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EFFECTS OF NOVEL BILE SALTS ON CHOLESTEROL METABOLISM IN RATS AND GUINEA-PIGS

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Abstract—Novel bile salts (quaternary ammonium conjugates) inhibited cholic acid binding and transport in everted ileal sacs *in vitro*. The cationic piperazine conjugate of lithocholic acid (di-iodide salt, compound 8, BRL 39924A) appeared most active, inhibiting binding by 29% and transport by 59% in guinea-pig ileum (200 μM). BRL 39924A also inhibited taurocholate uptake into guinea-pig ileal sacs and cholate uptake into rat ileal sacs and was selected for further study *in vivo*. In hyperlipidaemic rats, BRL 39924A significantly raised cholesterol 7α-hydroxylase activity and decreased hepatic accumulation of exogenous cholic acid. HDL cholesterol concentration in the serum increased and the level of VLDL plus LDL cholesterol decreased. In hyperlipidaemic guinea-pigs, BRL 39924A lowered serum total cholesterol and triglyceride levels. Although metabolic changes were less than those achieved with the bile acid sequestrant, cholestyramine, the doses of BRL 39924A used were much lower (100–500 mg/kg body wt). Selective inhibition of receptor mediated bile acid uptake may be associated with local side-effects but these novel bile salts are useful pharmacological tools to examine the effects of receptor blockade on lipoprotein metabolism.

Pharmacological interventions to decrease blood levels of cholesterol and triglycerides have had a long history that, until recently, was disappointing because of the lack of unequivocal evidence to demonstrate clinical benefit. It has now been shown, however, that in high-risk patients the bile acid sequestrant, cholestyramine, decreases coronary mortality and morbidity [1] and the progression of atherosclerosis [2] in association with a decrease in low-density lipoprotein (LDL) and, possibly, a small rise in high-density lipoprotein (HDL).

By binding bile acids, an agent such as cholestyramine reduces bile acid absorption with a concomitant derepression of bile acid biosynthesis, cholesterol depletion in the liver, induction of hepatic LDL receptor synthesis and, hence, lowering of the circulating concentration of LDL [3]. However, the insoluble, polymeric, resins are administered in large doses, they are unpalatable and patient compliance may be poor [4].

While drugs with alternative modes of action, such as inhibition of cholesterol synthesis, may lead to greater clinical efficiency [3], other approaches for disrupting the enterohepatic circulation of bile salts may also be desirable [5]. One method would be to identify a small-molecular weight substance that could inhibit the receptor-mediated active transport system in the ileum, the major route by which bile salts are (re-)absorbed [6]. In order to obtain receptor specificity for such an agent, we considered it worthwhile to study synthetic bile acid conjugates as potential structural leads in the development of competitive inhibitors. Although this pharmacological approach to LDL-lowering appears to be novel, some possibilities for inhibitory structural types were suggested by the work of Lack and colleagues [7–10] who characterized the properties of the ileal bile salt transport system. In these studies it was observed that quaternary ammonium conjugates of bile salts were not actively absorbed but were capable of reversibly inhibiting the active uptake of native bile salts.

It was the purpose of the present work to investigate in further detail the structure–activity relationships for inhibition of cholate uptake by the ileum *in vitro* and to determine if this mechanism for interference with bile salt transport was associated with effects on systemic lipid levels in hyperlipidaemic animals.

MATERIALS AND METHODS

Incubation of everted ileal sacs in vitro. The experimental procedure was adapted from that described by Lack and co-workers [7–10]. Ileum was obtained from guinea-pigs fasted overnight and tissue was washed in Krebs–Ringer bicarbonate buffer, pH 7.4 containing 2 mg/mL (w/v) glucose. Everted sacs approximately 6–8 cm long were prepared, containing 3 mL of glucose-enriched buffer in the serosal compartment. Because of the proximal-distal gradient in receptor-mediated bile salt uptake along the length of the ileum, sacs from several guinea-pigs were randomized into experimental groups (4 guinea-pigs providing sufficient tissue for 2 groups of 5 replicates). Everted sacs were incubated at 37° in 15 mL of glucose-enriched buffer containing ^{14}C-radiolabelled bile acid substrate (11.1 kBq, 5.6 μmol) in an atmosphere of O_2 (95%) : CO_2 (5%). Compounds were added in DMSO (1% final concentration); it was found in preliminary studies that this concentration of vehicle did not affect bile acid binding, an observation in agreement with previous results [8, 10].

At the end of the incubation period, sacs were washed with buffer, serosal fluid removed, sacs blotted dry and weighed. The tissue from each sac was homogenized in 10 mL buffer using a Polytron homogenizer (Lucerne, Switzerland). Duplicate samples

of homogenate (500 μL) and serosal fluid (100 μL) were incubated with Protosol at 37° and radioactivity was measured by counting in a scintillant containing ethoxyethanol.

