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Report

Effect of Anions on Adsorption of Bile Salts by Colestipol Hydrochloride

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The Langmuir affinity constant and adsorptive capacity for the adsorption of citrate anion or cholate anion by colestipol hydrochloride at pH 7.5, 37°C, were similar. Prior exposure of colestipol hydrochloride to citrate anion caused the adsorption of cholate anion to decrease slightly in comparison to a control utilizing only cholate anion. The concentration of citrate anion was found to be directly related to the decrease in cholate anion adsorption. Simultaneous exposure of colestipol hydrochloride to citrate and cholate anions at pH 7.5, 37°C, resulted in the same adsorption of cholate anion as sequential exposure to citrate anion followed by cholate anion. Sequential exposure of colestipol hydrochloride to simulated gastric fluid and simulated intestinal fluid containing cholate anion resulted in a small decrease in cholate adsorption which was attributed to competition with phosphate anion in simulated intestinal fluid. Pepsin in the simulated gastric fluid did not affect adsorption of cholate anion from simulated intestinal fluid. Preexposure to components of tomato juice and orange juice also slightly reduced the adsorption of cholate anion by colestipol hydrochloride.

KEY WORDS: colestipol hydrochloride; bile salt adsorption; competitive adsorption.

INTRODUCTION

Colestipol hydrochloride is effective orally in decreasing serum cholesterol levels because it is an anion-exchange copolymer which binds bile salts in the intestine, forming a complex that is excreted in the feces. The reduced level of bile salts stimulates the rate of conversion of cholesterol to bile acids in the liver to restore the bile salt concentration of the intestinal fluid (1,2). Competition between bile salts and anions may reduce the adsorption of bile salts. Anions which compete with bile salts may be encountered in the gastrointestinal tract or when the colestipol hydrochloride is dispersed in a beverage prior to ingestion. The possibility of competition with other anions was recognized by Ko and Royer (3), who demonstrated that the adsorption of taurocholate anion by colestipol hydrochloride at pH 7.5 was inversely related to ionic strength. They studied the effect of monovalent anions such as chloride but speculated that the effect of polyvalent anions could be substantial. Further evidence for competitive adsorption was that taurocholate adsorption at pH 7.5 was greater in a tromethamine buffer than in a phosphate buffer (3). In this study we investigated the effect of sequential or simultaneous exposure of anions on the adsorption of bile salts by colestipol hydrochloride.

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EXPERIMENTAL

Colestipol hydrochloride was obtained commercially (Upjohn). Cholate anion or citrate anion was quantified by high-pressure liquid chromatography (HPLC; Model M-45, Waters) using a UV detector at 214 nm (Model 441, Waters) and C-18 reverse-phase column (μBondapak C18, Waters) through modification of a published method (4). The mobile phase consisted of 0.01 *M* monobasic potassium phosphate adjusted to pH 3 with phosphoric acid and then diluted with an equal volume of acetonitrile. All standard curves had an R^2 value of 0.99 or greater.

The adsorption isotherm of citrate anion was determined by combining 1 g of colestipol hydrochloride and 100 ml of a sodium citrate solution (ranging from 0.006 to 0.041 *M*) in a 150-ml beaker which was covered and placed in a water jacket at 37°C. The mixture was stirred and the pH maintained at 7.5 using a pH-stat titrator (PHM 62, TTT 60, ABU 12, REA 160, Radiometer) with 1 *N* HCl. The volume of titrant added was less than 0.2 ml. After approximately 2 hr of stirring, a sample of supernatant was removed and centrifuged. The clear supernatant was used for HPLC analysis.

Sequential adsorption studies were performed by adding 100 ml of the first solution to 1 g of colestipol hydrochloride in a 150-ml jacketed beaker at 37°C. The solution was stirred and the pH maintained at 7.5 by a pH-stat titrator (PHM 62, TTT 60, ABU 12, REA 160, Radiometer) using 1 *N* HCl for 1 hr. The colestipol hydrochloride was allowed to settle and 75 ml of supernatant liquid was removed by pipette. The colestipol hydrochloride was washed with distilled water (75 ml minus the volume of titrant added by the pH-stat titrator).

