while the total concentration was varied. As the concentration was increased from 0.5 to 2 mmol, the amount of citrate bound by cholestyramine decreased from 0.39 to 0.24 mmol, whereas cholate binding increased from 0.46 to 1.40 mmol. The preferential binding of cholate over citrate may be related to the binding mechanisms. As noted in the effect of pH on binding (Table I), the binding of cholate anion to cholestyramine involves ion exchange and

Table IV. Binding of Cholate and Citrate by Cholestyramine Following Simultaneous Exposure at pH 7.5, 37°C (100-ml Solution)

mmol cholate: mmol citrate	Cholate anion bound, mmol (%)	Citrate anion bound, mmol (%)
0.5:0.5	0.46 (92)	0.39 (78)
1:1	0.78 (78)	0.29 (29)
2:2	1.40 (70)	0.24 (12)

Table V. Binding of Cholate or Citrate Anion by Cholestyramine Following Sequential Exposure at pH 7.5, 37°C

Experiment No.	First solution	mmol/g bound (%)	Second solution	mmol/g bound (%)
1	2.0 mmol citrate/100 ml	1.35 (68)	2.0 mmol cholate/100 ml	1.46 (77)
2	2.0 mmol cholate/100 ml	1.86 (93)	2.0 mmol citrate/100 ml	0.36 (18)
3	2.0 mmol citrate/100 ml	1.34 (67)	11.0 mmol cholate/100 ml	2.78 (24)
4	0.8 mmol citrate/100 ml	0.58 (73)	2.0 mmol cholate/100 ml	1.54 (81)

sorption. In contrast, binding of citrate anion by cholestyramine is primarily electrostatic.

The results of a series of sequential competitive binding studies are shown in Table V. Prior exposure to sodium citrate resulted in a decrease in cholate anion adsorption from 1.86 to 1.46 mmol. In contrast, prior exposure to sodium cholate caused citrate adsorption to decrease from 1.35 to 0.36 mmol. Thus, cholate binding is less sensitive to competing anions than citrate binding. At the higher concentration of sodium cholate in the second exposure, the adsorption of cholate anion was significantly increased. Therefore, competition with other anions may be less important when the concentration of bile salts in the intestinal fluid is high. Comparison of experiments 1 and 4 demonstrates that cholate anion binding is inversely related to the concentration of sodium citrate in the first exposure.

Sequential competitive adsorption of cholate and citrate anions was investigated further by determining the selectivity coefficient of cholestyramine for these anions. The selectivity of ion-exchange resins refers to the property of certain ion exchangers to exhibit a preferential activity for different ions. For the general reaction

$$bRA + aB^{b+} \rightleftharpoons aRB + bA^{a+}$$

where R represents the resin and A and B the exchangeable ions, the selectivity coefficient (5) is determined by the following quotient:

$$K_{ba} = \frac{[RB]^a[A]^b}{[RA]^b[B]^a}$$

where brackets represent the concentration and a and b are the absolute charges of ions A and B, respectively.

When cholestyramine was first saturated with cholate and then exposed to a solution containing citrate anions, a selectivity coefficient of 0.021 was obtained. Alternatively, when cholestyramine was saturated with citrate followed by exposure to cholate anions, the selectivity coefficient was 47.0. The two values are reciprocals, indicating that the adsorption process is reversible and that cholestyramine preferentially adsorbs cholate anion over citrate anion.

Competitive adsorption was further studied under conditions which more closely mimic those existing *in vivo*. Orally administered cholestyramine is first exposed to gastric fluid and then to intestinal fluid as it passes through the gastrointestinal tract. Thus, the effect of sequential exposure to solutions of different composition and pH on the binding of bile salts at pH 7.5, 37°C, by cholestyramine was studied. As shown in Table VI, exposure to simulated gastric fluid, rather than to water adjusted to pH 1.2, decreased the binding of cholate anion from simulated intestinal fluid from 1.84 to 1.36 mmol/g.

When used as an antihyperlipidemic agent, cholestyramine is frequently suspended in a liquid such as water, fruit juice, or carbonated beverage prior to administration. Since anions present in these liquids may compete with bile salts for adsorption, the effect of suspending cholestyramine in several liquids was studied (Table VII). Two types of tomato juice with regular and low sodium content were examined. The salt content of the tomato juice was not a factor, as both types of tomato juice reduced binding of cholate from 1.84 mmol in water to 1.39 mmol/g.