Alkaline phosphatase (EC 3.1.3.1) activity in an aliquot (1 mL in 0.9% NaCl, w/v) of a diluted (10^3-fold) homogenate of ileum was determined by measuring the release of *p*-nitrophenol from *p*-nitrophenylphosphate using diagnostic reagents obtained from BCL (Lewes, U.K.).

Animals, diets and treatment. Male Sprague–Dawley rats (140–160 g) were obtained and maintained on a cholesterol-supplemental sucrose-based diet as described previously [11]. Rats were allocated to experimental groups (8 rats/group, 4 rats/cage) so that the mean body weight of each group was similar. On the third day after the start of compound administration, each rat received an oral dose of [^{14}C]cholic acid (37 KBq, 2.5 μmol) in 0.1 mL of 1% methyl cellulose.

Guinea-pigs (250–340 g) were maintained on a stock diet supplemented with cholesterol (1.6%) and corn oil (15%), a dietary regimen known to induce high serum levels of lipoproteins of $d < 1.03$ [12]. Guinea-pigs were allocated to experimental groups (6 per group) so that the mean body weight of each group was similar.

All animals were killed 7 days after the start of compound administration, between 10:00 and 12:00 a.m. (daylight-dependent light cycle).

Biochemical analyses. Serum total cholesterol and triglycerides were measured by automated enzymatic methods [13]. High-density lipoproteins (HDL) were separated from low-density and very-low-density lipoproteins (LDL, VLDL) by chemical precipitation methods [13] and lipoprotein cholesterol was measured by the enzymatic procedure.

Residual radioactivity present in the liver of rats, 4 days after an oral dose of [^{14}C]cholic acid, was measured by total combustion (approximately 0.5 g samples in duplicate) using an Intertechnique 'Oxymat' tissue oxidizer and scintillation counting. Rat liver cholesterol 7α-hydroxylase (EC 1.14.13.17) activity in microsomes was measured as described elsewhere [11] using radiolabelled cholesterol substrate solubilized in Tween 80 and isolating the product by TLC.

Materials. [4-^{14}C]Cholesterol, [carboxyl-^{14}C] cholic acid, sodium salt, tauro[carbonyl-^{14}C]cholic acid, sodium salt and [^{14}C]polyethylene glycol 4000 were obtained from Amersham International plc (Little Chalfont, U.K.). 3-[(3-Cholamidiopropyl) dimethylammonia]-1-propanesulphonate (CHAPS) and other chemicals and biochemicals were obtained from Aldrich Chemical Co. (Gillingham, U.K.) or from Sigma Chemical Co. (Poole, U.K.) unless otherwise specified.

Synthesis of bile salt conjugates

Compounds **1–10** in Table 2 were synthesized in two or three steps starting from the appropriate bile acid, i.e. lithocholic acid (compounds **1–8**), deoxycholic acid (compound **9**) or cholic acid (compound **10**).

The amide side-chain was introduced (step A) by causing the appropriate bile acid to condense with

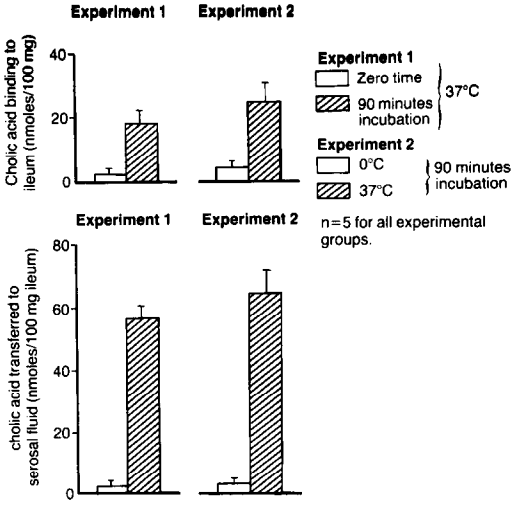


Fig. 1. Cholate binding to ileal sacs (guinea-pigs) and transfer to serosal fluid: effect of incubation time and temperature.

an aminoalkylamine in the presence of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). Commercially unavailable aminoalkylamines, required for the synthesis of extended chain derivatives (N = 3 in Table 2), were prepared by lithium aluminium hydride reduction of the corresponding nitriles [14] which were in turn prepared by reaction of the appropriate cyclic amines (piperidine, *N*-methylpiperazine) with acrylonitrile [15]. The purified free base from Step A was subsequently quaternized (step B) by reaction with iodomethane. To prepare compounds **1, 3, 5, 6, 9** and **10** the quaternary iodide was converted into the corresponding chloride by ion-exchange on Amberlite IRA-401 (Cl) resin (step C). The following general procedure was used.

