

Studies on Adsorption Characteristics of Bile Acids and Methotrexate to a New Type of Anion-Exchange Resin, Colestimide

Yoshiteru HONDA and Masahiro NAKANO*

Department of Pharmacy, Kumamoto University Hospital, 1-1-1 Honjo, Kumamoto 860-8556, Japan.

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The adsorption characteristics of various bile acids and methotrexate to a new type of anion-exchange resin, colestimide, were studied *in vitro* and compared with those to cholestyramine. For bile acids, colestimide was shown to have a higher capacity than cholestyramine. For example, approximately 1.4-fold higher for cholic acid and 2.0-fold for deoxycholic acid in water. In the presence of physiological anions, the degree of adsorption of cholic acid to both resins was greatly reduced, whereas adsorption of deoxycholic acid was only slightly reduced. Furthermore, the bed-volume of colestimide swelled about 6.8-fold in water, hence the anion-exchange groups of this resin are expected to be able to function effectively in adsorption of bile acids in the gut. In addition, colestimide was found to have high adsorption capacity for methotrexate, not only in water but also in media containing various physiological anions, and thus it is suggested that colestimide is a potential oral antidote to reduce possible toxicity by methotrexate through interruption of enterohepatic circulation.

Key words colestimide; anion-exchange resin; methotrexate; bile acid adsorption; methotrexate adsorption; methotrexate toxicity

Anion-exchange resins, a representative of which is cholestyramine, have been studied extensively and have been proven to be most effective in reducing cholesterol levels in patients with hypercholesterolemia.^{1,2)} It is also well known that these resins have rather low *in vivo* adsorption efficacy, in spite of high bile acid binding capacity *in vitro*.³⁾ This low *in vivo* adsorption appears to be due largely to poor dispersion of the resins in the intestine because of their insolubility in water and inaccessibility of bile acids to binding sites on the resins on account of their bulky steroid nucleus, and thus large doses (8—12 g/d) of these resins are required in order to produce hypolipidemic effects. Accordingly, clinical use of the conventional anion-exchange resins is limited, particularly in Japan, because of poor patient compliance. If a more potent resin can be developed, effective lowering of serum cholesterol levels may be achieved with a smaller dose and adverse effects such as constipation, nausea and meteorism associated with the resin may be less likely to occur.

Colestimide is a new type of anion-exchange resin with an imidazolium salt on an epoxide polymer skeleton that exhibits clinical pharmacological effects similar to those of cholestyramine at approximately a quarter (one-sixth on the basis of preparation) of the dose of cholestyramine⁴⁾ and thus may be expected to be useful as a bile acid sequestrant. However, little is known about the sorption characteristics of the resin. The present communication describes the results of an *in vitro* study on the sorption characteristics of colestimide for bile acids under various conditions.

From the standpoint of development of further applications other than hypercholesterolemia, the adsorption behavior of colestimide for methotrexate (MTX), an antifolate antineoplastic agent, was also examined. MTX is an anionic drug with two carboxyl groups in the structure and its molecular weight is similar to the bile acids. Additionally, it is known that the enterohepatic circulation plays an important role in the pharmacokinetics of the drug,⁵⁾ and that cholestyramine can accelerate excretion of the drug in patients suffering from MTX toxicity associated with high-dose MTX therapy and/or renal insufficiency, through binding of

MTX in the gut and interruption of the enterohepatic circulation of the drug.^{6,7)} Similarly with colestimide, the potential for application to modulating adverse reactions of MTX is suggested.

Experimental

Materials Colestimide is a new type of anion-exchange resin, and was a generous gift from Mitsubishi Chemical Co., Tokyo, Japan. Cholestyramine resin was purchased from Sigma Chemical Co., St. Louis, Missouri. The bile acids cholate, taurocholate, glycocholate, deoxycholate, chenodeoxycholate, ursodeoxycholate and glycodeoxycholate were kindly supplied as sodium salts by Mitsubishi Tokyo Pharmaceutical Co., Tokyo. Total Bile Acids-Test Wako for bile acid measurement was purchased from Wako Pure Chemical Industries, Osaka, Japan. Methotrexate was kindly supplied through Japan Wyeth Lederle Co., Tokyo, Japan. All other chemicals used were of the highest purity commercially available.

