# Effects of 2164U90 on ileal bile acid absorption and serum cholesterol in rats and mice

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**Abstract** 2164U90, [(3R,5R)-*trans*-3-butyl-3-ethyl-2,3,4,5-tetrahydro-5-phenyl-1,4-benzothiazepine 1,1-dioxide], was found to be a potent inhibitor of the ileal bile acid active transport system. In vitro, 2164U90 decreased uptake and active transport of taurocholic acid by rat everted ileal sacs with $IC_{50}$s of 4.0 $\mu$M and 1.5 $\mu$M, respectively. In vivo, 2164U90 produced dose-dependent increases in 23,25-$^{75}$Se-labeled homocholic acid taurine (SeHCAT) fecal excretion in rats and mice at doses of 3–30 mg/kg and 1–10 mg/kg, respectively. In rats, 30 mg/kg 2164U90 was equivalent to 500 mg/kg cholestyramine. Two days oral administration of 10 mg/kg 2164U90 to rats decreased the bile concentrations of total bile acids 42%, orally administered [$^3$H]taurocholic acid ([$^3$H]TC) 82%, and cholesterol 35%. Cholestyramine (500 mg/kg) had effects similar to 2164U90 on total bile acid and orally administered [$^3$H]TC concentrations but had no effect on biliary cholesterol. The hypocholesterolemic activity of 2164U90 was determined in cholesterol-cholic acid-fed rats and cholesterol-cholic acid-coconut oil-fed mice. 2164U90 inhibited the dietary-induced increase in dextran sulfate-precipitable lipoprotein cholesterol (VLDL + LDL) at doses comparable to doses needed to increase the fecal excretion of bile acids. These data indicate that 2164U90 decreases bile acid absorption by inhibiting the ileal bile acid active transport system, resulting in hypocholesterolemic activity.—**Lewis, M. C., L. E. Brieaddy, and C. Root.** Effects of 2164U90 on ileal bile acid absorption and serum cholesterol in rats and mice. *J. Lipid Res.* 1995. **36:** 1098–1105.

**Supplementary key words** active transport • enterohepatic circulation • hyperlipidemia • LDL • bile acid excretion • biliary lipids • taurocholic acid • cholestyramine • bile • 23,25-$^{75}$Se-labeled homocholic acid taurine

Plasma LDL cholesterol concentration is dependent on the rate of LDL clearance from plasma (1). Approximately two-thirds of plasma LDL cholesterol is cleared through the LDL receptor, principally by the liver (2). The rate of synthesis of LDL receptors is dependent on intracellular free cholesterol concentration. Cells respond to decreases in their free cholesterol content by stimulating the synthesis of LDL receptors, thus increasing the clearance of LDL cholesterol from plasma (3). Therefore, decreasing the concentration of hepatocyte free cholesterol is an effective way to lower LDL cholesterol. This is achieved pharmacologically by inhibiting cholesterol biosynthesis with HMG-CoA reductase inhibitors or increasing the conversion of cholesterol to bile acids with bile acid sequestrants.

Bile acids are synthesized in the liver and secreted into the small intestine where they facilitate fat and cholesterol absorption. More than 90% of the bile acids are reabsorbed either by passive diffusion along the entire intestine or actively at the terminal ileum. The small amount of bile acids not reabsorbed is excreted into feces and replenished by hepatic synthesis from cholesterol. Decreasing the enterohepatic flux of bile acids increases bile acid synthesis by induction of cholesterol 7$\alpha$-hydroxylase, the rate-limiting enzyme in bile acid synthesis. Bile acid sequestrants, such as cholestyramine, bind bile acids in the intestinal lumen decreasing their reabsorption. The decrease in the bile acid pool returning to the liver stimulates bile acid synthesis from cholesterol, reduces hepatocyte free cholesterol concentration which in turn results in a compensatory increase in LDL receptor synthesis and enhanced clearance of LDL from the plasma (4).

Clinical studies have shown that bile acid sequestrants alone or in combination with other hypolipidemic agents decrease plasma low density lipoprotein (LDL) cholesterol, significantly reduce the morbidity and mortality due to coronary heart disease (CHD) and can slow the rate of progression and, in some cases, produce regression of atherosclerotic lesions (5–8). However, large doses of bile acid sequestrants (12 to 24 g/day) are necessary for effective LDL cholesterol lowering. In vivo, bile acid sequestrants increase fecal bile acid excretion much less than would be expected from the in vitro bile acid binding capacity of sequestrants (9). Suggested reasons for the low in vivo potency of bile acid sequestrants are low affinity

Abbreviations: VLDL, very low density lipoprotein; HDL, high density lipoprotein; LDL, low density lipoprotein; TC, total cholesterol; HMG, 3-hydroxy-3-methylglutaryl; CHD, coronary heart disease.