Step A. The starting acid (1 mol) was suspended in ethyl acetate and the appropriate amine (1.2 mol) in ethyl acetate was added with stirring at room temperature. EEDQ (1.1 mol) dissolved in ethyl acetate was added and the mixture was stirred under reflux overnight. Cooling gave the crude amide (free base) which was recrystallized from ethanol (yield: 25–35%).

Step B. The product (1 mol) from Step A was dissolved in dichloromethane and excess of iodomethane (for example 1.5 mol) in dichloromethane was added. The mixture was stirred under reflux for several hours, cooled, and the product was collected. Recrystallization from ethanol gave the quaternary iodide in high yield. To prepare the di-iodide salt **6** this step was carried out with a large excess (10 mol) of iodomethane in refluxing methanol.

Step C. Amberlite IRA-401 (Cl) resin (ex BDH) was regenerated by treating it with 4% (w/v) sodium hydroxide solution, washing well with deionized water, and then immersing it in 4% (w/v) sodium chloride solution. The resin was finally washed well with deionized water before use.

The iodide salt from step B, dissolved in aqueous ethanol, was stirred for several hours in the presence of the regenerated Amberlite resin and the resin was

Table 1. Effect of standard agents on cholic acid uptake by guinea-pig ileal sacs

Agent	Concentration (μM)	% Change from control value: Binding to tissue	% Change from control value: Accumulation in serosal fluid
Antimycin A	100	−15	−41*
Lithocholic acid	200	−32*	−35*
Sulphobromophthalein	400	+2	−3
Ouabain	100	−47*	−89*
Dinitrophenol	100	−28*	−78*
NaN_3	3000	−1	−41*
	7000	−19	−43*
	13,000	−28*	−46*

Cholic acid (370 μM) plus agent were incubated with everted sacs, for 90 min at 37°. Five sacs were included per experimental group, average binding to tissue in control sacs (mean ± SE) = 35 ± 2.5 nmol/100 mg, transfer to serosal fluid in control sacs = 64 ± 3.0 nmol/100 mg.

* $P < 0.05$.

% Change was calculated as the difference between mean value (N = 5) in the presence of agent and in the absence.

then filtered off and washed with aqueous ethanol. Evaporation of the filtrate gave the required quaternary chloride in good yield.

The structures of compounds **1–10** were confirmed by i.r., NMR and elemental analysis.

RESULTS

Characterization of everted gut sac preparation

The procedure, using ileum from guinea-pigs, was adapted from that described by Lack and his colleagues [7–10] with the modification that cholic acid was not present in the serosal compartment at the start of the incubation. In the present experimental conditions, there was reproducible tissue binding and transfer to the serosal fluid of cholic acid (sodium salt) after 90 min incubation at 37° and little binding at zero time or on incubation in ice (Fig. 1). In preliminary validation studies, polyethylene glycol, which is not subject to absorption [16, 17], was used as a marker for the extent of diffusion into the extracellular space plus extent of tissue damage and contamination of samples with solution from the mucosal compartment. Apparent binding of polyethylene glycol to ileum (90 min incubation at 37°) was approx. 1 nmol/100 mg tissue (<5% cholate binding) and the apparent concentration of polyethylene glycol in serosal fluid was approx. 0.6 nmol/100 mg tissue (1% of cholate uptake).

The effect of standard agents (Table 1) on cholate uptake was examined in order to evaluate whether an inhibition of active transport would be detected in the measurements of total uptake into tissue and fluid. Significant reductions in the accumulation of cholate in the serosal compartment were obtained for antimycin A (depleting ATP stores), lithocholic acid (competitive inhibitor of binding), ouabain (disrupting active transport), dinitrophenol and sodium azide (disrupting oxidative phosphorylation). The effect of these standard inhibitors on cholate binding to ileal tissue was generally less than the effect on the transfer of bile acid to the serosal fluid. Sulphobromophthalein was without apparent effect. In corresponding studies using everted ileal sacs (2 cm long) from rats, neither dinitrophenol (200 μM) nor ouabain (100 μM) exerted a significant inhibitory effect. This lack of action of ouabain is in agreement with previous results [18], reflecting the resistance of rats to cardiac glycosides.