Orange juice with and without pulp was studied to determine if pulp affects binding of cholate anion. Both types of orange juice reduced the binding of cholate to 1.24 mmol/g, a decrease of 30% in comparison to distilled water. Nonnutritive fiber has been shown to adsorb bile salts (9); how-

Table VI. Effect of Sequential Exposure to Simulated Gastric and Simulated Intestinal Fluid on the Binding[a] of Cholate Anion by Cholestyramine

Simulated gastric fluid		Simulated intestinal fluid		Cholate anion bound, mmol/g (%)
Pepsin	NaCl	Pancreatin	KH_2PO_4	
–[b]	–	–	–	1.84 (92)
+[c]	+	+	+	1.36 (68)

[a] 2.0 mmol sodium cholate exposed to 1 g cholestyramine in 100 ml.
[b] Omitted.
[c] Included.

Table VII. Binding of Cholate by Cholestyramine[a] Suspended in Typical Beverages

Beverage	mmol/g bound (%)
Distilled water	1.84 (92)
Tomato juice[b]	1.40 (70)
Tomato juice, low salt	1.38 (69)
Orange juice, with pulp	1.24 (62)
Orange juice, without pulp	1.24 (62)
Orange juice, with pulp but no cholestyramine added	0.016 (0.8)

[a] 2.0 mmol sodium cholate exposed to 1 g cholestyramine in 100 ml.
[b] 450 mg sodium and 570 mg potassium per 6 oz.
[c] 20 mg sodium per 6 oz.

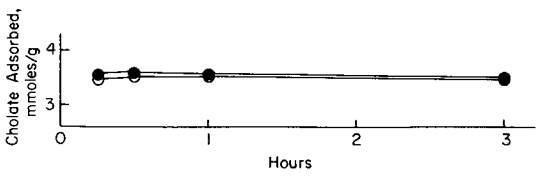

Fig. 1. Rate of adsorption of cholate anion by cholestyramine at pH 7.5, 37°C. (○) Cholate anion equal to 140% of the adsorptive capacity/g; (●) cholate anion equal to 230% of the adsorptive capacity/g. The adsorptive capacity was 3.49 mmol/g.

ever, the binding of cholate anion by the pulp in orange juice was only 0.016 mmol/g, indicating that cholestyramine is the major cholate-binding agent. These results demonstrate that the liquid in which cholestyramine is suspended influences cholate binding.

The rate of cholate diffusion to the binding sites of cholestyramine was studied because cholestyramine presence within the gastrointestinal tract in limited. At both of the sodium cholate concentrations examined (140 or 230% of the adsorptive capacity), binding of cholate anion was rapid, reaching equilibrium within 15 min. (Fig. 1). Thus, during the residence time in the ileum, it is expected that cholestyramine reaches equilibrium with the surrounding solution to exert maximum therapeutic effect.

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REFERENCES

1. *The Merck Index,* 10th ed., Merck & Co., Rahway, N.J., 1983, p. 312.
2. T. B. Van Itallie, S. A., Hashim, R. S. Crampton, and D. M. Tennent. The treatment of pruritus and hypercholesterolemia of primary biliary cirrhosis with cholestyramine. *N. Engl. J. Med.* 265:469–473 (1961).
3. T. J. Konechnik, R. Kos, J. L. White, S. L. Hem, and M. T. Borin. In-vitro adsorption of bile salts by colestipol hydrochloride. *Pharm. Res.* 6:619–623 (1989).
4. A. Martin, J. Swarbrick, and A. Cammarata. *Physical Pharmacy,* 3rd ed., Lea & Febiger, Philadelphia, 1983, pp. 461–464.
5. M. Marhol. *Ion Exchangers in Analytical Chemistry. Their Properties and Use in Inorganic Chemistry,* Elsevier, New York, 1982, pp. 106–115.
6. *The United States Pharmacopeia,* United States Pharmacopeial Convention, Rockville, Md., 1985, p. 1424.
7. F. Helfferich. *Ion Exchange,* McGraw–Hill, New York, 1962, pp. 72–78.
8. W. A. Phillips, J. R. Schultz, and W. W. Stafford. Effects of colestipol hydrochloride on drug absorption in the rat I: aspirin, L-thyroxine, phenobarbitol, cortisone, and sulfadiazine. *J. Pharm. Sci.* 63:1097–1103 (1974).
9. D. Kritchevsky and J. A. Story. Binding of bile salts in vitro by nonnutritive fiber. *J. Nutr.* 104:458–462 (1974).