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for glycine-conjugated and trihydroxy bile acids (10–12), competition for binding sites by other anions (12–14), and dissociation of bile acids from the sequestrants in the terminal ileum with reabsorption of the unbound bile acids (15). In addition, bile acid sequestrants are unpalatable and produce gastrointestinal side effects resulting in poor patient compliance (16).

The bile acid active transport system located in the terminal ileum is the major site of intestinal bile acid reabsorption (17–19). Therefore inhibiting the ileal bile acid active transport system could be an alternative method to bile acid sequestrants for decreasing bile acid reabsorption. Partial ileal bypass surgery (PIB) demonstrates the effectiveness of decreasing the ileal absorption of bile acids on plasma LDL cholesterol concentration. In a randomized clinical trial PIB decreased plasma LDL cholesterol concentration 38% (20). Mortality due to CHD was reduced and coronary arteriograms showed less disease progression. In addition, recent studies with ursodeoxycholic acid in humans and a series of novel bile salts in guinea pigs have shown that inhibiting the ileal bile acid active transport system decreases plasma LDL cholesterol concentrations (21–24).

The purpose of this study is to demonstrate that 2164U90, a chemically novel compound, decreases plasma cholesterol concentrations by decreasing bile acid absorption by inhibiting the ileal bile acid active transport system.

## METHODS

### Materials

2164U90 [(3R,5R)-*trans*-3-butyl-3-ethyl-2,3,4,5-tetrahydro-5-phenyl-1,4-benzothiazepine 1,1-dioxide] (**Fig. 1**) was synthesized at Burroughs Wellcome Co. 23,25-$^{75}$Se-labeled homocholic acid taurine was supplied by Amersham International. [$^3$H]taurocholic acid was supplied by New England Nuclear.

### Rat everted ileal sacs

*Preparation of tissue.* Everted ileal sacs were prepared from male Sprague-Dawley rats (CD, Charles River, 250–325 g) by the method of Wilson and Wiseman (25). Briefly, animals were killed, the ileum was removed, flushed with 60 ml cold glucose-free Krebs-Ringer bicarbonate buffer, pH 7.3, and everted using a blunt-tipped metal rod. Starting at the distal end, four everted ileal sacs, approximately 5–6 cm long, were prepared from one ileum. For each experiment, ileal sacs from four rats were arranged according to a Latin square into four groups to minimize the effect of the distal to proximal gradient in bile acid transport.

*Uptake studies.* The uptake and active transport of taurocholic acid were determined by a modification of the procedure described by Lack and Weiner (26). Everted sacs were filled with 0.75 ml glucose-free Krebs-Ringer bicarbonate buffer, pH 7.3, containing 0.37 mM [$^3$H]taurocholic acid ([$^3$H]TC) (serosal fluid) and incubated in 10 ml (mucosal fluid) of the [$^3$H]TC containing buffer with or without test compounds for 30 min in an orbital shaker water bath (90 rpm) at 37°C under an atmosphere of 95% $O_2$ and 5% $CO_2$. Test compounds were dissolved in 100% DMSO and added to the mucosal fluid giving a final DMSO concentration of 3%. Controls received DMSO only. At the end of the incubation, sacs were removed from the incubation medium, blotted, and opened to drain out the serosal fluid. Three hundred $\mu$l of mucosal fluid sampled before and after incubation and 300 $\mu$l serosal fluid were placed in scintillation vials with Scintiverse BD and total radioactivity was determined using a Packard TRI-CARB 1900TR liquid scintillation analyzer. Uptake of taurocholic acid was determined from the disappearance of [$^3$H]TC from the mucosal fluid. Active transport of taurocholic acid was determined from the final serosal/mucosal ratio of [$^3$H]TC in excess of unity.

