Biochemical Pharmacology, Vol. 52, pp. 1177–1186, 1996.

ISSN 0006-2952/96/$15.00 + 0.00
PII S0006-2952(96)00455-8

Hypolipidaemic Properties of a Potent and Bioavailable Alkylsulphinyl-diphenylimidazole ACAT Inhibitor (RP 73163) in Animals Fed Diets Low in Cholesterol

*David Riddell, Colin P. Bright, Brenda J. Burton, Raymond C. Bush, Neil V. Harris, David Hele, Una M. Moore, Kuni Naik, David P. Parrott, Christopher Smith and Robert J. Williams**

RHÔNE-POULENC RORER LT., DAGENHAM RESEARCH CENTRE, DAGENHAM, ESSEX RM10 7XS, U.K.

ABSTRACT. RP 73163 ((S)-2-[5-(3,5-dimethyl-1-pyrazolyl)pent-1-yl)-sulphinyl]-5,6-diphenylimidazole) has been shown to be a potent and specific inhibitor of acyl-CoA:cholesterol acyltransferase (EC 2.3.1.26; ACAT) *in vitro* using the tissues of experimental animals as sources of the enzyme. The concentrations of RP 73163 required to produce 50% inhibition of ACAT activity (IC_{50} values) in microsomal preparations ranged from 86 nM for rat liver to 370 nM for rabbit intestine. In whole cell assays using human hepatic (HepG2), intestinal (Caco2), and monocytic (THP-1) cell lines, RP 73163 inhibited ACAT activity with IC_{50} values of 266, 158, and 314 nM, respectively. The addition of RP 73163 (0.03–1.0 μM) to the medium of cultured HepG2 cells produced a concentration-dependent decrease in apolipoprotein B (apoB) secretion. The compound has high systemic bioavailability. Using a bioassay, a concentration of active inhibitor equivalent to 29 μM of parent compound was present in plasma 1 hr after oral administration of RP 73163 (50 $mg \cdot kg^{-1}$). In rats that had been fed a basal diet *ad libitum* or starved for 18 hr prior to blood sampling, the administration of RP 73163 (50 $mg \cdot kg^{-1}$ *b.i.d.* for 7 days) reduced plasma triglyceride levels by 50% without affecting the concentration of cholesterol. This hypotriglyceridaemic effect was associated with reductions in plasma very-low-density-lipoprotein (VLDL) and low-density-lipoprotein (LDL) levels. RP 73163 decreased the rate of VLDL secretion by 24% in Triton WR-1339-treated rats that had been fasted overnight but did not affect the secretion rate in animals fed *ad libitum*, indicating that ACAT was only important in regulating VLDL secretion under certain nutritional conditions. RP 73163 reduced the accumulation of intraperitoneally administered [^{3}H]leucine into the plasma VLDL–apoB pool in both fed and fasted states. The results suggest that, in fed animals at least, an increase in the clearance of VLDL from the bloodstream may contribute to the hypolipidaemic activity of the compound. In rabbits with casein-induced endogenous hypercholesterolaemia, RP 73163 specifically reduced the levels of cholesterol carried by LDL. In conclusion, the hypolipidaemic actions of RP 73163, a potent and systemically bioavailable ACAT inhibitor, are consistent with a reduction in the secretion of apoB containing lipoproteins by hepatic tissue and possibly with an increase in the clearance of these particles. BIOCHEM PHARMACOL 52;8:1177–1186, 1996.

KEY WORDS. cholesterol; acyl-CoA:cholesterol acyltransferase; plasma lipoproteins; apoB; VLDL secretion; fasting

Hypercholesterolaemia is a major risk factor for the development of coronary heart disease [1]. Clinical studies have demonstrated that cholesterol lowering therapy reduces the incidence of coronary events [2] in association with a reduction in the size of atherosclerotic lesions [3]. Two important determinants of the plasma cholesterol concentration are the efficiency of cholesterol absorption from the intestine [4] and the rate of secretion of VLDL§ by the liver [5]. In studies carried out in experimental animals [6, 7] and in tissue culture using human cell lines [8, 9], both these processes have been shown to be regulated by the activity of ACAT, the enzyme responsible for the intracellular esterification of cholesterol [10]. Therefore, inhibitors of ACAT are expected to lower plasma cholesterol levels. Numerous studies have demonstrated a hypocholesterolaemic response to the administration of ACAT inhibitors in animals fed

* Corresponding author. TEL: 0181 919 3852; FAX: 0181 919 2005.

§ *Abbreviations:* VLDL, very low density lipoprotein; ACAT, acyl-CoA:cholesterol acyltransferase; CI-976, 2,2-dimethyl-N-(2,4,6-trimethoxyphenyl)dodecanamide; RP 73163, ((S)-2-[5-(3,5-dimethyl-1-pyrazolyl) pent-1-yl)sulphinyl]-4,5-diphenylimidazole); CL 277,082 N-(2,4-difluorophenyl)-N-(4-neopentylbenzyl)-N-(*n* heptyl)urea; apoB, apolipoprotein B; DMEM, Dulbecco's modified Eagles medium; LDL, low density lipoprotein; PCEH, pancreatic cholesterol ester hydrolase; LCAT, lecithin cholesterol acyltransferase; IDL, intermediate density lipoprotein; HDL, high density lipoprotein; DMSO, dimethylsulphoxide; TLC, thin layer chromatography.

