EFFECTS OF COLESTIPOL HYDROCHLORIDE ON CHOLESTEROL AND BILE ACIDS ABSORPTION IN THE RAT INTESTINAL TRACT

TOSHIAKI FUJIIHASHI, KATSUNORI MUNEKIYO AND TERUHIKO MESHI

Pharmacology Function, Japan Upjohn Research Laboratories, 168 Ohyagi, Takasaki, Gunma 370, Japan

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It was clearly shown that colestipol hydrochloride inhibits lymphatic absorption of not only endogenous but also exogenous cholesterol and triglyceride in thoracic duct cannulated rats. Inhibition of cholesterol absorption by colestipol hydrochloride was effectively blocked by administration of cholic acid. It was also suggested that colestipol hydrochloride inactivates bile acids which are essential for cholesterol absorption in the intestinal tract. Administration of colestipol hydrochloride to fasted rats decreased bile flow and biliary secretion of cholesterol and bile acids and increased fecal excretion of bile acids bound to colestipol hydrochloride. These results show that colestipol hydrochloride binds bile acids in the intestinal tract and interferes with bile acids reabsorption.

Keywords—colestipol hydrochloride; cholesterol; bile acids; intestinal tract; absorption; thoracic duct cannulation; binding

INTRODUCTION

Colestipol hydrochloride (Cp) is an anion-exchange resin polymerized with diethylene-triamine and 1-chloro-2,3-epoxypropane. It is not absorbed from the gastrointestinal tract.¹⁾ Cp is known to effectively lower serum cholesterol in dogs, cockerels and humans.^{2–4)} No toxic effects have been detected in experimental and clinical usage.⁵⁾

One *in vitro* study indicated that Cp binds with cholic acid, a major bile acid in human bile.²⁾ It has been reported that fecal excretion of bile acids and sterols,^{3,6)} hepatic syntheses of cholesterol and bile acids (unpublished data), and the metabolic turnover rate of cholesterol⁷⁾ are increased after Cp administration for long periods. Parkinson *et al.*^{2,4)} concluded that increased fecal loss of bile acids due to Cp administration leads to increased conversion of cholesterol to bile acids and decreased serum cholesterol levels.

Whether Cp binds with cholic acid *in vivo* has not been clearly established. The mechanism of Cp action in the intestinal tract also remains unclear. Interference with dietary cholesterol

absorption may be a factor in the reduction of serum cholesterol levels; in fact, Cp is known to counteract increases in serum cholesterol due to cholesterol and cholic acid feeding.⁶⁾ In the present study, the effect of Cp on the absorption of cholesterol and the interaction of Cp with bile acids in the intestinal tract were investigated in rats.

MATERIALS AND METHODS

Male Sprague-Dawley rats (CD-CRJ) weighing approximately 300 g were used.

Effects of Cp on Cholesterol Absorption from the Intestinal Tract—In order to determine the direct effect of Cp on the intestinal absorption of cholesterol, the thoracic duct was cannulated and thoracic lymph was assayed for cholesterol. Cannulation was carried out by the modified method of Bollman *et al.*⁸⁾ Animals were subsequently restrained in Bollman cages and fasted until the end of the experiment with free access to 0.9% saline solution. About 20 h after the operation, lymph was collected from cannulated rats in which the lymph flow was consistently greater

than 1 ml/h.

Cp suspended in 5 ml of 0.25% methylcellulose was orally administered in doses of 150 and 300 mg/rat. Cholestyramine, a quaternary ammonium styrenedivinylbenzene copolymer,⁹⁾ was also administered in a dose of 150 mg/rat (340 mg as Questran[®], Mead Johnson Laboratories). The control group received 0.25% methylcellulose.

After administration of Cp, lymph was collected at intervals of 2 h for 10-h period and thereafter for 14 h. Collected lymph was frozen until cholesterol and triglyceride analyses. At the end of the experiment, rats were laparotomized for inspection of the intestinal tract and the cannulated area for abnormalities.