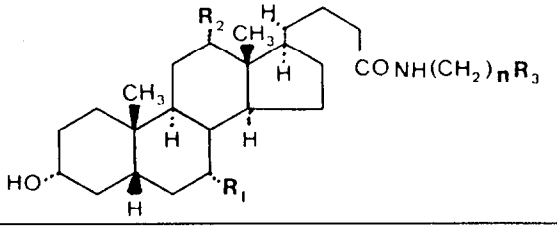
Primary evaluation of synthetic bile acid conjugates

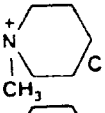
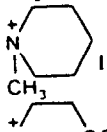
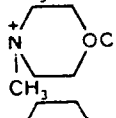
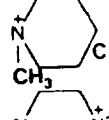
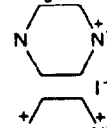
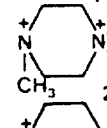
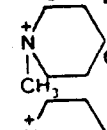
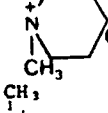
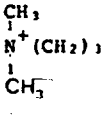
The synthetic bile acid conjugates were evaluated in the same experimental conditions as the standard inhibitors: 370 μM cholate was incubated at 37° for 90 min. Compounds were added in DMSO to give a concentration of 200 μM (Table 2).

The lithocholic acid analogue, lithocholamine, described by Lack and his colleagues [10] (compound **1**, Table 2) significantly inhibited the uptake of cholate into serosal fluid although the inhibition of binding to the tissue did not achieve statistical significance. A series of novel cationic bile acid conjugates was then synthesized in order to examine structure–activity relationships with particular regard to the nature of the anion, the number of hydroxyl substituents on the steroid nucleus, the length of the side-chain and the nature of the end group (Table 2).

When comparing pairs of compounds, iodides appeared less active than the corresponding chlorides: compound **2** versus **1** and compound **4** versus **3** (piperidines). The lithocholic acid analogue, compound **3**, was more active than the corresponding deoxycholic acid analogue, compound **9** and cholic acid analogue, compound **10**. Activity was not confined to compounds where $n = 2$ in $—(CH_2)_n—$ for example, compound **6** versus **3**. With regard to the nature of the end group, all of the heterocycles (piperidine, morpholine in compound **5**, piperazine in compound **8**) were active as lithocholic acid conjugates (chloride) but compound **8**, bearing a double charge, appeared the most active. Compound **8** (BRL 39924A) was selected for further evaluation.

Table 2. Effect of synthetic bile acid conjugates on cholic acid uptake by guinea-pig ileal sacs



Compound	R_1	R_2	n	R_3	Molecular formula	Melting point (°C)	% Change from control value: Binding to tissue	% Change from control value: Accumulation in serosal fluid
1	H	H	2	$N^+(CH_3)_3Cl^-$	$C_{29}H_{53}ClN_2O_2$	226–228	−18	−30*
2	H	H	2	$N^+(CH_3)_3I^-.H_2O$	$C_{29}H_{55}IN_2O_3$	222	−15	+6
3	H	H	2		$C_{32}H_{57}ClN_2O_2$	255–257	−37*	−32*
4	H	H	2		$C_{33}H_{57}IN_2O_2$	230–235	−14	−26
5	H	H	2		$C_{31}H_{55}ClN_2O_3$	232–233	−19*	−36*
6	H	H	3		$C_{33}H_{59}ClN_2O_2$	240–242	−26*	−29*
7	H	H	3		$C_{33}H_{60}IN_3O_2$	238–243†	−27*	−45*
8	H	H	3		$C_{34}H_{63}I_2N_3O_2$	200–203	−29*	−59*
9	H	OH	2		$C_{32}H_{57}ClN_2O_3$	174–180	−6	−1
10	OH	OH	2		$C_{32}H_{57}ClN_2O_4$	169–175	−16	−23
11	OH	OH	3		$C_{32}H_{58}N_2O_7S$	157†	+15	+8

Cholic acid (370 μM) plus synthetic bile acid (200 μM) were incubated with everted sacs for 90 min at 37°. At least 5 sacs were included per experimental group, average binding to tissue in control sacs = 39 ± 2.9 nmol/100 mg, transfer to serosal fluid in control sacs = 64 ± 3.0 nmol/100 mg.

* $P < 0.05$.

† Melts with decomposition.

Compound **1** = lithocholamine, compound **8** = BRL 39924A.

The commercially-available synthetic bile acid and zwitterionic detergent CHAPS (compound **11**) was inactive. This finding suggests that the activity of the cyclized analogues cannot be attributed merely to non-specific membrane effects and cytotoxicity.

Further evaluation of BRL 39924A in vitro

Lower concentrations of BRL 39924A (for example, 50 μM) were not consistently inhibitory (result not shown) so 200 μM was chosen as the standard concentration for further work *in vitro*.