### Cholesterol and bile acid concentrations in rat bile

Male Sprague-Dawley rats (CD, Charles River, 180–250 g) were used. Animals were housed individually with free access to food and water. Test compounds were administered by gavage (1 ml/100 g body weight) as a suspension in 0.5% methycellulose. Three treatment groups of four animals each were studied. The rats received 10 mg/kg 2164U90, 500 mg/kg cholestyramine, or 0.5% methyl cellulose at 9:00 AM and 3:00 PM on days 1 and 2 and at 9:00 AM on day 3. Each rat was given 10 $\mu$Ci of [$^3$H]TC (2.1 $\mu$Ci/nmol) in 1 ml of saline p.o. at 11:30 AM on day 1. At 11:30 AM on day 3 the rats were anesthetized with pentobarbital sodium (60 mg/kg ip), a midline incision was made, and the bile duct was exposed and cannulated with PE-10 tubing. Bile was collected for 15 min in 20-ml glass scintillation vials. Total radioactivity, bile acid, and cholesterol concentrations in bile were determined.

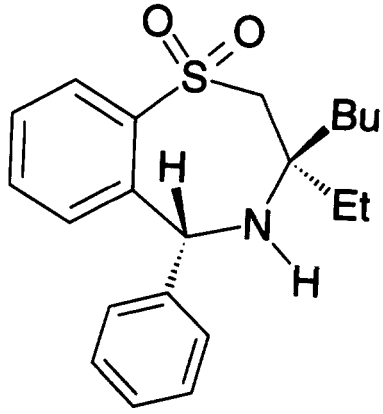


**Fig. 1.** Chemical structure of 2164U90; $C_{21}H_{27}NO_2S$; mol wt 357.51.

### Fecal excretion of bile acids

*Rat.* Male Sprague-Dawley rats (CD, Charles River, 220–260 g) were housed individually and fed normal chow. The rats were divided into six treatment groups of 10 to 12 rats per group. The rats were dosed by oral gavage (1 ml/100 gm body weight) with test compounds as a suspension in 0.5% methylcellulose at 9:00 AM and 3:30 PM for two days. The control group received 0.5% methylcellulose. Two hours after the morning dose on day two, the rats were given a trace amount (1.3 nmoles) of 23,25-$^{75}$Se-labeled homocholic acid taurine ($^{75}$SeHCAT) in 1.0 ml saline orally. $^{75}$SeHCAT, a synthetic gamma emitting bile acid analog, is absorbed by the ileal bile acid active uptake system similar to taurocholic acid and has been used clinically to measure ileal bile acid absorption (27–30). Feces were collected over the 24 h after $^{75}$SeHCAT administration. Fecal content of $^{75}$SeHCAT was determined using a Packard Auto-Gamma 5000 Series gamma-counter.

*Mouse.* Male CD-1 mice (Charles River, 22–28 gm) were housed individually and fed normal chow. The mice were divided into four treatment groups of six mice per group. The procedure was the same as described for rats except that the mice were given $^{75}$SeHCAT (1.3 nmol) in 0.2 ml saline orally.

### Hypolipidemic activity

*Rat.* Hypercholesterolemia was produced in male Sprague-Dawley rats (CD, Charles River, 250–300 g) by administration of Wayne Laboratory meal enriched in cholesterol (0.4%) and cholic acid (0.2%). Blood samples were collected under halothane anesthesia by cardiac puncture prior to diet administration. Serum was obtained for analysis of total cholesterol (TC), high density lipoprotein (HDL) cholesterol, dextran-precipitable lipoprotein (VLDL + LDL) cholesterol, and total triglyceride concentrations. The rats were divided into groups so that each group had similar baseline serum lipid concentrations. Five days after the initial sampling for serum lipids, the rats were fed ad lib the cholesterol-cholic acid-enriched diet. For acute (3.5 days) and chronic (4 weeks) dosing studies, test compounds and diet administration were initiated on the same day. Test compounds were administered by gavage (1 ml/100 g body weight) as a suspension in 0.5% methycellulose b.i.d. at 9:00 AM and 3:00 PM. Animals in the control group received only 0.5% methycellulose. Four hours after the last morning dose, rats were anesthetized and bled for the determination of serum lipids.

To determine the effects of 2164U90 in preexisting hypercholesterolemia, rats were given the enriched diet for 7 days prior to administration of 2164U90. After 7 days on diet, serum was obtained and analyzed for lipids. The rats were then given 25 mg/kg 2164U90 b.i.d. by gavage for 6 days and once on day 7. Four hours after the last dose, serum was obtained and analyzed as before. 2164U90 administration was discontinued and the diet was continued for an additional 7 days at which time serum samples were obtained. All blood collections were done after a 4-h fast.