Received 20 October 1995; accepted 3 June 1996.

cholesterol-supplemented diets [reviewed in refs. 11, 12]. Many of the inhibitors used in these studies show poor systemic bioavailability, and inhibition of intestinal cholesterol absorption has been demonstrated to be their primary mode of action. Furthermore, ACAT inhibitors have been reported to be ineffective in reducing cholesterol absorption in animals fed diets that are not supplemented with cholesterol, leading to the hypothesis that ACAT only has a role in regulating cholesterol absorption during administration of a high exogenous load of the sterol [13]. More recently, CI-976, a systemically bioavailable inhibitor of ACAT, has been shown to reduce plasma lipid levels in models of endogenous hyperlipidaemia in which animals are fed diets that are cholesterol free but are supplemented with casein or sucrose [14, 15]. The actions of CI-976 in these models are thought to be the result of direct inhibition of hepatic ACAT.

A series of 2-(alkylthio)-4,5-diphenyl-1-H-imidazoles have been shown to be potent inhibitors of ACAT [16]. In this paper, we describe some of the properties of RP 73163, a systemically bioavailable member of this series [17], with particular reference to studies carried out in animals fed diets that contained low concentrations of cholesterol.

MATERIALS AND METHODS

Materials

RP 73163, CI-976, and CL 277,082 were synthesised by the Discovery Chemistry Department, Dagenham Research Centre (Rhône-Poulenc Rorer Ltd., U.K.). Radiochemicals were purchased from Amersham International plc. (Little Chalfont, U.K.). Cell lines for tissue culture were obtained from ECACC (Porton Down, U.K.). All culture media were obtained from Life Technologies (Paisley, U.K.). Affinity purified human apoB, polyclonal mouse anti-human apoB, and anti-mouse IgG were purchased from Chemicon International Inc. (Temecula, U.S.A.). "FAST" ELISA plates and all tissue culture ware were obtained from Falcon, Becton Dickinson (Oxford, U.K.). Foetal bovine serum was purchased from Globepharm (Esher, U.K.). Other biochemicals and Tyloxapol were purchased from Sigma Chemical Co. Ltd. (Poole, U.K.). Scintillation fluids were obtained from Lumac (Groningen, Holland). Polygram Sil G plates (Machery-Nagel) were obtained from Camlab (Cambridge, U.K.). Sep-Pak cartridges were obtained from Millipore Corporation (Milford, MA, U.S.A.).

Animals and Diets

Male Sprague-Dawley rats weighing approximately 150–175 g were obtained from Charles River U.K. Ltd. (Margate, U.K.). Male Golden Syrian hamsters weighing 90–110 g were obtained from Wrights of Essex (Chelmsford, U.K.). Both rats and hamsters were fed a pelleted rodent diet (ERD supplied by SDS, Witham, U.K.) or the same diet supplemented with 0.5% cholesterol and 0.25% cholic acid. According to the manufacturer, the concentration of cholesterol in the basal diet was 0.006%. Male New Zealand White rabbits (1.5–2.0 kg) were obtained from Froxfield Rabbits Ltd. (Petersfield, U.K.). The rabbits were fed either a standard rabbit diet that contained no cholesterol (R14, SDS), the standard diet supplemented with 0.5% cholesterol, or a diet containing casein (Teklad, Madison, WI, USA). The composition of the casein diet was 40% casein, 15% sucrose, 13% corn starch, 14% coconut oil, 1% corn oil, 11% cellulose, and 6% vitamins and inorganic salts. Unless stated otherwise, RP 73163 was administered to rats by gavage as a suspension in 1% sodium carboxymethylcellulose and 0.2% Tween 80 (2 mL · kg^{-1}). Rabbits received the compound by stomach tube as a suspension in 0.5% tragacanth.

Microsomal ACAT Assays

Microsomes were prepared from tissues of animals that had been fed cholesterol-supplemented diets to saturate the substrate pool for ACAT. Rats and hamsters were fed the appropriate cholesterol diet for 5 days. Rabbits were fed cholesterol-supplemented diet for 7–8 weeks to increase ACAT activity in arterial tissue. In addition, microsomes were prepared from three human cell lines: HepG2 (liver), Caco2 (intestine), and THP-1 (monocyte/macrophage). Prior to the preparation of microsomes, the cells were preloaded with cholesterol as described previously [18].