In Vitro Adsorption Studies Adsorption studies were carried out at 37°C by batch methods.⁸⁾ Accurately weighed amounts (5—10 mg) of colestimide or cholestyramine resin were placed in 50-ml screw-capped polypropylene test-tubes. Bile acid solutions of known concentrations (10 ml) were added to each of the test-tubes and the tubes were mechanically shaken for 30 min at 37°C. Since adsorption equilibrium to both resins was rapidly established within several minutes of incubation (results not shown), it was decided that a 30 min incubation time would guarantee adsorption equilibrium. After equilibration, the contents of each tube were filtered through a membrane filter (Millex-HA, 0.45 μm), appropriately diluted, and assayed for the concentration of unbound (*i.e.*, free) bile acids by means of the enzymatic color-comparative method with 3α-hydroxysteroid dehydrogenase. The amount of bile acid adsorbed was calculated as the difference between the total amount of bile acid introduced into the system and the amount of unadsorbed bile acid. The maximum binding capacities of the resins were calculated according to the Langmuir adsorption equation.⁸⁾ In the methotrexate adsorption test, incubation was similar to that for bile acids, with the exception of the determination method, *i.e.*, spectrophotometric analysis at 370 nm. Water, saline, 2nd fluid of JPXIII Disintegration Test and 0.05 M bicarbonate buffer containing 0.15 M NaCl were used as the incubation media. Absorbance was determined using a Shimadzu model UV-240 double beam spectrophotometer.

Swelling Test Swelling of the resins in water was examined. One gram of each resin was placed in a 50-ml measuring cylinder and an appropriate amount of water was added. The changes of the bed-volume of the resin were measured at predetermined times.

Results and Discussion

Adsorption of Bile Acids Figure 1 presents the adsorp-

* To whom correspondence should be addressed. e-mail: nakano@kaiju.medic.kumamoto-u.ac.jp

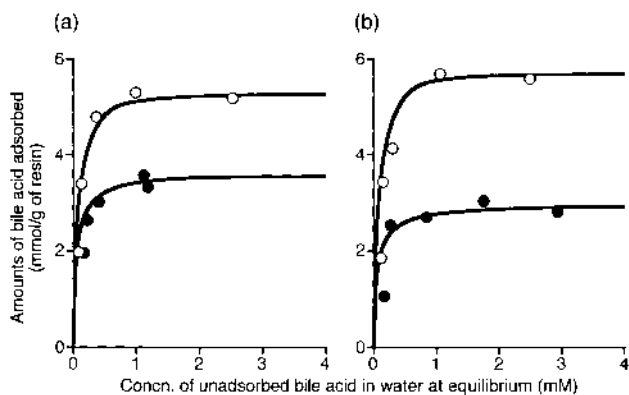


Fig. 1. Adsorption Isotherms for Binding of (a) Chololate and (b) Deoxychololate to Colestimide and Cholestyramine in Water at 37°C

○, colestimide; ●, cholestyramine.