Effects of Cp on Cholesterol Absorption with Varying Amounts of Bile Acids — The effects of Cp on lymphatic absorption of cholesterol after test emulsion administration were determined at 4 stages where bile acids levels in the intestinal tract were artificially varied. No Bile Acid (Stage 1): the common bile duct was cannulated and all bile diverted; Normal Bile Acid (Stage 2): no treatment with intact bile circulation; Increasing Bile Acid (Stage 3): 80 mg cholic acid was orally given simultaneously with a test emulsion; and Excess Bile Acid (Stage 4): 240 mg cholic acid was orally given simultaneously with a test emulsion.

Effects of Cp on the Enterohepatic Circulation of Bile Acids — In order to study the interaction of CP with bile acids *in vivo*, Cp was orally administered in a dose of 1 g/kg/d for 2 days under fasting conditions or in powdered laboratory chow (Oriental Yeast Industrial Co.) containing 2.5% of Cp for 7 days.

Feces were collected for the last 2 days of the Cp treatment period and frozen until cholesterol and bile acids analyses. After Cp treatment, rats were anesthetized with urethane (1 g/kg *i.p.*) and cannulated in the common bile duct for the collection of bile for 2 h in an air-conditioned room (temperature: 27–29°C; relative humidity: 40–45%). At the end of the experiment, the blood and liver were removed for cholesterol and bile acids analyses.

Chemical Analyses — Total cholesterol was

extracted with *n*-hexane after alcoholic KOH hydrolysis of body fluid and measured with *o*-phthalaldehyde (Cholesterol B-Test Wako). Serum cholesterol was assayed enzymatically (Cholesterol C-Test Wako). Triglyceride in lymph was assayed using acetylacetone (Triglyceride B-Test). Total bile acids were determined enzymatically (Sterognost-3 α Daiichi); bile was assayed after appropriate dilution with pure water, and other tissues after extraction with boiling ethanol followed by washing with a mixture of ether and *n*-heptane (1:1). Feces and the liver were homogenized in 95% ethanol by using a Polytron homogenizer (Kinematica). A portion of the homogenate was assayed for cholesterol and bile acids. Fecal bile acids were extracted with boiling ethanol (free bile acids fraction) and later reextracted with 1 N HCl (bound bile acids fraction). The bound bile acids fraction is actually considered to be that bound to Cp, since cholic acid bound to Cp is easily released in 1 N HCl and not in boiling ethanol.

Student's *t*-test was used to establish significant differences in mean values between the control and treated groups.

RESULTS

Effects of Cp on Cholesterol Absorption from the Intestinal Tract

Total cholesterol and triglyceride contents in lymph collected from 18 to 44 h after cannulation were measured (Fig. 1, control group). These levels actually indicate the lymphatic reabsorption rate of endogenous cholesterol and triglyceride and maintenance of constant values throughout the experimental period of 24 h. Cp (150 mg/rat) significantly reduced the reabsorption of cholesterol from 2 to 10 h after administration, but this value returned to the control level within 24 h (Fig. 1). Cp had a similar effect on the reabsorption of endogenous triglyceride.

Rats were orally given 3 ml of a test emulsion containing 50 mg cholesterol, 300 mg triolein and 50 mg bovine serum albumin in 0.25% methylcellulose to determine the effect of Cp on exogenous cholesterol absorption. Absorption of both

cholesterol and triglyceride peaked 2 to 4 h after test emulsion administration and returned to normal values within 24 h (Fig. 2, control group). When administered simultaneously with the test emulsion, Cp (150 mg/rat) significantly inhibited the absorption of exogenous cholesterol. Cholestyramine (150 mg/rat) was somewhat more potent than an equal dose of Cp. At 300 mg/rat Cp, the inhibition was the greatest (Fig. 2).

Effects of Cp on Cholesterol Absorption with Varying Amounts of Bile Acids

This series of experiments was carried out to determine how Cp exerts inhibitory action on cholesterol absorption. Cholesterol absorption for 24 h after test emulsion administration increased as the bile acids level in the intestinal tract was artificially increased, as shown in Fig. 3. Cholesterol absorption was the highest at stages 3 and 4. Two doses of Cp inhibited cholesterol absorption at stages 2 and 3. The amounts of cholesterol absorption inhibited by Cp were the same although the amounts of cholesterol

FIG. 1. *Effects of Colestipol Hydrochloride on Lymphatic Reabsorption of Endogenous Cholesterol*

Rats were orally administered with Cp (150 mg/rat) about 20 h after thoracic duct cannulation (indicated by the arrow). Lymph was collected every 2 h for 10 h and thereafter for 14 h. Each point represents the mean $\pm$ S.E. of cholesterol in lymph collected for 2 h. ● — ●: control group ($n = 7$), ○ --- ○: colestipol treated group ($n = 4$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, significantly different from the control group.

absorbed varied significantly. Cp failed to inhibit cholesterol absorption at the excess bile acids level of stage 4.