ACAT activity was determined according to standard methodology. Briefly, approximately 60 μg microsomal protein was added to 0.1 M sodium phosphate buffer, pH 7.4, containing fatty-acid-free bovine serum albumin and dithiothreitol to produce a volume of 170 μl. Compounds were added in 1 μl of DMSO and preincubated for 5 min at 37°C. The reaction was started by the addition of 30 μl of [1-^{14}C]oleoyl-CoA (0.15 μCi). Final concentrations in the incubations were 90 μM oleoyl-CoA, 90 μM albumin, and 2 mM dithiothreitol. After 5 min, the reaction was stopped by the addition of 4 mL of dichloromethane:methanol 2:1 containing 10 mg · L^{-1} cholesterol oleate as carrier. Tritiated cholesterol oleate was added to determine recovery. Lipids were extracted according to Folch *et al.* [19] and separated by TLC on 0.25-mm silica gel plates using *n*-heptane:diethyl ether:glacial acetic acid 90:10:1 as the solvent system. The concentrations of protein in the microsomal preparations were determined by the method of Lowry *et al.* [20]. ACAT activity was calculated as pmol cholesterol oleate formed/mg microsomal protein/min. Each inhibitor was evaluated at five concentrations in triplicate, and IC_{50} values were determined from computer-derived sigmoidal log concentration-response curves.

The microsomal assay was also used to determine the systemic bioavailability of RP 73163 by measuring the ACAT inhibitory activity of plasma prepared from the blood of treated rats. Aliquots of plasma (5–20 μL) were preincubated for 20 min with hepatic microsomes in 0.1 M phosphate buffer, pH 7.4, containing bovine serum albumin and dithiothreitol plus 1 μl DMSO. Radiolabelled oleoyl-

CoA was added and ACAT activity determined as described above. A standard concentration-response curve was prepared by incubating microsomes with known concentrations of RP 73163 in 1 μl DMSO in the presence of appropriate volumes of plasma from untreated rats.

ACAT Assays in Human Cell Lines

ACAT activity in HepG2, Caco2, and THP-1 cell lines was determined using methodology described previously [18].

Specificity of Inhibition

The ability of RP 73163 to inhibit PCEH and LCAT mediated cholesterol esterification was evaluated by previously described methods [21, 22] using rat PCEH (extracted from fresh tissue according to [21]), bovine PCEH (Sigma), and rat plasma LCAT.

Hypolipidaemic Activity

The hypolipidaemic activity of RP 73163 was investigated in normocholesterolaemic rats and in casein-fed rabbits. RP 73163 was administered by gavage to rats at a dose level of 50 mg · kg^{-1} *b.i.d.* (09.00 and 17.00 hr) for 7 days. The animals were allowed to eat a basal rodent diet *ad libitum* or were fasted for 18 hr prior to blood sampling. Sixty minutes after the final dose of compound, the rats were anaesthetised, and blood was sampled from the abdominal aorta. Plasma was separated by centrifugation at 1500*g* for 10 min. When required, lipoprotein fractions were separated by sequential ultracentrifugation [23] using density cuts of d < 1.006, 1.006–1.063, and 1.063–1.21 for VLDL, LDL (+ IDL), and HDL, respectively.

Rabbits were rendered hypercholesterolaemic by feeding a mixture of standard diet:40% casein diet 3:1 for 7 days followed by the 40% casein diet for another 14 days. During the induction period and for the remainder of the study, the animals were bled from the central ear artery at weekly intervals for plasma cholesterol analysis. After 3 weeks on the casein diet, the plasma cholesterol levels were ranked, and the ranking was used to allocate the rabbits to treatment groups. The diet of one group was replaced by the standard diet, and the other two groups continued to be maintained on the 40% casein diet. RP 73163 was administered to one group of casein-fed rabbits at a dose level of 50 mg · kg^{-1} *b.i.d.* for 4 weeks. At the end of the study, plasma lipoprotein analyses were carried out using density cuts of d < 1.020, 1.020–1.063, 1.063–1.21, and >1.21 for VLDL (+IDL), LDL, HDL, and VHDL, respectively. After rats were killed, aliquots of liver were removed and lipids extracted according to the method of Folch *et al.* [19]. Following concentration of the extract, cholesterol and cholesterol esters were separated on silica columns (Sep-Pak cartridges) using sequential solvent elution [24]. All lipid and protein analyses were carried out by conventional enzymatic assays.

ApoB Secretion by HepG2 Cells

HepG2 cells were cultured in DMEM supplemented with 10% fetal bovine serum in 96-well plates (2×10^4 cells, 200 μL medium/well) for 3 days until approximately 75% confluent. The medium was replaced with DMEM containing 1% bovine serum albumin (BSA) plus RP 73163 in DMSO (or vehicle alone) to produce a final solvent concentration of 0.15% v/v. At different time intervals over 18 hr, the medium was removed and stored at 4°C until analysis. FAST lids, precoated with polyclonal mouse anti-human apoB and blocked with BSA, were shaken with the cell supernatants or standard apoB 100 solutions in phosphate buffered saline, pH 7.2, containing 3% BSA for 2 hr at 4°C. Following three washes with 0.5% Tween 20 in phosphate buffered saline, the lids were incubated with anti-mouse IgG conjugated with alkaline phosphatase for 2 hr at 4°C and again washed three times with buffer. The lids were shaken with *p*-nitrophenyl phosphate (2 g · mL^{-1} in 1 M diethanolamine buffer, pH 9.8, containing 0.5 mmol · L^{-1} $MgCl_2$) for 30 min at room temperature. The reaction was stopped by the addition of 100 μL of 0.3 M NaOH and absorbance read at 405 nm. The cells in each well were washed twice with 200 μL phosphate buffered saline and dissolved in 100 μL of 0.5% SDS. Total cell protein was determined [20] and apoB accumulation in the medium was measured as μg/mg total cell protein.