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The wash was repeated by removing a second 75-ml portion of the supernatant liquid and replacing it with 75 ml of distilled water. An appropriate quantity of sodium citrate or sodium cholate was added to produce the desired concentration of the final suspension.

Simulated gastric and intestinal fluids were prepared according to USP standards (5). A sample of simulated gastric fluid was also prepared without pepsin. The pH was 1.1 compared to a pH of 1.2 for the USP standard. A sample of simulated intestinal fluid was prepared by omitting the dibasic potassium phosphate. The pH was adjusted to 7.5 with 0.2 *N* NaOH as required for the USP standard. One gram of colestipol hydrochloride was mixed with 100 ml of the appropriate simulated gastric fluid for 1 hr at 37°C. The simulated gastric fluid was removed as completely as possible by pipette. One hundred milliliters of the appropriate simulated intestinal fluid was added to the colestipol hydrochloride. Sodium cholate was then added to the simulated intestinal fluid to produce a dispersion of colestipol hydrochloride in 100 ml of simulated intestinal fluid containing 0.02 *M* cholate anion. The pH-stat titrator with 1 *N* NaOH was used to maintain the pH at 7.5 for 2 hr. A sample of the supernatant liquid was taken and centrifuged prior to HPLC analysis.

The effect of juices on adsorption of cholate anion was determined by dispersing 1 g of colestipol hydrochloride in 50 ml of the juice to be investigated. Fifty milliliters of a 0.04 *M* sodium cholate solution was added and the solution was maintained at 37°C. After 30 min, the supernatant liquid was sampled, and the pH measured and analyzed for cholate anion by HPLC. The adsorption of cholate anion by 1 g of colestipol hydrochloride dispersed in 100 ml of 0.02 *M* sodium cholate adjusted to the same pH as the juice sample was also determined.

RESULTS AND DISCUSSION

Citrate was selected to study the competition between bile salts and anions for adsorption by colestipol hydrochloride because this trivalent anion is expected to be adsorbed by the anion exchange resin and it is present in gastrointestinal fluids (6) and in many foods. The Langmuir equation was applied to the adsorption of citrate anion by colestipol hydrochloride at pH 7.5, 37°C. This pH was selected as it is the pH of the distal portion of the ileum, where bile salt adsorption is believed to occur (7). As seen in Table I, the affinity constant and adsorptive capacity were 4.77×10^3 liter/mol and 1.54 mmol/g. The affinity constant is the same order of magnitude but approximately three times larger than found for three model bile salts (8). The affinity constant reflects the magnitude of forces involved in adsorbing citrate anion and its larger value might be expected due to the larger negative charge of the citrate anion at pH 7.5 in comparison to the bile salts. In contrast, the adsorptive capacity for citrate anion is approximately one-third of that exhibited by the bile salts.

Adsorption, according to the Langmuir model, depends on both the affinity constant and the adsorptive capacity. The data in Table I suggest that the higher negative charge of the citrate anion results in stronger binding in comparison to bile salts. However, the more highly charged citrate anions will interfere with the adsorption of other citrate anions on adjacent adsorption sites and yield a lower adsorptive capacity. On balance, it appears that the adsorption characteristics of colestipol hydrochloride for bile salt anions or citrate anions are comparable and the presence of citrate anions will not prevent adsorption of bile salts. This hypothesis was tested by exposing colestipol hydrochloride to citrate and cholate anions either simultaneously or sequentially.

Table II summarizes the results of a series of competitive adsorption experiments at pH 7.5, 37°C. As seen in experiments 1 and 2, when 2.0 mmol citrate anion was exposed to 1 g of colestipol hydrochloride in 100 ml, 1.4 mmol of the citrate anion was adsorbed. In comparison, exposure of 1 g of colestipol hydrochloride to 2.0 mmol sodium cholate in 100 ml resulted in adsorption of 1.9 mmol of the cholate anion. The lower fraction of citrate anion adsorbed in comparison to cholate anion is consistent with the adsorptive capacities determined in Table I. Prior exposure to 2.0 mmol sodium citrate caused the adsorption of cholate anion to decrease from 1.9 to 1.46 mmol. Likewise, prior exposure to 2.0 mmol sodium cholate caused the adsorption of citrate anion to decrease from 1.4 to 0.86 mmol. Thus, cholate adsorption is reduced but is still substantial when colestipol hydrochloride is first exposed to citrate anion.