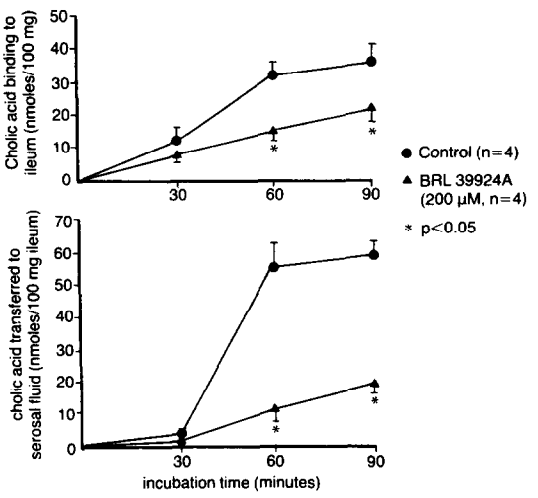


Fig. 2. Effect of time on inhibition of cholate uptake into ileal sacs (guinea-pigs) by BRL 39924A.

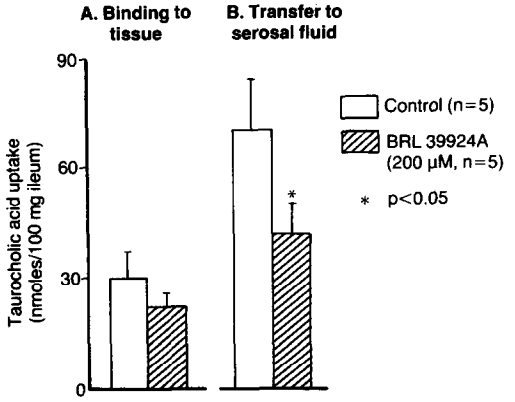


Fig. 3. Effect of BRL 39924A on taurocholate uptake into ileal sacs (guinea-pigs).

When the time course for inhibition was examined, there was little effect after 30 min incubation but a marked decrease in both binding and transfer of cholate was observed after 60 min (Fig. 2). In control sacs there was relatively little further uptake of bile acid between 60 and 90 min but the effect of BRL 39924A at 90 min was still considerable. Alkaline phosphatase was measured as a marker enzyme for non-specific cell damage. There was no change in the ileal concentration of alkaline phosphatase activity after incubation with 200 μM BRL 39924A (2580 ± 210 units/g wet wt/min vs control value of 2600 ± 180 units/g wet wt/min, N = 5 in both groups).

BRL 39924A also reduced the uptake of taurocholate into everted ileal sacs from guinea-pigs although only the inhibition of transfer to the serosal fluid achieved statistical significance (Fig. 3).

The uptake of cholate by everted ileal sacs from rats was also inhibited by BRL 39924A (Fig. 4) although, again, only the reduction in transfer to the serosal compartment achieved statistical significance.

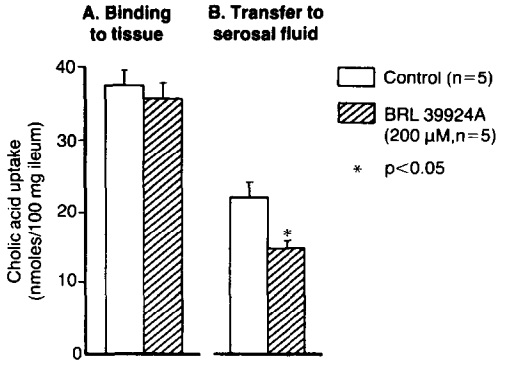


Fig. 4. Effect of BRL 39924A on cholate uptake into ileal sacs (rats).

Evaluation in hyperlipidaemic rats

Results *in vitro* had established that BRL 39924A consistently inhibited bile acid uptake. In order to determine whether such activity will influence systemic lipoprotein levels it was necessary to evaluate BRL 39924A *in vivo*. The effect of BRL 39924A and other inhibitors on bile acid uptake by ileal sacs might be less for rats than guinea-pigs but because lipid metabolism is better characterized in rats, it was decided to conduct the initial study *in vivo* using a rat model previously established in this laboratory [11].

BRL 39924A at two dietary levels was compared with lithocholamine (compound **1**) and cholestyramine at a high level. At this dose, cholestyramine did not affect serum total cholesterol or triglycerides (Table 3) but there was a significant increase in HDL and a significant decrease in VLDL plus LDL. Cholestyramine significantly increased the hepatic activity of cholesterol 7α-hydroxylase, the rate-limiting enzyme in bile acid synthesis, secondary to a decreased absorption of bile acid measured as the hepatic accumulation of exogenous cholic acid.