VLDL Secretion In Vivo

The effect of RP 73163 on the rate of VLDL secretion was determined by measuring the accumulation of triglyceride in the plasma lipoprotein fraction (d < 1.006) following intravenous administration of Triton WR-1339 (Tyloxapol) to rats [25]. RP 73163 (50 mg · kg^{-1} *b.i.d.*) was administered by gavage for 7 days. Thirty minutes after the final dose, 400 mg · kg^{-1} Triton WR-1339, as a 10% solution in 0.9% NaCl, was administered by tail-vein injection under light ether anaesthesia. After another 90 min, the rats were anaesthetised with sodium pentobarbitone, and blood was sampled from the abdominal aorta. VLDL was separated and analysed as described above.

Incorporation of [^{3}H]leucine Into VLDL ApoB

RP 73163 was administered to rats at a dose level of 50 mg · kg^{-1} *b.i.d.* for 7 days. Thirty minutes after the final dose of compound, 2 mL · kg^{-1} of an aqueous solution of [^{3}H]leucine (50 μCi, 0.75 μmol · kg^{-1}) was administered by the intraperitoneal route. One hour later, the animals were anaesthetised with isoflurane, and blood samples collected from the abdominal aorta. After separation of VLDL as described above, apoB was selectively precipitated [26] by mixing equal volumes of VLDL sample and isopropanol.

The samples were incubated overnight at 4°C and centrifuged at 2000*g* for 15 min. The supernatant was discarded carefully and the pellet washed with ether:ethanol 2:1 v/v to remove lipid. The pellet was dissolved in 500 μL of Lumasolve, and the radioactivity present determined by liquid scintillation counting.

Statistical Analysis

Statistical analyses were performed using one-way analysis of variance followed by Student's *t*-test to determine degree of significance.

RESULTS

In Vitro Potency and Selectivity

In hepatic microsomes prepared from cholesterol-fed rats, ACAT activity was of the order of 1 nmol · mg protein^{-1} · min^{-1} and was not increased appreciably by the addition of exogenous cholesterol to the incubation, suggesting that the membranes were saturated with cholesterol. In this assay system, RP 73163 had an IC_{50} value of 86 nM (Table 1). For comparison, the IC_{50} values of the standard ACAT inhibitors CI-976 and CL 277,082 were 430 nM and 710 nM, respectively, which are in reasonable agreement with the original published values of 180 nM and 740 nM for these two inhibitors [14, 27]. RP 73163 had similar inhibitory potency against ACAT preparations obtained from tissues of cholesterol-fed hamsters and rabbits (Table 1).

To determine the potency of RP 73163 against human ACAT, HepG2, Caco2, and THP-1 cell lines were used as sources of the enzyme. These cells were loaded with cholesterol to maximise enzyme activity. RP 73163 displayed similar potency in inhibiting ACAT activity in these three cell lines and in microsomes prepared from them with IC_{50} values in the range of 108–314 nM (Table 1).

RP 73163 was examined for its ability to inhibit two other enzymes that esterify cholesterol. At 100 μM (>1000-fold the IC_{50} concentration required to inhibit hepatic ACAT), RP 73163 had no effect on either PCEH or LCAT activity using rat pancreas and rat plasma as enzyme sources, respectively. Using bovine PCEH, cholesterol esterification was not reduced by RP 73163 at a concentration of 200 μM.

TABLE 1. Inhibitory potency of RP 73163 against ACAT activity in microsomal and whole cell preparations

Tissue	IC_{50} (nM)	
	Microsome	Whole cell
Rat liver	86	—
Hamster liver	88	—
Rabbit liver	122	—
Rabbit intestine	370	—
Rabbit artery	245	—
HepG2	108	266
Caco2	170	158
THP-1	164	314

ACAT activity was determined by measuring the incorporation of [^{14}C]oleoyl-CoA into cholesterol esters. In microsomal preparations, IC_{50} values were determined by using five concentrations of RP 73163 in triplicate. In whole cell assays, four concentrations of drug in quadruplicate were used.

Systemic Bioavailability

Systemic bioavailability of RP 73163 was determined by using a bioassay that provided rapidly information on the level of ACAT inhibitory activity present in the plasma following acute oral administration of the compound. Fresh plasma samples from treated rats were incubated with hepatic microsomes and the degree of ACAT inhibition measured. Using a standard curve prepared by adding known amounts of RP 73163 and plasma from untreated animals to enzyme assays, an estimate of systemic bioavailability was obtained that was expressed as equivalent concentration of RP 73163. Maximal ACAT inhibitory activity was present in plasma approximately 1 hr after acute oral administration of RP 73163. Increasing the dose level of RP 73163 from 3 to 100 mg · kg^{-1} resulted in a dose-related increase in ACAT inhibitory activity present in the plasma (Table 2).