*Mice.* Hypercholesterolemia was produced in male CD-1 mice (Charles River, 20–30 g) by administration of Wayne Laboratory meal enriched in cholesterol (0.4%), cholic acid (0.2%), and coconut oil (5%). The mice were divided into groups of eight and diet and compound administration were initiated. 2164U90 was administered by gavage as a suspension in 0.5% methylcellulose (1 ml/100 g body weight) at 9:00 AM and 3:00 PM for 9 days and at 9:00 AM on day 10. Control animals received 0.5% methylcellulose only. Four hours after the last dose, blood was collected from mice under halothane anesthesia by cardiac puncture. Serum was obtained for analysis of total TC, HDL cholesterol, and VLDL + LDL cholesterol.

### Biochemical analysis

Total cholesterol and triglyceride concentrations were determined enzymatically using reagents obtained from Seragen Diagnostics. HDL cholesterol was determined

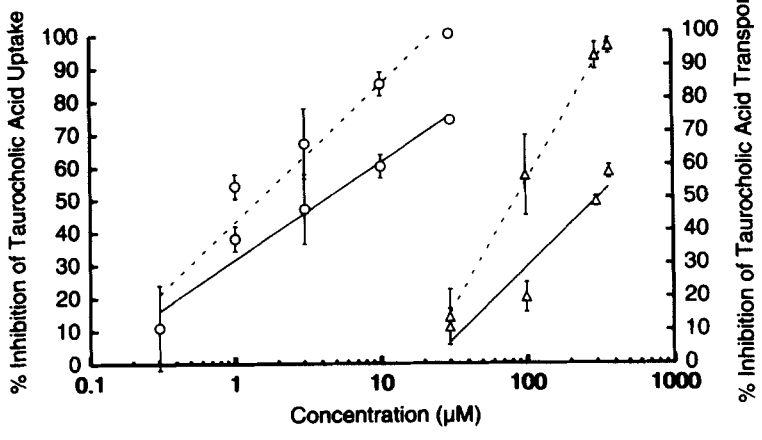


**Fig. 2.** Concentration-dependent effect of 2164U90 (○) and chenodeoxycholic acid (△) on taurocholic acid uptake (—) and active transport (--) by rat everted ileal sacs. Distal ileal segments (5–6 cm) from rats were everted and filled with 0.75 ml of buffer containing 0.37 mM taurocholic acid (TC). The sacs were incubated in 10 ml of buffer (mucosal fluid) containing 0.37 mM [$^3$H]TC with or without 2164U90, at concentrations indicated, for 30 min at 37°C. Uptake of TC was determined by measuring the disappearance of [$^3$H]TC from the mucosal fluid. Active transport of TC was determined from the increase in the serosal/mucosal ratio of [$^3$H]TC in excess of unity. The data are expressed as mean ± SEM for four determinations.

TABLE 1. Effect of 2164U90 and cholestyramine on biliary bile acid and cholesterol concentrations

| | Control | 2164U90 10 mg/kg | Cholestyramine 500 mg/kg |
|---|---|---|---|
| Total bile acid, μmol/ml bile | 33.6 ± 5.9 | 19.5 ± 2.5[a] | 20.6 ± 2.6[a] |
| [$^3$H]taurocholic acid, nmol/ml bile | 0.825 ± 0.154 | 0.147 ± 0.036[b] | 0.237 ± 0.069[b] |
| Total cholesterol, μmol/ml bile | 0.132 ± 0.008 | 0.086 ± 0.004[b] | 0.123 ± 0.003 |

Rats were dosed orally with 2164U90, cholestyramine, or vehicle at 9:00 AM and 3:30 PM for 2.5 days. [$^3$H]taurocholic acid (4.76 nmol, 10 μCi) was given orally at noon on day 1. Bile was collected 48 h after dosing with [$^3$H]TC. Values are given as the mean ± SEM for four rats/group.
[a] $P < 0.05$ (treated vs. control by one-way analysis of variance).
[b] $P < 0.01$ (treated vs. control by one-way analysis of variance).

after selective precipitation of VLDL and LDL with dextran sulfate and magnesium sulfate. VLDL + LDL cholesterol was determined as the difference between total and HDL cholesterol. Total bile acid concentration was determined enzymatically using reagents from Sigma Diagnostics.