Lithocholamine was apparently without effect but the high dose of BRL 39924A significantly raised cholesterol 7α-hydroxylase activity and decreased hepatic accumulation of exogenous cholic acid. Although these effects were less than achieved by cholestyramine, the dose of BRL 39924A was much lower in terms of unit dose per body weight (g/kg). Both levels of BRL 39924A raised serum HDL concentration and the higher dose of BRL 39924A also lowered VLDL + LDL concentration. However, BRL 39924A also decreased serum total triglyceride and decreased the gain in body weight in a dose-dependent manner and some diarrhoea was noted at the high dose. It is possible, therefore, that BRL 39924A may be inducing other effects, for example on triglyceride absorption.

Evaluation in hyperlipidaemic guinea-pigs

Guinea-pigs are usually considered to be a poor

Table 3. Comparison of effects of cholestyramine, compound 1 and BRL 39924A in hyperlipidaemic rats

	Control	Cholestyramine (2000 mg/kg body wt)	Compound 1 (250 mg/kg body wt)	BRL 39924A (250 mg/kg body wt)	BRL 39924A (500 mg/kg body wt)
Food intake (g/day)	16.2	15.4	15.9	15.4	13.2
Gain in body weight (g)	39.6 ± 2.1	38.3 ± 2.7	39.9 ± 4.3	26.3 ± 3.4†	7.5 ± 3.6†
Serum lipid (mg/100 mL)					
Cholesterol	123 ± 8.0	106 ± 5.1	120 ± 4.9	148 ± 11	126 ± 7.3
Triglyceride	155 ± 20	142 ± 23	131 ± 14	82 ± 7.3†	53 ± 2.6†
HDL cholesterol	48 ± 2.0	76 ± 4.7†	44 ± 2.0	62 ± 3.5†	69 ± 4.6†
VLDL + LDL cholesterol	76 ± 4.9	31 ± 2.0†	76 ± 3.0	86 ± 4.5	57 ± 2.7*
Hepatic cholesterol 7α-hydroxylase (pmol/min/mg protein)	5.4 ± 0.9	30.6 ± 1.5†	4.1 ± 0.8	5.4 ± 1.2	14.0 ± 1.5*
Hepatic cholic acid uptake (dpm/liver)	9890 ± 1280	900 ± 260†	9200 ± 1160	12,270 ± 1440	4840 ± 660*

Results are the means ± SE for eight analyses; each analysis being of tissue from a single rat. Compounds were administered as a supplement to the diet for 7 days. Individual food intakes per day were calculated as the average from each cage of 4 rats.

Compound 1 = lithocholamine.

* Indicates a significant difference from the control group ($P < 0.02$).

† Indicates a significant difference from the control group ($P < 0.001$).

species for the study of drugs affecting lipoprotein metabolism because dietary cholesterol can induce haemolytic anaemia. However, because of the activity observed for BRL 39924A *in vitro* it was considered worthwhile to attempt a further comparison with lithocholamine and cholestyramine *in vivo*.

At the end of the study, the gross appearances of ileum and liver were similar in all groups. Cholestyramine markedly decreased serum cholesterol and triglyceride concentrations and promoted food intake and weight gain (Table 4). This improvement in physiological status may arise as a consequence of the avoidance of hypercholesterolaemia-induced haemolysis. Lithocholamine at 250 mg/kg body wt and BRL 39924A at 100 and 250 mg/kg both significantly decreased serum cholesterol and triglyceride levels. These effects were less than the response to cholestyramine but were achieved at substantially smaller doses.

DISCUSSION

The observations of Lack and co-workers [8, 10] were used as the starting point in our design of novel, synthetic bile acid conjugates as inhibitors of bile salt absorption but our choice of assay system, *in vitro*, differed in one important respect. Our omission of bile acid substrate from the serosal compartment at the start of the incubation period ensured an initial chemical gradient so that the measurement of binding and transport from mucosal to serosal compartments will reflect passive as well as active transport. These conditions were chosen in order to simulate, as closely as possible, conditions *in vivo* and the determination of transport along a chemical gradient is a method adopted by other workers [17, 19–21].

Cholate was chosen as the principal substrate as it is known to be the predominant bile acid in adult guinea-pigs. Validation studies using standard inhibitors of active transport [6, 9, 18] together with the use of polyethylene glycol as a non-absorbable marker indicated that an inhibition of active transport could be monitored primarily as a reduction in transfer of substrate into the serosal compartment. Using the standardized system, the principal inhibitor described by Lack (lithocholamine, compound **1** in Table 2) was active but some of the novel, cyclized analogues were probably more inhibitory. Although only a limited number of structural types was synthesized it was possible to make certain conclusions regarding structure–activity relationships and the activities were not explained by general cytotoxicity—there was no effect on alkaline phosphate activity and CHAPS was not inhibitory. It is likely that the degree of inhibition of cholate uptake is directly related to the affinity of the inhibitor for the receptor. The inhibition is probably competitive but the inhibitors are not transported [8, 10].