Hypolipidaemic Response in Rats

In rats fed a basal diet *ad libitum*, the administration of RP 73163 (50 mg · kg^{-1} *b.i.d.* for 7 days) did not affect plasma levels of free or esterified cholesterol but it reduced significantly the mean triglyceride concentration from 1.03 to 0.53 mmol · L^{-1} (Fig. 1). This study also contained groups of rats that had been fasted for 18 hr prior to blood sampling. Fasting *per se* lowered significantly the plasma concentrations of free and esterified cholesterol and triglyceride. As in fed animals, RP 73163 lowered triglyceride but not cholesterol levels.

Lipoprotein analysis of the plasma samples showed that overnight fasting reduced significantly the total mass of lipoprotein in the $d < 1.006$ fraction by 44% from 122 to 82 mg · dL^{-1} (Table 3). Because the concentration of chylo-

TABLE 2. Effect of dose on systemic bioavailability of RP 73163

RP 73163 (mg · kg^{-1})	ACAT inhibitory activity in plasma equivalent to RP 73163 (μM)
3	n.d.
10	4.0 ± 0.8
30	21.7 ± 2.4
50	29.1 ± 1.9
100	82.0 ± 1.8

Blood samples were obtained 60 min after the acute oral administration of RP 73163. Plasma was assayed for ACAT inhibitory activity, which is expressed as the concentration of RP 73163 required to produce the same degree of inhibition. Results are mean ± SEM (n = 4/group). n.d. = not detected.

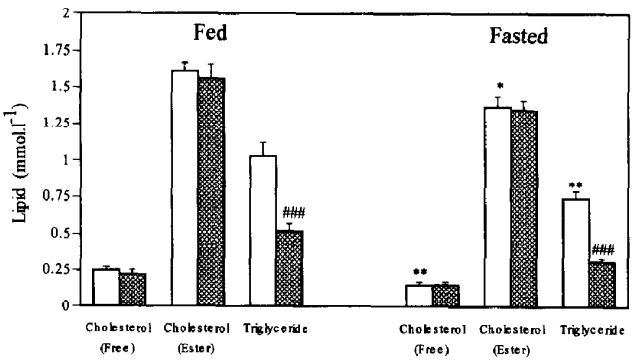

FIG. 1. The effects of RP 73163 (50 mg · kg^{-1} *b.i.d.* for 7 days, shaded bars) or vehicle (open bars) on the concentrations of plasma lipids in rats fed a basal diet. Bars represent mean + SEM (n = 10/group). Using one-way analysis of variance *$P < 0.05$ and **$P < 0.01$ for control fasted animals were compared with control fed animals and $^{\#\#\#}P < 0.001$ for RP 73163-treated animals were compared with corresponding fed or fasted controls.

microns and their remnants in the plasma of basal fed rats have been reported to be quantitatively unimportant [28], we have referred to this lipoprotein fraction as VLDL in both fed and fasted animals. The concentrations of all components of the VLDL fraction, with the exception of that of cholesterol ester, were reduced by fasting. Irrespective of the nutritional state of the rats at the time of blood sampling, RP 73163 treatment reduced the concentrations of all components of VLDL, with the greatest effect (in terms of percentage reduction) being on cholesterol ester.

Fasting reduced the concentration of plasma LDL from 56.8 to 35.9 mg · dL^{-1} without affecting the concentration of HDL (Table 3). RP 73163 treatment reduced the mass of LDL in fed and fasted rats by 34% and 29%, respectively, but the inhibitor had no significant effect on the mass or composition of HDL.

Using these treatment conditions, RP 73163 lowered significantly ACAT activity by 55% and 43% in hepatic microsomes prepared from fed and fasted animals, respectively (Fig. 2). Fasting *per se* increased hepatic ACAT activity by 83%.

Effect on ApoB Secretion in Vitro

The effects of RP 73163 on the secretion of apoB by HepG2 cells were examined over an 18-hr time period. The inhibitor, in the concentration range of 0.03 to 1.00 μM, produced a concentration-related decrease in the accumulation of apoB in the medium (Fig. 3). At higher concentrations of the drug, a significant reduction in apoB output was detected initially between 4 and 6 hr, and this decreased rate of accumulation continued for the remainder of the study.