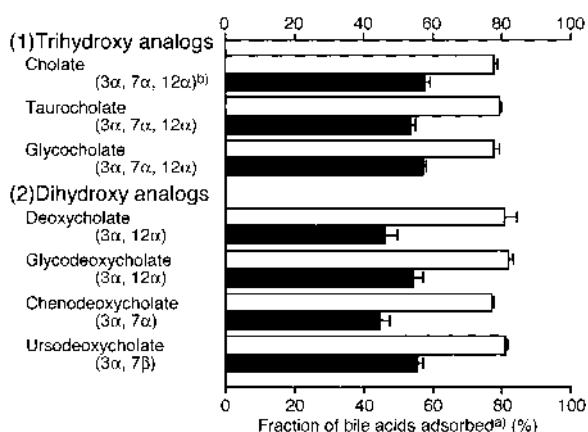


Fig. 2. Comparison of Bile Acid Adsorptive Powers of Colestimide and Cholestyramine in Water at 37°C

a) The percent adsorbed was determined for 5 mg of either resin equilibrated with 10 ml of 3 mM of each bile acid. Each value represents the mean+S.E. of three determinations. b) The position and orientation of hydroxyl groups are shown in parentheses. □, colestimide; ■, cholestyramine.

tion isotherms for the binding of two representative bile acids in water at 37°C. For both bile acids; chololate, a representative of bile acids which possess three hydroxyl groups, and deoxychololate, a dihydroxy analog, colestimide displayed greater adsorptive power than cholestyramine. The maximum binding capacities of colestimide for chololate and deoxychololate calculated according to the Langmuir equation were 5.38 and 5.86 mmol/g, and those of cholestyramine were 3.83 and 2.92 mmol/g, respectively. Thus, the adsorptive power of colestimide was shown to be approximately 1.4 times greater for cholic acid, and 2.0 times greater for deoxycholic acid than cholestyramine.

Adsorption behavior of the resins for a selected series of bile acids, under the following conditions, 10 ml of 3 mM bile salts solution; 5 mg resin, are also shown in Fig. 2. It was found that the rate of uptake of bile salts from solutions by colestimide is generally larger than that with cholestyramine, and is especially pronounced for dihydroxy analogs. With colestimide, there was no significant difference between unconjugated bile salt anions and their corresponding amino acid conjugates, *i.e.*, among the cholates or deoxycholates; chololate, taurochololate and glycochololate, deoxychololate, glycodeoxychololate, chenodeoxychololate and ursodeoxychololate.

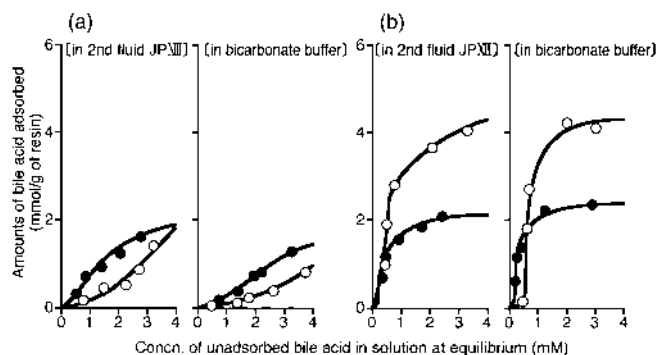


Fig. 3. Effects of Media on the Adsorption of (a) Chololate and (b) Deoxychololate to Colestimide and Cholestyramine at 37°C

○, colestimide; ●, cholestyramine.

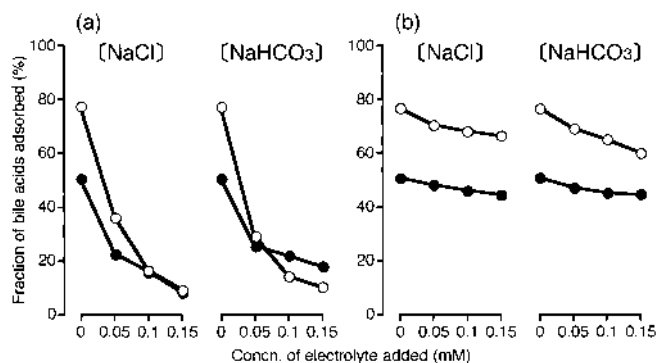


Fig. 4. Effects of Sodium Chloride and Sodium Bicarbonate on the Adsorption of (a) Chololate and (b) Deoxychololate to Colestimide and Cholestyramine in Water at 37°C

The percent adsorbed was determined for 5 mg of either resin equilibrated with 10 ml of 3 mM of each bile acid.