Effects of Cp on the Enterohepatic Circulation of Bile Acids

Administration of Cp to fasted rats (1 g/kg, for 2 days) reduced bile flow. This was accompanied by a reduction in bile acids contents in the liver and bile (Table I). Cp also reduced biliary cholesterol secretion. In order to determine whether this reduction was attributable to the inhibition of cholesterol absorption, 10 rats were cannulated in the thoracic duct (Lymph fistula group), 6 rats in the common bile duct (Bile fistula group), and 6 rats received an abdominal wall incision with no cannulation (sham opera-

FIG. 2. *Effects of Colestipol Hydrochloride on Lymphatic Absorption of Cholesterol after Test Emulsion Administration*

Thoracic duct cannulated rats were orally administered with a test emulsion containing 50 mg cholesterol, 300 mg triolein and 50 mg serum albumin with Cp at the time indicated by the arrow. ● — ●: control group (the test emulsion alone, $n = 7$), ■ --- ■: colestipol treated group (150 mg/rat, $n = 6$), ▲ ▲: colestipol treated group (300 mg/rat, $n = 6$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, significantly different from the control group.

FIG. 3. Effects of Colestipol Hydrochloride on Lymphatic Absorption of Cholesterol with Varying Amounts of Bile Acids

Rats were administered a test emulsion with or without Cp 20 h after thoracic duct cannulation. Lymph was collected for the succeeding 24 h period. Bile acids contents in the intestinal tract were artificially manipulated as described in "Method." Each column represents the mean $\pm$ S.E. The values in parentheses are the number of rats used in each experiment. White column: control group; striped column: colestipol treated group (150 mg/rat); hatched column: colestipol treated group (300 mg/rat).

tion group). Treatment with lymph fistula produced no changes in bile flow and bile acids secretion while bile fistula produced a reduction in bile flow and bile acids secretion. Fecal excretion of bile acids, especially those of the bound bile acids fraction, was significantly increased by Cp treatment.

Table II shows that daily treatment of Cp for 7 days resulted in increased fecal bile acids and decreased biliary bile acids contents. Increased fecal cholesterol was also noted. Food consumption during the Cp treatment period did not change compared to the control group (the averages for 7 days, control group: 22 ± 1 g/d; Cp treated group: 23 ± 1 g/d), although the quantity of excreted feces in the Cp treated group increased significantly ($p < 0.01$) due to excreted Cp (control group: 4.9 ± 0.2 g/d; Cp treated group: 5.9 ± 0.2 g/d).

DUSCUSSION

Essentially all absorbed cholesterol is transferred to thoracic lymph,¹⁰ which can be collected at any time interval. Bile acids secretion, which is

TABALE I. Effects of Colestipol Hydrochloride on Bile Acids Secretion under Fasting Conditions