Effect of VLDL Secretion In Vivo

As a measure of VLDL secretion *in vivo*, the accumulation of lipoprotein and triglyceride in the d < 1.006 fraction of plasma was determined over a 90-min period following the intravenous administration of Triton WR-1339. The administration of Triton WR-1339 alone resulted in an approximate six-fold increase in the total lipoprotein mass present in this density fraction. RP 73163 (50 mg · kg^{-1} *b.i.d.* for 7 days) reduced VLDL mass by 29% in rats that had been fasted overnight prior to Triton administration

TABLE 3. Effects of RP 73163 on mass and composition of plasma lipoproteins in fed and fasted rats

		Lipoprotein component (mg · dL^{-1})					
Lipoprotein fraction	**Treatment**	**Free cholesterol**	**Cholesterol ester**	**Triglyceride**	**Phospholipid**	**Protein**	**Total mass**
VLDL	Fed						
	Vehicle	3.4 ± 0.4	5.8 ± 0.6	84.5 ± 10.2	15.1 ± 1.9	13.4 ± 1.4	122.2 ± 14.3
	RP 73163	2.1 ± 0.3**	2.5 ± 0.3***	47.5 ± 3.6**	7.8 ± 1.0**	8.6 ± 0.9**	68.5 ± 5.3**
	Fasted						
	Vehicle	2.2 ± 0.1‡	7.1 ± 0.6	53.5 ± 4.1§	9.5 ± 0.9‡	10.0 ± 0.6†	82.3 ± 5.3‡
	RP 73163	0.8 ± 0.1***	1.0 ± 0.1***	18.6 ± 2.7***	1.8 ± 0.5***	6.3 ± 0.7**	28.5 ± 3.4***
LDL	Fed						
	Vehicle	2.2 ± 0.2	20.3 ± 1.3	9.0 ± 0.7	9.5 ± 0.8	15.8 ± 1.1	56.8 ± 3.8
	RP 73163	1.8 ± 0.2	13.7 ± 1.4**	4.9 ± 0.5***	6.2 ± 0.7**	11.0 ± 0.8**	37.6 ± 3.4**
	Fasted						
	Vehicle	1.0 ± 0.2§	11.6 ± 1.1§	7.6 ± 0.8	4.5 ± 0.5§	11.2 ± 0.9‡	35.9 ± 3.1§
	RP 73163	0.8 ± 0.2	7.2 ± 1.1**	4.6 ± 0.3**	3.3 ± 0.5	9.6 ± 0.6	25.5 ± 2.4*
HDL	Fed						
	Vehicle	2.6 ± 0.3	61.0 ± 4.7	1.8 ± 0.2	37.7 ± 3.6	70.0 ± 4.7	173.1 ± 12.3
	RP 73163	3.2 ± 0.4	72.7 ± 5.2	2.0 ± 0.2	35.6 ± 3.0	73.9 ± 4.5	187.4 ± 12.9
	Fasted						
	Vehicle	2.2 ± 0.2	61.8 ± 3.0	1.9 ± 0.2	32.9 ± 1.3	63.4 ± 2.2	162.2 ± 6.7
	RP 73163	2.0 ± 0.4	68.4 ± 4.8	1.3 ± 0.2	33.8 ± 2.3	64.7 ± 3.5	170.2 ± 10.7

RP 73163 (or vehicle) was administered at a dose level of 50 mg · kg^{-1} *b.i.d.* for 7 days. Results are expressed as mean ± SEM (n = 10/group). One-way analysis of variance: †$P < 0.05$, ‡$P < 0.01$, and §$P < 0.001$ compares control fed and control fasted animals; *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ for RP 73163-treated animals versus the appropriately fed or fasted control group.

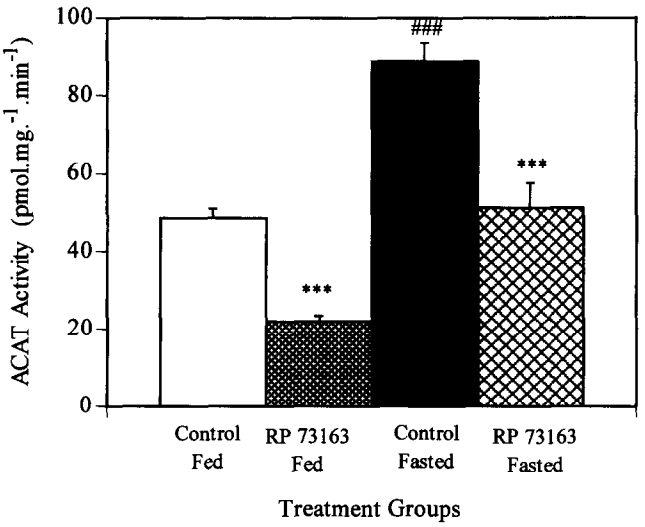

FIG. 2. Hepatic ACAT activity in fed and fasted rats treated with RP 73163 (50 mg · kg^{-1} *b.i.d.* for 7 days). Bars represent mean + SEM (n = 10/group). Using one-way analysis of variance, ***$P < 0.001$ for RP 73163 groups were compared with appropriate control animals and $^{\#\#\#}P < 0.001$ for control fasted compared with control fed animals.

and blood sampling (Table 4). The concentrations of all components of the lipoprotein fraction were decreased significantly, with the greatest effect, in percentage terms, being the reduction in cholesterol ester. Assuming a plasma volume of 4.1% [25] and using body weights of the rats prior to fasting, RP 73163 reduced the VLDL-triglyceride secretion rate from 11.3 to 8.6 mg · 100 g body wt^{-1} · hr^{-1}, a 24% reduction. In rats fed *ad libitum*, however, RP 73163 had no effect on the accumulation or composition of VLDL apart from reducing the cholesterol ester content by 52%. Fasting *per se* did not affect total VLDL secretion, but it did increase specifically the amount of cholesterol ester carried by the lipoprotein.