## RESULTS

### Effect of 2164U90 on taurocholic acid uptake and active transport by rat everted ileal sacs

2164U90 decreased mucosal uptake and active transport of taurocholic acid by rat everted ileal sacs in a concentration-dependent manner with $IC_{50}$s of 4.0 μM and 1.5 μM, respectively (**Fig. 2**). $IC_{50}$ values for chenodeoxycholic acid were 296 μM for uptake and 123 μM for transport.

### Effect of 2164U90 and cholestyramine on biliary concentrations of orally administered [$^3$H]TC, total bile acids, and cholesterol

Two days administration of 10 mg/kg 2164U90 p.o. b.i.d decreased the bile concentrations of total bile acids 42%, orally administered [$^3$H]TC 82%, and cholesterol 35% (**Table 1**). Cholestyramine, at 500 mg/kg b.i.d, decreased the concentrations of total bile acid and orally administered [$^3$H]TC 39% and 71%, respectively, with no effect on biliary cholesterol.

### Effect of 2164U90 on the fecal excretion of bile acids

2164U90 increased the fecal excretion of $^{75}$SeHCAT in a dose-dependent manner in rats and mice (**Fig. 3**). The lowest dose of 2164U90 in rats that produced a statistically significant increase on excretion was 3 mg/kg b.i.d. The 30 mg/kg dose of 2164U90 produced activity comparable to 500 mg/kg cholestyramine. In mice, 2164U90 significantly increased the fecal excretion of $^{75}$SeHCAT at doses of 1 mg/kg to 10 mg/kg.

### Hypolipidemic effects of 2164U90

The effects of 2164U90, administered orally for 3.5 days, on serum total cholesterol, VLDL + LDL cholesterol, HDL cholesterol, and triglycerides in dietary-induced hypercholesterolemic rats are summarized in **Table 2.** 2164U90 significantly decreased serum VLDL + LDL cholesterol 60%, 37%, and 28% at doses of 12.5, 5, and 1 mg/kg b.i.d., respectively. 2164U90 was substantially

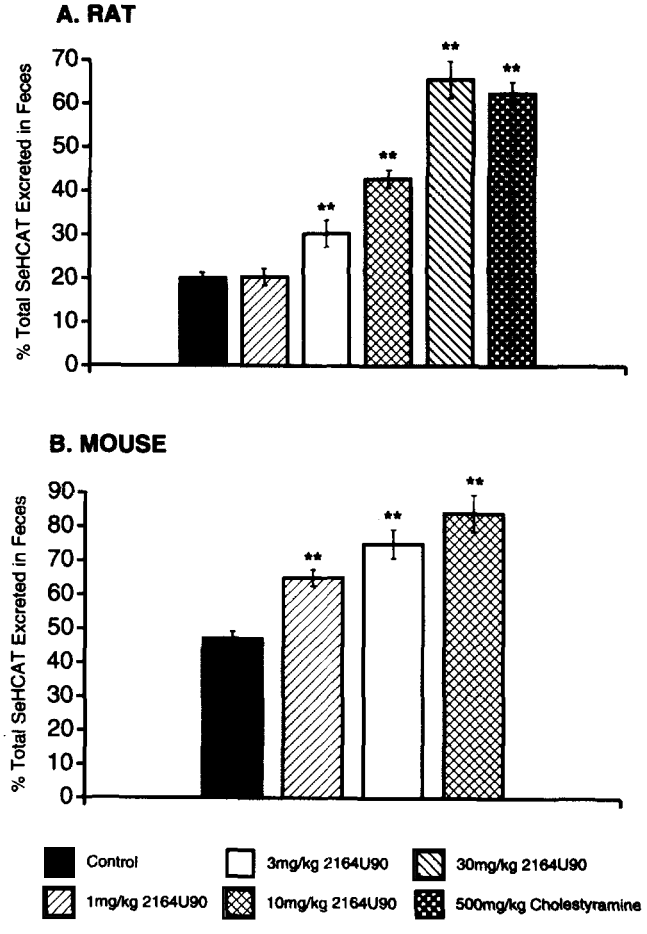


**Fig. 3.** Dose-dependent effect of 2164U90 on fecal excretion of $^{75}$SeHCAT in rats (A) and mice (B). Animals were dosed orally with test compound at 9:00 AM and 3:30 PM for 2 days. At midday on the second day the animals were given a trace (1.3 nmol) of $^{75}$SeHCAT. Total radioactivity in feces excreted over the next 24 h was determined. Each data point represents the mean ± SEM for 10–12 animals. $^{**}P < 0.01$ (treated vs. control by one-way analysis of variance).