Since the primary mechanism of the interaction between bile acids and anion-exchange resins is most probably electrostatic in nature (*i.e.*, between the negatively charged carboxyl groups of the bile salt anions and the positively charged anion-exchange groups on the resin),⁹⁾ bile acid adsorption seems to be readily influenced by the various anions in the gut. Figure 3 presents the adsorption isotherms of the resins in solutions containing various electrolytes. All isotherms showed sigmoidal curves and thus no attempt was made to calculate the maximum binding capacities. Using the 2nd fluid of JPXIII and bicarbonate buffer, which are similar to physiological conditions in the gut,¹⁰⁾ the adsorption capacity of both resins for chololate was dramatically reduced in contrast to that in water. However, the reduction in the capacity of both resins for deoxycholic acid was not as great and colestimide exhibited nearly twice as high a capacity as that of cholestyramine, similar to the case when water was used as a medium.

In view of the possible influence of other anions on the adsorptive powers of the resins for bile acids, two physiological anions were introduced separately into the adsorption system. The effects of sodium chloride and sodium bicarbonate were investigated in the adsorption of chololate and deoxychololate in water (Fig. 4). As expected from the preceding results, the binding of chololate to both resins decreased markedly with increasing concentration of electrolyte. The observed reduction can best be attributed to the existence of

a competition between the chloride or bicarbonate anion and the bile salt anion for the available binding sites on the resin particle. However, in the case of deoxycholate, the resin capacities were not reduced much by the addition of inorganic salts. These findings indicate the importance of hydrophobicity of the bile salt anions in the adsorption to anion-exchange resins, and suggests that the sequestration of bile salts by the resin in the gut is greater for dihydroxy bile acids and negligible for trihydroxy analogs. Therefore, the serum cholesterol-lowering effects of anion-exchange resins are proposed to be based on the preferential adsorption of dihydroxy bile acids, which are the main components of bile acids in the gut.

The results of these adsorption experiments indicate that colestimide exhibited superior binding activities towards bile acids compared to cholestyramine. One of the reasons for the superiority of colestimide seems to be that the ion exchange capacity per gram of colestimide is 1.6 times higher than that of cholestyramine, *i.e.*, the former is 4.9 mEq/g and the latter is 3.0 mEq/g.⁴⁾ It has been demonstrated that the forces involved in the bile salt anion-cholestyramine interaction are primarily electrostatic in nature and are reinforced by an additional nonelectrostatic interaction of the steroid nucleus of bile acids with the hydrophobic portion of the resin, and the strength of the latter force is dependent on the degree of hydrophobicity of the adsorbate molecule.⁹⁾ From our observation that addition of electrolytes to the test system led to a marked reduction in binding for trihydroxy bile acids, but little reduction for dihydroxy analogs, this secondary hydrophobic binding force appears to contribute greatly to the adsorption of dihydroxy bile acids, but not for trihydroxy analogs. Furthermore, the contribution of this nonelectrostatic force to binding of bile salt anions is proposed to be much greater with colestimide compared with cholestyramine.

The phenomenon of a larger difference in adsorption capacity between colestimide and cholestyramine with the more hydrophobic dihydroxy bile salts than with the more polar trihydroxy derivatives, may be explained to some degree by the above-mentioned secondary interaction. Because the additional 7 α -hydroxyl group causes increased polarization of the cyclopentanophenanthrene ring system and increased steric hindrance of accessibility to binding sites in the resin matrix, dihydroxy derivatives are expected to be more readily adsorbed to the resin. Especially in the case of colestimide, steric hindrance due to the hydroxyl group on the bulky steroid ring seems to be a serious limiting factor for adsorption of bile salts, since it possesses many hydroxyl groups in its polymer matrix. Accordingly, it appears that the ratio of the adsorptive capacity for deoxycholate for colestimide and cholestyramine is larger than, and the ratio for cholate is smaller than, the ratio of ion exchange capacity between the resins.