Treatment		Methylcellulose (control)	Colestipol	Sham operation ^{a)}	Lymph fistula ^{a)}	Bile fistula ^{a)}
No. of rats		<i>n</i> = 10	<i>n</i> = 10	<i>n</i> = 6	<i>n</i> = 10	<i>n</i> = 6
Serum	Cholesterol (mg/dl)	27.8 ± 2.6	30.1 ± 3.4	55.7 ± 0.6	68.4 ± 6.2	56.5 ± 3.5
	Bile acids (mg/dl)	3.1 ± 0.3	2.3 ± 0.4	2.7 ± 0.7	1.8 ± 0.3	ND
Liver	Cholesterol (mg/g)	5.94 ± 0.18	5.15 ± 0.35	4.51 ± 0.30	4.82 ± 0.22	3.71 ± 0.20
	Bile acids (mg/g)	0.40 ± 0.03	$0.32 \pm 0.02^*$	0.34 ± 0.03	0.35 ± 0.02	$0.25 \pm 0.03^{\#}$
Bile flow	(ml/h)	0.69 ± 0.05	$0.44 \pm 0.03^{***}$	0.69 ± 0.06	0.80 ± 0.05	$0.40 \pm 0.07^{\#}$
Bile	Cholesterol (μ g/h)	178 ± 17	$86 \pm 12^{**}$	169 ± 15	148 ± 12	$52 \pm 31^{##}$
	Bile acids (mg/h)	14.0 ± 1.5	$5.4 \pm 0.7^{***}$	12.2 ± 1.6	12.9 ± 1.3	$1.5 \pm 0.3^{##}$
Fecal ^{b)}	Cholesterol (mg/d)	1.36 ± 0.29	1.76 ± 0.36	0.96 ± 0.17	0.74 ± 0.11	ND
	Total bile acids (mg/d)	7.94 ± 1.82	$14.72 \pm 2.28^*$	7.00 ± 0.88	4.68 ± 0.94	ND
	Bound bile acids ^{c)} (mg/d)	0.81 ± 0.30	$4.57 \pm 0.78^{***}$	0.60 ± 0.22	0.20 ± 0.13	ND

Rats were administered Cp (1 g/kg p.o.) once a day for 2 days, and allowed only tap water. These values represent the mean $\pm$ S.E. ND: not determined.

a) The surgical operation was carried out 2 days before the experiment; rats were allowed only tap water.

b) Average 2-day value.

c) These values are for bile acids bound in residue after boiling ethanol extraction.

Significantly different from the control group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Significantly different from the sham operation group, # $p < 0.05$, ## $p < 0.01$.

TABLE II. *Effect of Colestipol Hydrochloride on Bile Acids Secretion*

Treatment			Control		Colestipol	
No. of rats			<i>n</i> = 6		<i>n</i> = 6	
Serum	{	Cholesterol (mg/dl)	60.5 ± 3.0		56.3 ± 2.4	
	{	Bile acids (mg/dl)	2.9 ± 0.2		2.4 ± 0.4	
Liver	{	Cholesterol (mg/g)	3.58 ± 0.12		2.99 ± 0.07***	
	{	Bile acids (mg/g)	0.37 ± 0.04		0.29 ± 0.02	
Bile flow		(ml/h)	0.69 ± 0.07	0.61 ± 0.03 ^{c)}	0.73 ± 0.04	0.62 ± 0.06 ^{c)}
Bile	{	Cholesterol (μg/h)	157 ± 20	140 ± 10 ^{c)}	209 ± 15	142 ± 34 ^{c)}
	{	Bile acids (mg/h)	13.9 ± 0.7	9.3 ± 0.7 ^{c)}	11.4 ± 0.7*	4.4 ± 0.6 ^{c)} **
Fecal ^{a)}	{	Cholesterol (mg/d)	9.43 ± 0.60		11.23 ± 0.66*	
	{	Total bile acids (mg/d)	19.13 ± 0.60		31.23 ± 1.55***	
	{	Bound bile acids ^{b)} (mg/d)	2.58 ± 0.14 ^{b)}		9.11 ± 0.61***	

Rats were maintained on powdered laboratory chow containing 2.5% Cp for 7 days; bile was collected for 2 h on day 8. These values represent the mean ± S.E.

a) Feces were collected on days 6 and 7 of the treatment period; the value shown is the 2-day average.

b) These values are for bile acids bound in residue after boiling ethanol extraction.

c) These values are for bile collected in the later period (from 6 to 8 h after cannulation).