TABLE 2. Effect of 2164U90 on serum lipid components of cholesterol-cholic acid-fed rats

| Group | Dose | TC | VLDL + LDL | HDL | TG |
|---|---|---|---|---|---|
| | *mg/kg* | | *mg/dl* | | |
| Control | | 139 ± 16 | 123 ± 17 | 16 ± 2 | 109 ± 9 |
| 2164U90 | 25.0 | 83 ± 4[a] | 55 ± 4[a] | 28 ± 2[a] | 90 ± 5 |
| 2164U90 | 12.5 | 77 ± 3[a] | 49 ± 3[a] | 28 ± 3[a] | 69 ± 6[a] |
| 2164U90 | 5.0 | 98 ± 4[a] | 77 ± 3[a] | 20 ± 1 | 83 ± 8 |
| 2164U90 | 1.0 | 110 ± 8[a] | 88 ± 10[a] | 22 ± 3 | 93 ± 13 |

Rats were fed a diet enriched in cholesterol (0.4%) and sodium cholate (0.2%) and were dosed orally at 9:00 AM and 3:00 PM for 3.5 days. Values are from bleeding done 4 h after last dose on 4th day of study and given as the mean ± SEM for 5 rats/group. TC, total cholesterol; VLDL + LDL, very low density + low density lipoprotein cholesterol; HDL, high density lipoprotein cholesterol; TG, triglycerides.

[a] $P < 0.05$ (treated vs. control by one-way analysis of variance).

more potent than cholestyramine which decreased VLDL + LDL cholesterol 65% at 500 mg/kg b.i.d. with no effect at 150 mg/kg (data not shown). 2164U90 attenuated the dietary-induced decrease in HDL cholesterol at doses of 12.5 mg/kg and higher. No significant dose-dependent effect on serum triglyceride concentration was seen.

The effects of 4 weeks administration of 2164U90 on serum VLDL + LDL cholesterol, HDL cholesterol, and triglycerides in cholesterol-cholic acid-fed rats are summarized in **Table 3.** 2164U90 dose-dependently decreased VLDL + LDL cholesterol throughout the 4 weeks of treatment. HDL cholesterol was 25% to 35% higher in the 25 mg/kg group relative to controls throughout the 4-week dosing period. Serum triglycerides were 29% lower than controls following 1 week of dosing with 25 mg/kg 2164U90. At 2 weeks, a 25% decrease was seen in both 2164U90 treatment groups. No significant effects were seen after 4 weeks of dosing.

2164U90, at 25 mg/kg b.i.d., decreased VLDL + LDL cholesterol concentration by 50% in rats when compound administration was begun 7 days after the rats had been consuming the cholesterol-cholic acid diet (**Table 4**). After withdrawal of 2164U90, VLDL + LDL cholesterol returned towards its pre-2164U90 values. 2164U90 attenuated the dietary-induced decrease in HDL cholesterol. This was most evident in the large decrease in HDL cholesterol after discontinuing treatment with 2164U90. A small but significant decrease was seen in serum triglyceride concentration.

The effects of 2164U90 on serum VLDL + LDL and HDL cholesterol concentrations in mice fed a diet enriched in cholesterol, cholic acid, and coconut oil are summarized in **Table 5.** 2164U90, at 0.3 mg/kg to 10 mg/kg b.i.d., significantly decreased serum VLDL + LDL concentration in a dose-dependent manner. HDL cholesterol was significantly increased by 12.5% to 16% at doses of 1 mg/kg and greater.

## DISCUSSION

Inhibiting intestinal bile acid absorption with bile acid sequestrants or surgically by partial ileal bypass surgery

TABLE 3. Effects of chronic administration of 2164U90 on serum lipid components of cholesterol-cholic acid-fed rats