Swelling in Water Since each bile acid consists of a bulky hydrophobic steroid nucleus and an ionic group, the extent of adsorption of bile acids to resins is considered to be greatly dependent on the accessibility of the anionic sites on the bile salts to the anion-exchange sites of the resins.¹¹⁾ Previously, we reported that the introduction of a spacer arm between the polymer backbone and a functional group makes binding sites readily accessible to the functional group, and thereby leads to improvement in the *in vivo* adsorptive efficiency of the resin.¹²⁾ Thus, it appears that the swelling prop-

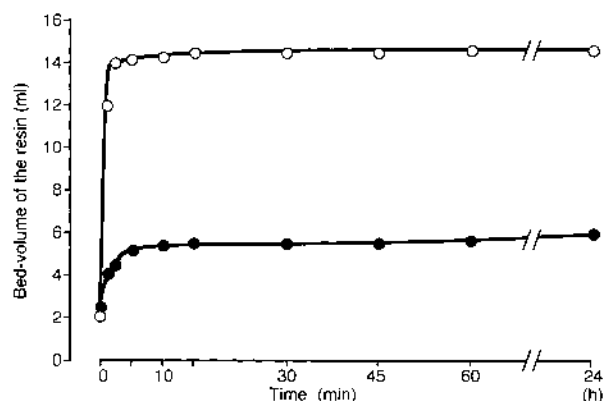


Fig. 5. Swelling Patterns of Colestimide and Cholestyramine in Water at 37°C

○, colestimide; ●, cholestyramine.

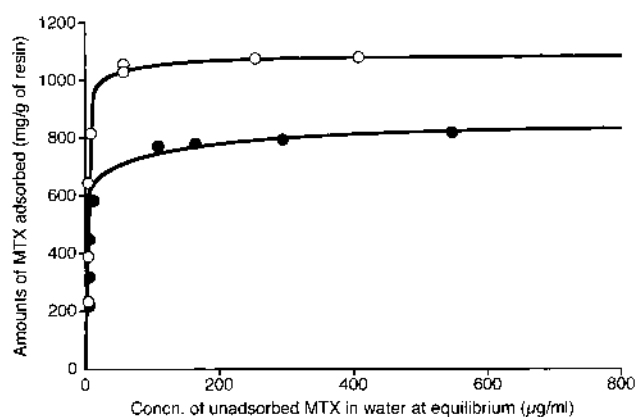


Fig. 6. Adsorption Isotherms for Binding of Methotrexate (MTX) to Colestimide and Cholestyramine in Water at 37°C

○, colestimide; ●, cholestyramine.

erty of the resin is one of the factors that influences accessibility to the binding sites.

Figure 5 presents the swelling patterns of both resins in water. Cholestyramine swelled to about 2.4 volumes within a few minutes, whereas colestimide showed much more swelling capacity, *i.e.*, the bed-volume increased about 6.8 fold. Consequently, the difference in the swelling property seems to indicate colestimide has easier accessibility to bile acids and thus more efficient adsorption of bile acids in the gut.

Adsorption of Methotrexate Because the renal clearance of MTX is markedly lower with an increase in MTX dose,¹³⁾ high-dose MTX therapy may be followed by extremely prolonged MTX elimination not only in patients with renal failure but also even in patients with normal renal function. Thus, if colestimide demonstrates a large adsorptive capacity for MTX and thereby can remove a large portion of MTX from the enterohepatic circulation, the total clearance of MTX would be greatly improved by an oral dose of colestimide.