Significantly different from the control group; **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

believed to be essential for cholesterol absorption,¹¹⁾ was not changed as a result of thoracic duct cannulation (see Table I). Thoracic lymph contains hepatic lymph and cholesterol synthesized in the intestinal walls.¹²⁾ However, in bile diverted-thoracic duct cannulated rats, lymphatic cholesterol levels were consistently low, indicating that they contribute very little to total cholesterol in thoracic lymph. For this reason, it is possible to determine the direct effects of Cp on cholesterol absorption in the intestinal tract by determining cholesterol levels in thoracic lymph. Cp inhibited the absorption of exogenous cholesterol at two dose levels. Inhibition was greater at the higher dose. The same results were obtained when cholesterol absorption was promoted by the addition of cholic acid. Also, the increased levels of fecal cholesterol suggested that administration of chronic doses of Cp inhibited cholesterol absorption in rats. Cp administration in a dose of 150 mg/rat also reduced the reabsorption of cholesterol beginning 2 h after administration. This inhibitory action of Cp on cholesterol absorption was consistent for more than 10 h but had completely disappeared within 24 h. Since a

preliminary experiment showed that most Cp had passed through the small intestinal tract about 10 h after oral administration (unpublished observation), Cp may exert inhibitory action on cholesterol absorption only when it exists in the intestinal tract and its action is not the result of an irreversible dysfunction of the intestine.⁵⁾

Bile acids are essential for cholesterol absorption¹¹⁾ and Cp binds with cholic acid *in vitro* (1.1 meq cholic acid/g Cp).²⁾ The hypocholesterolemic effects of cholestyramine⁹⁾ and neomycin^{13,14)} are thought to be the result of binding with bile acids and fatty acids in the intestinal tract, respectively. The binding of Cp with cholic acid in the intestinal tract would be a cause of the inhibition of cholesterol absorption by Cp. Cp reduced bile flow and biliary secretion of bile acids and cholesterol in fasted rats. Although lymph fistula did not affect bile secretion, bile fistula greatly reduced bile secretion in this study. Interruption of bile acids circulation caused a decrease in total bile acids pool under fasting conditions. The decrease in bile secretion due to Cp may be attributable to the interruption of bile acids circulation, and not to the inhibition of cholesterol

absorption. Fecal excretion of bile acids in the Cp treated group significantly increased; especially the bound bile acids fraction, most of which is thought to be bound to Cp. These findings suggest that bile acids bind to Cp *in vivo* so firmly that they are excreted in the form of a bile acids-Cp complex which has already been demonstrated *in vitro*.⁶⁾

After repeated administration of Cp to normally feeding rats, bile acids secretion again decreased and fecal excretion of cholesterol and bile acids increased. The reduction in bile acids secretion after repeated administration of Cp was less marked. However, bile acids content was dramatically reduced when bile was collected at later periods (Table II). Bile acids secretion following Cp treatment was thought to be different from that of the control. The serum cholesterol level was not altered by Cp treatment because the rate of hepatic cholesterol synthesis is rather high in rats¹⁵⁾ and the turnover rate for cholesterol increases during Cp treatment.⁷⁾ These observations indicate that compensatory activity is initiated as a result of low cholesterol and bile acids levels after Cp treatment.

Cholesterol absorption was promoted by the addition of cholic acid in a previous study.¹⁶⁾ Cholesterol absorption was also dependent on the bile acids level in the intestinal tract under the present experimental conditions (Fig. 3); *i.e.*, bile acids in the intestinal tract primarily promoted cholesterol absorption. It was also shown that Cp suppressed this promotive action of cholesterol absorption by bile acids. It seems reasonable to conclude from the data presented above that Cp in the intestinal tract inhibits cholesterol absorption by binding with bile acids. The inhibition of cholesterol absorption by cholestyramine was greater than that by Cp. This may be attributable to the fact that cholestyramine binds more bile acids than Cp *in vitro*.²⁾

Cp inhibited triglyceride absorption to the same extent as cholesterol (data not shown). It has been reported that triglyceride and fatty acids promote cholesterol absorption.¹⁷⁾ However, cholesterol absorption for 24 h did not decrease

when triolein was omitted from the test emulsion in the present study and the cholesterol absorption rates at certain times did not closely reflect the rate of triglyceride absorption. It cannot be proposed that Cp reduces cholesterol absorption due to the inhibition of triglyceride absorption by another mechanism; *e.g.*, inhibition of esterase. The inhibitory action of Cp was completely blocked by addition of cholic acid. Cp probably inhibits triglyceride absorption which is also partially mediated by bile acids^{11,17)} by the same mechanism; *i.e.*, binding with bile acids.

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