As a further measure of VLDL metabolism, the incorporation *in vivo* of radiolabel into VLDL-apoB over 60 min following the intraperitoneal administration of [^{3}H]leucine was determined. RP 73163 reduced significantly [^{3}H]levels in VLDL-apoB in fed and fasted animals by 33% and 64%, respectively (Fig. 4). Fasting alone had no significant effect on accumulation of [^{3}H] into VLDL.

Hypocholesterolaemic Activity in Casein-Fed Rabbits

Feeding rabbits a diet that contained 10% casein for 1 week followed by a 40% casein diet for another 2 weeks resulted in a 72% increase in the mean plasma cholesterol concentration from 1.23 ± 0.06 to 2.21 ± 0.18 mmol · L^{-1} (mean ± SEM, n = 27, $P < 0.001$ using paired *t*-test). Cholesterol levels of untreated animals remaining on the casein diet for another 4 weeks stayed relatively constant throughout, whereas those of animals that were returned to the standard diet fell to typical normocholesterolaemic levels within 7 days (Fig. 5). In casein-fed rabbits treated with RP 73163 (50 mg · kg^{-1} *b.i.d.*), plasma cholesterol levels fell slowly and were significantly lower than those of casein-fed controls and within the normal basal range after 3–4 weeks of treatment. Lipoprotein analyses carried out on the plasma samples obtained on the final day of the study showed that the casein-induced hypercholesterolaemia was associated with a selective increase in the concentration of cholesterol carried by LDL (Table 5). The LDL particles of casein-fed rabbits were relatively rich in cholesterol ester and poor in triglyceride (Table 6). RP 73163 reversed these changes.

The livers of rabbits that had been fed casein throughout the study contained a significantly higher concentration of cholesterol ester as compared with those of rabbits that had been fed the standard diet for the final 4 weeks (0.81 ± 0.16 vs. 0.22 ± 0.04 mg · g^{-1}, $P < 0.01$). The increase in hepatic-free cholesterol produced by the casein diet just failed to reach significance (1.90 ± 0.21 vs. 1.35 ± 0.15 mg · g^{-1}, $P = 0.055$). RP 73173 treatment reduced free and esterified cholesterol levels in the livers of casein-fed rabbits to basal values.

DISCUSSION

A systemically bioavailable ACAT inhibitor may be of value in the treatment of atherosclerosis by reducing the secretion of atherogenic lipoproteins from hepatic and intestinal tissues and also by directly reducing the accumulation of cholesterol ester in foam cells of the arterial wall [11]. The studies described in this report have shown RP 73163 to be a potent inhibitor of ACAT in microsomes prepared from hepatic, intestinal, and arterial tissues of experimental animals and equivalent human cell lines. RP 73163 possesses similar inhibitory activity against ACAT in whole cell preparations. Bioassays have demonstrated that, following the oral administration of RP 73163, a bio-

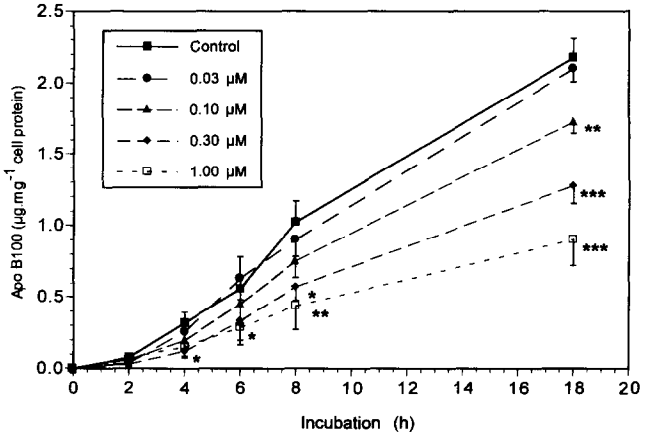

FIG. 3. RP 73163 reduces the accumulation of apoB in the medium of HepG2 cells. Cells were incubated in DMEM in the presence of 0–1.0 µM RP 73163. At different time intervals, the medium was removed and assayed for apoB. Each point represents mean ± SEM of five replicates. Using one-way analysis of variance, *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ for cells incubated with RP73163 compared with cells exposed to vehicle alone.