Experiment 3 (Table II) illustrates the effect of increasing the concentration of the sodium cholate solution in the second exposure. When the second solution contained 12.0 mmol cholate anion, 4.32 mmol was adsorbed. Cholate adsorption increased by a factor of 2.9 when the concentration of cholate anion in the second solution was increased from 2 to 12 mmol/100 ml (experiment 1 vs 3). Thus, colestipol hydrochloride will be more effective in removing cholate anion at higher concentrations of bile salts in intestinal fluid, even after prior exposure to citrate anion.

Experiment 4 (Table II) illustrates the effect of reducing the concentration of sodium citrate in the first exposure from 2.0 to 0.80 mmol/100 ml. At this lower level, almost all of the citrate anion was adsorbed, but subsequent exposure to 2 mmol cholate anion in 100 ml resulted in the adsorption of cholate anion increasing from 1.46 mmol (experiment 1) to 1.66 mmol (experiment 4). Thus, the concentration of the competing anion to which the colestipol hydrochloride is first exposed is also an important factor.

Colestipol hydrochloride was exposed to 100 ml of a solution containing 2.0 mmol of both cholate and citrate anions in experiment 5 (Table II). The results show greater adsorption of cholate anion (1.38 mmol) than citrate anion

Table I. Adsorption Parameters from the Langmuir Equation for the Absorption of Citrate and Bile Salt Anions by Colestipol Hydrochloride at pH 7.5, 37°C

	Affinity constant (liter/mol)	Adsorption capacity (mmol/g)
Citrate anion[a]	4.77×10^3	1.54
Cholate anion[b]	1.60×10^3	4.72
Glycocholate anion[b]	1.90×10^3	4.28
Taurocholate anion[b]	1.15×10^3	4.72

[a] Five points; $R^2 > 0.99$.
[b] From Ref. 8

Table II. Adsorption of Citrate or Cholate Anion by Colestipol Hydrochloride at pH 7.5, 37°C, Following Sequential or Simultaneous Exposure

Expt No.	First solution	mmol/g adsorbed (% bound)	Second solution	mmol/g absorbed (% bound)
1	2.0 mmol citrate/100 ml	1.40 (70)	2.0 mmol cholate/100 ml	1.46 (73)
2	2.0 mmol cholate/100 ml	1.90 (95)	2.0 mmol citrate/100 ml	0.86 (43)
3	2.0 mmol citrate/100 ml	1.34 (67)	12.0 mmol cholate/100 ml	4.32 (36)
4	0.80 mmol citrate/100 ml	0.79 (99)	2.0 mmol cholate/100 ml	1.66 (83)
5[a]	2.0 mmol citrate and 2.0 mmol cholate	0.92 (46) citrate and 1.38 (69) cholate		

[a] Citrate and cholate anions simultaneously exposed to colestipol hydrochloride.

(0.92 mmol). Thus, competition with citrate anion will not prevent colestipol hydrochloride from adsorbing significant quantities of bile salts.

It is interesting to compare the results of experiment 1 with experiment 5. Simultaneous exposure to citrate and cholate anions results in virtually the same adsorption of cholate anion (1.38 mmol) as sequential exposure of citrate anion followed by cholate anion (1.46 mmol).

When used as an antihyperlipidemic agent, colestipol hydrochloride will first be exposed to gastric fluid and then to intestinal fluid as it passes through the gastrointestinal tract. It is possible that anions present in these fluids may alter cholate adsorption. Thus, the effect of sequential exposure to simulated gastric fluid and simulated intestinal fluid on the adsorption of cholate anion at pH 7.5, 37°C, was studied. Colestipol hydrochloride (1 g) was treated with simulated gastric fluid and then dispersed in simulated intestinal fluid before exposure to 2.0 mmol sodium cholate per 100 ml and determination of cholate anion adsorption.