| Serum Lipid | Group | Dose | Initial | Week 1 | Week 2 | Week 4 |
|---|---|---|---|---|---|---|
| | | *mg/kg* | | *mg/dl* | | |
| VLDL + LDL cholesterol | Control | 0 | 40 ± 4 | 113 ± 5 | 114 ± 5 | 136 ± 12 |
| | 2164U90 | 5 | 33 ± 2 | 71 ± 3[a] | 60 ± 5[a] | 80 ± 4[a] |
| | 2164U90 | 25 | 32 ± 3 | 43 ± 4[a] | 48 ± 4[a] | 48 ± 6[a] |
| HDL cholesterol | Control | 0 | 38 ± 1 | 26 ± 1 | 19 ± 1 | 21 ± 1 |
| | 2164U90 | 5 | 34 ± 1 | 28 ± 1 | 20 ± 1 | 18 ± 1 |
| | 2164U90 | 25 | 35 ± 2 | 35 ± 2[a] | 25 ± 2[a] | 26 ± 2[a] |
| Triglyceride | Control | 0 | 65 ± 7 | 121 ± 12 | 103 ± 6 | 107 ± 9 |
| | 2164U90 | 5 | 61 ± 5 | 124 ± 10 | 77 ± 6[a] | 84 ± 13 |
| | 2164U90 | 25 | 61 ± 5 | 85 ± 8[a] | 77 ± 7[a] | 89 ± 15 |

Rats were fed a diet enriched in cholesterol (0.4%) and sodium cholate (0.2%) and were dosed orally at 9:00 AM and 3:00 PM for 4 weeks. Values are from bleeding done 4 h after morning dose at the indicated times and given as the mean ± SEM for five rats/group. TC, total cholesterol; VLDL + LDL, very low density + low density lipoprotein cholesterol; HDL, high density lipoprotein cholesterol; TG, triglycerides.

[a] $P < 0.05$ (treated vs. control by one-way analysis of variance).

TABLE 4. Effects of 2164U90 on serum lipid components in hypercholesterolemic rats

| Treatment Period | Treatment | VLDL + LDL | HDL | TG |
|---|---|---|---|---|
| | | | *mg/dl* | |
| Initial | | 42 ± 7 | 36 ± 3 | 62 ± 6 |
| I | Diet | 137 ± 10[a] | 24 ± 2[a] | 106 ± 5[a] |
| II | Diet + 2164U90 | 68 ± 5[a] | 27 ± 2[a] | 78 ± 5[a] |
| III | Diet | 107 ± 11[a] | 20 ± 2[a] | 75 ± 8 |

Rats were fed a diet enriched in cholesterol (0.4%) and sodium cholate (0.2%) for 21 days. 2164U90 was administered orally at 9:00 AM and 3:00 PM at 25 mg/kg on days 7 thru 14. Values are from bleeding done 4 h after the end of each treatment period and given as the mean ± SEM for five rats/group. VLDL + LDL, very low density + low density lipoprotein cholesterol; HDL, high density lipoprotein cholesterol; TG, triglycerides.
[a] $P < 0.05$ (paired $t$ test comparing values found at one period to those of the preceding period).

is an effective way to decrease plasma LDL cholesterol concentration. Another approach to decreasing bile acid absorption is inhibiting the ileal bile acid active transport system. The results of these studies demonstrate that 2164U90 is a potent inhibitor of the ileal bile acid active transport system and that this inhibition results in hypocholesterolemic activity.

Rat everted ileal sacs were used in our initial studies to evaluate the effect of 2164U90 on the bile acid active transport system. Everted ileal sacs have been used extensively to study the transport of bile acids by the bile acid active transport system (24, 31–33). In these sac preparations, equal concentrations of taurocholic acid were present in both mucosal and serosal fluids at the start of incubation. Under this condition, mucosal to serosal transport of taurocholic acid is due to active transport whereas uptake is both passive and active. The results of our study in everted ileal sacs indicate that 2164U90 is a potent inhibitor of the ileal bile acid active transport system. 2164U90 decreased mucosal uptake and active transport of taurocholic acid with $IC_{50}$s of 4.0 and 1.5 $\mu$M, respectively. 2164U90 was greater than 70 times more potent than chenodeoxycholic acid. In addition, 2164U90 was more potent than bile acid analogs reported by other investigators, which require concentrations greater than 100 $\mu$M to inhibit transport 50% (24, 34, 35).