Adsorption isotherms for the binding of MTX in water are presented for the two anion-exchange resins, colestimide and cholestyramine (Fig. 6). The curve with colestimide shows a tendency to reach a plateau at high values, *i.e.*, the amount of MTX adsorbed was over 1000 mg/g of resin. Adsorption data of the two resins fitted well to the Langmuir equation and it

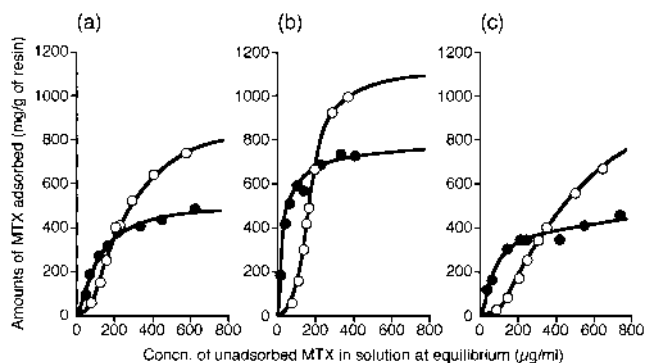


Fig. 7. Effects of Media on the Adsorption of Methotrexate (MTX) to Colestimide and Cholestyramine at 37 °C

(a), in 2nd fluid JPXIII; (b), in bicarbonate buffer; (c), in saline. ○, colestimide; ●, cholestyramine.

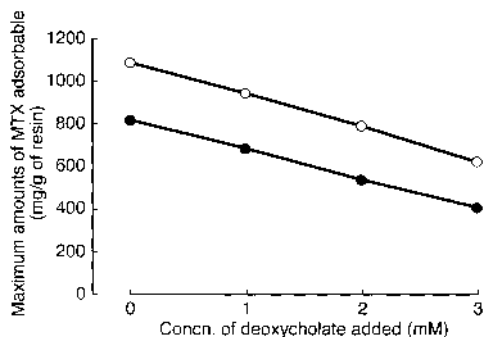


Fig. 8. Comparison of Methotrexate (MTX) Adsorptive Capacities of Colestimide and Cholestyramine in the Presence of Deoxycholate

○, colestimide; ●, cholestyramine.

was found that the maximum binding capacity of colestimide for MTX is 1087 mg/g, *i.e.*, 1.3 times as much as that of cholestyramine (820 mg/g). Not only in water but also in saline, 2nd fluid of JPXIII and the bicarbonate buffer, colestimide exhibited greater adsorptive powers as the concentration of MTX was increased. (Fig. 7). Furthermore, supposing that colestimide is used as an MTX-adsorbent in the gut, the adsorption behavior for MTX in the presence of deoxycholate is shown in Fig. 8. Although the adsorption of MTX by colestimide was smaller with increasing concentration of deoxycholate, probably through competitive binding, colestimide exhibited superior capacity compared to cholestyramine. Based on the results of these adsorption experiments

and the swelling test, it is indicated that colestimide is more suitable as an intestinal adsorbent for the early treatment of possible toxicity by MTX, and may be of clinical value in patients receiving high-dose MTX.

Conclusions

Colestimide, a new type of anion-exchange resin, had greater adsorptive capacities than cholestyramine for bile acids, by a factor of 1.4—2.0 fold in water. Although the adsorptive power of this resin for cholate, a trihydroxy bile salt anion was dramatically reduced by the addition of chloride or bicarbonate anions to the system, that for deoxycholate, a dihydroxy bile analog was almost retained. In addition, colestimide showed superior swelling properties in water, and this is expected to lead to efficient bile salt adsorption in the gut. On the other hand, since colestimide also had a higher binding capacity for MTX, a folic acid antagonist, the oral administration of colestimide may improve total body clearance of MTX by enhancing extrarenal excretion. The results obtained in this study suggest the potential of colestimide as a modulator of adverse reactions due to MTX through binding of the drug in the gut, in patients with delayed MTX elimination and/or evidence of acute renal injury. Further animal and clinical studies are indicated to assess the benefits of oral colestimide, particularly in addition to leucovorin rescue, in patients receiving high-dose MTX therapy.

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