As seen in Table III, sequential exposure to simulated gastric fluid and simulated intestinal fluid reduced cholate anion adsorption from 1.90 to 1.44 mmol/g (control vs experiment 1). Pepsin is present in simulated gastric fluid and will be negatively charged as its isoelectric point is approximately 1 (9). However, comparison of experiments 1 and 2 indicates that the presence of pepsin in simulated gastric fluid has no effect on cholate adsorption. This may be due to the denaturation of pepsin which occurs above pH 4 (10). Another potentially competing anion arises from the potassium phosphate, monobasic present in simulated intestinal fluid. A small competitive effect was seen for the monobasic phosphate anion as adsorption increased from approximately 1.5 to approximately 1.6 mmol/g when potassium phosphate, monobasic, was omitted from the simulated intestinal fluid (experiments 3 vs 1 and 4 vs 2). Thus, competing anions in the simulated intestinal fluids reduced the adsorption of bile salts, although other components of the simulated intestinal fluids had a greater effect.

Colestipol hydrochloride is frequently mixed 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 colestipol hydrochloride in several different liquids was studied. In this series of experiments, the pH was not adjusted to 7.5 but was dependent upon the beverage. The adsorption of cholate anion in the presence of these beverages was compared to adsorption from water at the same pH. The results are presented in Table IV. In distilled water, exposure of 1 g of colestipol hydrochloride to 2.0 mmol of cholate anion in 100 ml resulted in adsorption of 1.74 mmol. Two types of tomato juice were examined. The standard tomato juice contained 450 mg sodium and 270 mg potassium per 6-oz serving, according to the label. For each of these cations, an equivalent amount of anions would have to be present. The low-salt tomato juice contained only 20 mg of sodium per 6 oz according to the label. The amount of cholate adsorbed was approximately 1.6 mmol/g for each tomato juice, but this value was lower than the 1.9 mmol/g adsorption observed in water at the same pH. Thus, competition with components of tomato juice slightly reduced adsorption of cholate anion.

Table III. Adsorption of Cholate Anion by Colestipol Hydrochloride at pH 7.5, 37°C, Following Sequential Exposure to Simulated Gastric Fluid and Simulated Intestinal Fluid

Experiment	Simulated gastric fluid	Simulated intestinal fluid	mmol/g adsorbed (% bound)[a]
Control[a]	—	—	1.90 (95)
1	With pepsin	With KH_2PO_4	1.44 (72)
2	Without pepsin	With KH_2PO_4	1.46 (73)
3	With pepsin	Without KH_2PO_4	1.60 (80)
4	Without pepsin	Without KH_2PO_4	1.62 (81)

[a] One gram colestipol hydrochloride exposed to 2.0 mmol sodium cholate in 100 ml water at pH 7.5.

Table IV. Adsorption of Cholate Anion by Colestipol Hydrochloride Suspended in Typical Beverages[a]

Beverage	Final pH	mmol/g adsorbed (% bound)	mmol/g adsorbed (% bound) from water at Same pH
Distilled water	8.5	1.74 (87)	—
Tomato juice	7.5	1.58 (79)	1.90 (95)
Low-salt tomato juice	7.4	1.60 (80)	1.88 (94)
Orange juice with pulp	5.9	1.50 (75)	1.94 (97)
Orange juice without pulp	5.6	1.70 (85)	1.94 (97)

[a] One gram colestipol hydrochloride exposed to 2.0 mmol sodium cholate in 100 ml.

Orange juice with and without pulp was studied to determine if pulp affects adsorption of cholate anion. Nonnutritive fiber has been shown to adsorb bile salts (11,12). Colestipol hydrochloride dispersed in orange juice with pulp adsorbed 1.50 mmol/g of the cholate anion. This is 22% less than exhibited by an aqueous solution at the same pH. It was surprising to note that when colestipol hydrochloride was dispersed in orange juice without pulp, more cholate anion (1.70 mmol/g) was adsorbed than when it was dispersed in the orange juice with pulp. The composition of the two orange juices was not known and other factors, such as anion composition, may override the effect of pulp. The beverage in which colestipol hydrochloride is suspended does affect adsorption of cholate anion. Bile salt adsorption will not be affected if colestipol hydrochloride is dispersed in water. Bile salt adsorption may be reduced if colestipol hydrochloride is dispersed in tomato or orange juice. However, the *in vitro* data in Table IV suggest that significant adsorption of bile salts will still occur even if colestipol hydrochloride is administered in juices.

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