Our identification of novel, synthetic inhibitors of bile salt transport is of interest, but it was of even greater interest to determine if such inhibition could be translated into effects on cholesterol metabolism *in vivo*. We did not directly study ileal bile acid binding *in vivo* and this would be a difficult task if

Table 4. Comparison of effects of cholestyramine, compound **1** and BRL 39924A in hyperlipidaemic guinea-pigs

	Control	Cholestyramine (2000 mg/kg body wt)	Compound **1** (250 mg/kg body wt)	BRL 39924A (100 mg/kg body wt)	BRL 39924A (250 mg/kg body wt)
Food intake (g/day)	15.4 ± 1.9	19.9 ± 1.0†	14.8 ± 1.3	15.3 ± 1.1	14.5 ± 1.7
Gain in body weight (g)	16.7 ± 6.8	39.0 ± 3.4†	15.2 ± 11.3	22.8 ± 4.7	18.2 ± 8.1
Serum lipid (mg/100 mL)					
Cholesterol	1037 ± 139	42 ± 7‡	584 ± 85†	479 ± 90†	650 ± 83*
Triglyceride	1008 ± 251	38 ± 6‡	401 ± 131*	366 ± 139*	350 ± 58*

Results are the means ± SE for six analyses; each analysis being from a single guinea-pig. Compounds were administered as a supplement to the diet for 7 days.

* Indicates a significant difference from the control group ($P < 0.05$).

† Indicates a significant difference from the control group ($P < 0.02$).

‡ Indicates a significant difference from the control group ($P < 0.001$).

the inhibition is reversible. However, we infer an effect on bile acid absorption from the measurements of bile acid biosynthesis (cholesterol 7α-hydroxylase) and hepatic bile acid content. Thus, in rats, both cholestyramine and BRL 39924A interfered with the enterohepatic circulation of bile acids and, in consequence, lowered LDL plus VLDL, raising HDL. Our observation that inhibition of ileal active transport leads to changes in serum lipoproteins contradicts the suggestion [17] that passive transport in the jejunem is the major determinant of bile acid absorption in rats. It was not possible to separate lipoprotein fractions in guinea-pigs but the qualitative effects of BRL 39924A (and lithocholamine) on serum lipids (probably mainly representing lower-density lipoproteins) resembled the effects of cholestyramine.

When comparing the responses, *in vivo*, to BRL 39924A and cholestyramine, two points are noteworthy. First, the effect of cholestyramine was greater than the effect of BRL 39924A. Although this difference may partly reflect the great discrepancy in doses there was some evidence that the maximum effect of BRL 39924A in guinea-pigs had been achieved (equi-active at 100 and 250 mg/kg body wt). BRL 39924A may exert less net effect because it is specific, that is it interferes only with the receptor-mediated uptake of cholic acid, whereas cholestyramine will impair both passive and active transport of all bile acids. The second point is that BRL 39924A may have other actions in the gut—expressed as changes in fat absorption and growth—possibly secondary to the high levels of free bile acid that will be excreted. Idiopathic bile acid catharsis has been observed in man [22] and high concentrations of bile acid can damage the wall of the large intestine. When considering the potential therapeutic merits of the present novel approach, the potential for other deleterious consequences of high fluxes of bile acid, for example colorectal cancer [23], must also be appreciated. It is conceivable, however, that a specific inhibitor of ileal bile acid transport might be used in conjunction with low-dose enterocoated cholestyramine to sequester bile acid subsequently in the colon [24].

In conclusion, therefore, although we have demonstrated that novel, non-absorbable, synthetic bile acid conjugates can exert beneficial effects on circulating lipids and lipoproteins, there are other issues to address before this can be viewed as a useful therapeutic approach. Furthermore, an excellent clinical response to cholestyramine can be achieved in high-risk patients when the resin is used in low-dose combination with the hypolipidaemic agent Complamin, that acts in a complementary manner [25]. Thus, the problem of the palatability of the resins may be solved more readily by combination therapy than by the design of more selective agents. Nonetheless, the present results are useful in showing what can be achieved by receptor blockade and it may well be possible to design more potent agents, for example with irreversible binding to the receptor.