To determine whether the inhibition of the bile acid active transport system observed in vitro resulted in inhibition of bile acid absorption in vivo, we investigated the effect of 2164U90 on fecal excretion of $^{75}$SeHCAT and the effects of 2164U90 on biliary concentrations of bile acids and orally administered [$^3$H]TC. The results from these studies indicate that 2164U90 decreases the intestinal adsorption of bile acids by inhibiting the ileal bile acid active transport system. 2164U90 increased the fecal excretion of orally administered $^{75}$SeHCAT and decreased the bile concentrations of total bile acids and orally administered [$^3$H]TC. Doses of cholestyramine that produced similar effects were 15–50 times greater than those for 2164U90. Decreasing bile acid absorption should result in a compensatory increase in bile acid synthesis by induction of cholesterol 7$\alpha$-hydroxylase. Though no direct measurement of bile acid synthesis was made, the smaller decrease seen in total bile acids relative to [$^3$H]TC for both 2164U90 and cholestyramine indicates an increase in bile acid synthesis. In addition to the effects on bile acids, 2164U90 decreased biliary cholesterol concentration 35%. Similar decreases in biliary cholesterol in humans have been reported with ursodeoxycholic acid, which inhibits the ileal absorption of bile acids (36). In our study, no effect on biliary cholesterol concentration was seen with cholestyramine. These results are similar to those previ-

TABLE 5. Effects of 2164U90 on serum lipid components of cholesterol-cholic acid-coconut oil-fed mice

| Group | Dose | VLDL + LDL | % Change | HDL | % Change |
|---|---|---|---|---|---|
| | | *mg/dl* | | *mg/dl* | |
| Control | | 95 ± 5.1 | | 104 ± 3.8 | |
| 2164U90 | 10.0 | 31 ± 3.9[a] | −67 | 117 ± 5.4[a] | +12.5 |
| 2164U90 | 3.0 | 40 ± 3.2[a] | −58 | 121 ± 4.3[a] | +16.3 |
| 2164U90 | 1.0 | 58 ± 4.7[a] | −39 | 120 ± 3.0[a] | +15.3 |
| 2164U90 | 0.3 | 71 ± 5.4[a] | −25 | 112 ± 3.0 | +7.7 |

Mice were fed a diet enriched in cholesterol (0.4%), sodium cholate (0.2%), and coconut oil (5%) for 10 days. 2164U90 was administered orally by gavage at 9:00 AM and 3:00 PM for 9 days and at 9:00 AM on day 10. Values are from bleeding done 4 h after the last dose on 10th day of study and given as the mean ± SEM for eight mice/group. VLDL + LDL, very low density + low density lipoprotein cholesterol; HDL, high density lipoprotein cholesterol.
[a] $P < 0.05$ (treated vs. control by one-way analysis of variance).

ously reported by Imai et al. (37). However, Robins et al. (38) report decreases in bile cholesterol after cholestyramine treatment in rats. The reason for the different effect of cholestyramine and 2164U90 on bile cholesterol concentration seen in our study is not known. Possible explanations could be differences between 2164U90 and cholestyramine in duration of action, different mechanisms of inhibiting bile acid absorption, or an activity of 2164U90 not related to its inhibition of bile acid absorption.

The hypocholesterolemic activity of 2164U90 was demonstrated in cholesterol-cholic acid-fed rats and cholesterol-cholic acid-coconut oil-fed mice. In rats and mice, 2164U90 decreased VLDL + LDL cholesterol at doses comparable to doses that increased bile acid fecal excretion. The increased potency of 2164U90 relative to cholestyramine was similar whether examining decreases in serum cholesterol or increases in bile acid excretion. Additionally, 2164U90 was effective during chronic dosing and in decreasing VLDL + LDL cholesterol concentrations in rats with established hypercholesterolemia. The close similarity between cholesterol-lowering doses and those required for increasing bile acid excretion strongly indicates that the hypocholesterolemic activity of 2164U90 is due to its inhibitory effect on the ileal bile acid active transport system.

Interfering with the enterohepatic circulation of bile acids is effective in reducing plasma LDL cholesterol concentrations. However, bile acid sequestrants are associated with adverse effects that limit patient compliance. The major adverse effects are: *1)* gastrointestinal, *2)* altered absorption of some co-administered drugs, vitamins, or minerals, and *3)* unpalatability (16). Most of these effects can be attributed to the nonspecific binding properties, physical properties, and the large dose requirements of bile acid sequestrants. By potently and selectively inhibiting the ileal bile acid active transport system, 2164U90 may not have the adverse effects of bile acid sequestrants.

In conclusion, 2164U90 decreases plasma LDL cholesterol concentration by decreasing the intestinal absorption of bile acids. However, unlike bile acid sequestrants that decrease bile acid absorption by binding bile acids, 2164U90 inhibits the ileal bile acid transport system. By selectively inhibiting the ileal bile acid transport system, 2164U90 may not have the limitations of bile acid sequestrants.

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