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REFERENCES

1. Lipid Research Clinics, The Lipid Research Clinics coronary primary prevention trial results. 1. Reduction in incidence of coronary heart disease. *J Am Med Assoc* **251**: 351–364, 1984.
2. Brensike JF, Levy RI, Kelsey SF, Passamani ER, Richardson JM, Loh IK, Stone NJ, Aldrich RF, Battaglini JW, Moriarty DJ, Fisher MR, Friedman L, Friedewald W, Detre KM and Epstein SE, Effects of therapy with cholestyramine on progression of coronary arteriosclerosis: results of the NHLBI type II coronary intervention study. *Circulation* **69**: 313–324, 1984.
3. Fears R, Mode of action of lipid-lowering drugs. *Baillière's Clin Endocrinol Metabol* **1**: 727–754, 1987.
4. Schucker BH and Goor RS, A behavioural approach for enhancing adherence to resin therapy. In: *Pharmacological Control of Hyperlipidaemia* (Ed. Fears R), pp. 91–109. Prous, Barcelona, 1986.
5. Kern F Jr, Davis RA and Grundy SM, The regulation of bile and synthesis. Report of a conference. *J Lip Res* **27**: 570–576, 1986.
6. Dietschy JM, Mechanisms for the intestinal absorption of bile acids. *J Lip Res* **9**: 297–309, 1968.
7. Lack L, Properties and biological significance of the ileal bile salt transport system. *Environ Health Perspect* **33**: 79–89, 1979.
8. Lack L, Tantawi A, Halevy C and Rockett D, Positional requirements for anionic charge for ileal absorption of bile salt analogues. *Am J Physiol* **246**: G745–G749, 1984.
9. Lack L and Weiner IM, *In vitro* absorption of bile salts by small intestine of rats and guinea pigs. *Am J Physiol* **200**: 313–317, 1961.
10. Firpi A, Walker JT and Lack L, Interactions of cationic bile salt derivations with the ileal bile salt transport system. *J Lip Res* **16**: 379–385, 1975.
11. Rush WR and Fears R, The role of cholesterol 7α-hydroxylase in the hypobetalipoproteinaemic activity of SKF-525A in rats. *Biochem Pharmacol* **31**: 2423–2426, 1982.
12. Mills GL, Chapman MJ and McTaggart F, Some effects of diet on guinea pig serum lipoproteins. *Biochim Biophys Acta* **260**: 401–412, 1972.
13. Fears R, Rush WR, Walker P and Ferres H, Hyperalphalipoproteinaemic activity of BRL 26314. 1. Enhanced cholesterol turnover in rats. *Biochem Pharmacol* **33**: 209–217, 1984.
14. Nystrom RF and Brown WG, Reduction of organic compounds by lithium aluminium hydride. III Halides, Quinones, miscellaneous nitrogen compounds. *J Am Chem Soc* **70**: 3738–3740, 1948.
15. Corse J, Bryant JT and Shonle HA, 2-Methoxy-6-chloro-9-(N-substituted-amino) acridines. II Compounds derived from cyclic secondary amines. *J Am Chem Soc* **68**: 1911–1913, 1946.
16. Little JM and Lester R, Ontogenesis of intestinal bile salt absorption in the neonatal rat. *Am J Physiol* **239**: G319–G323, 1980.
17. McClintock C and Shiau Y-F, Jejunum is more important than terminal ileum for taurocholate absorption in rats. *Am J Physiol* **244**: G507–G514, 1983.
18. Lack L and Weiner IM, Intestinal bile salt transport: structure–activity relationships and other properties. *Am J Physiol* **210**: 1142–1152, 1966.
19. Lester R, Smallwood RA, Little JM, Brown AS, Piasecki GJ and Jackson BT, Fetal bile salt metabolism. *J Clin Invest* **59**: 1009–1016, 1977.
20. Reymer MO, Montet JC, Crott C, Marteau C and

Gerolami A, Intestinal cholesterol uptake from mixed micelles. *Biochim Biophys Acta* **664**: 616–619, 1981.
21. Thomson ABR, Uptake of bile acids into rat intestine. *Diabetes* **32**: 900–907, 1983.
22. Hess Thaysen E and Pedersen L, Idiopathic bile acid catharsis. *Gut* **17**: 965–970, 1976.
23. Hill MJ, Lennard-Jones JE, Melville DM, Neale K and Ritchie JK, Faecal bile acids, dysplasia and carcinoma in ulcerative colitis. *Lancet* **ii**: 185–186, 1987.
24. Jacobsen O, Hojgaard L, Moller EH, Wielandt TO, Thale M, Jarnum S and Krag E, Effect of enterocoated cholestyramine on bowel habit after ileal resection: a double blind crossover study. *Br Med J* **290**: 1315–1318, 1985.
25. Fears R, Ferres H, Haacke H, Mäder C and Parwaresch MR, Decrease in LDL and increase in HDL concentrations in type II hyperlipoproteinaemic patients on low-dose combination therapy of cholestyramine and Complamin. *Atherosclerosis* **69**: 97–101, 1988.