TABLE 4. Effects of RP 73163 on mass and composition of VLDL in rats following Triton WR-1339 treatment

	Concentration ($mg \cdot dL^{-1}$)			
	Fed		Fasted	
VLDL component	Vehicle control	RP 73163 treated	Vehicle control	RP 73163 treated
Free cholesterol	32.3 ± 1.9	35.5 ± 1.1	31.5 ± 0.5	25.6 ± 1.2**
Cholesterol ester	23.0 ± 2.1	11.0 ± 1.0**	40.0 ± 2.2***	12.7 ± 0.6**
Triglyceride	459 ± 35	473 ± 20	444 ± 16	321 ± 19**
Phospholipid	96.7 ± 6.4	98.2 ± 4.2	93.2 ± 3.6	69.9 ± 4.2**
Protein	21.3 ± 0.9	21.1 ± 0.8	21.5 ± 1.1	15.8 ± 1.1*
Lipoprotein (mass)	632 ± 44	639 ± 25	630 ± 20	445 ± 24**

RP 73163 (or vehicle) was administered at a dose level of 50 $mg \cdot kg^{-1}$ *b.i.d.* for 7 days. Blood samples were obtained 90 min after Triton WR-1339 administration (400 $mg \cdot kg^{-1}$ i.v.). Results are expressed as mean ± SEM (n = 9/group). Using one-way analysis of variance, $^*P < 0.01$ and $^{**}P < 0.001$ for treated groups versus appropriate control values and $^{***}P < 0.001$ for control fasted versus fed values.

logically active material is present in the plasma. At doses used to examine hypolipidaemic activity, peak plasma levels of "active inhibitor" are up to three orders of magnitude greater than the IC_{50} values of RP 73163 for inhibiting cellular ACAT in tissue culture assays and are similar to those quoted for parent compound when measured by a specific HPLC assay. For instance, after acute administration of RP 73163 at a dose level of 30 $mg \cdot kg^{-1}$ to rats, addition of plasma obtained 60 min later to the bioassay resulted in ACAT inhibition equivalent to that produced by plasma from untreated rats spiked with RP 73163 at a concentration of 22 μM. In a separate study that used identical conditions of animal experimentation, HPLC analysis detected a plasma concentration of 18 μM RP 73163 plus a small amount of the biologically active sulphone metabolite that was not quantified [17].

In common with other systemically bioavailable ACAT inhibitors [14, 29, 30], the oral administration of RP 73163 to rats fed a basal diet produces a reduction in plasma triglyceride levels. This hypotriglyceridaemic action of RP 73163 is associated with a reduction in the plasma concentration of VLDL and is consistent with the hypothesis that ACAT activity and cholesterol ester levels in the liver are involved in the regulation of VLDL secretion. This suggestion is supported by the results of *in vitro* studies using HepG2 cells. In experiments described here, RP 73163 reduced significantly the accumulation of apoB in the medium of HepG2 cells. Cianflone *et al.* demonstrated that oleate-induced secretion of apoB is closely linked to the synthesis rate of cholesterol ester but not to that of triglyceride [9, 31]. The addition of 25-hydroxycholesterol to HepG2 cells has also been shown to increase apoB synthesis and secretion [32]. These increases were positively correlated with the concentrations of cellular and secreted cholesterol esters but not with the mass of triglyceride in the cells. Furthermore, the rates of secretion of apoB and VLDL lipids by perfused rat livers are increased by the addition of cholesterol to the diet of the donor animals and reduced by the addition of lovastatin [33, 34]. The rate of secretion of VLDL was correlated with the concentration of cholesterol ester in the liver [33]. As the incubation medium used in our studies was not supplemented with fatty acid, it is clear that ACAT inhibition can reduce apoB secretion in basal as well as oleate-stimulated HepG2 cells.

The effects of RP 73163 on VLDL metabolism *in vivo* is dependent upon the nutritional state of the animal. In rats that have been fasted overnight, RP 73163 clearly reduces VLDL secretion as measured by lipoprotein accumulation following Triton WR-1339 administration. However, in rats that have been fed *ad libitum*, RP 73163 reduces specifically the cholesterol ester content of VLDL but has no effect on the mass of lipoprotein secreted. Fasting *per se*

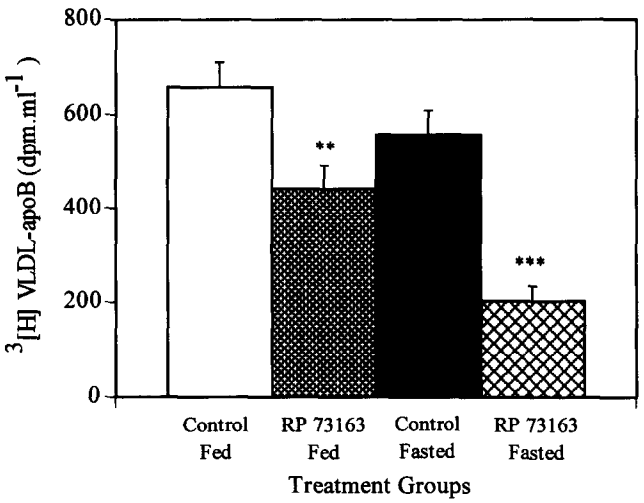

FIG. 4. The effects of RP 73163 (50 $mg \cdot kg^{-1}$ *b.i.d.* for 7 days) on the incorporation of [^{3}H]leucine into VLDL-apoB in fed and fasted rats. Blood samples were obtained 1 hr after the intraperitoneal injections of [^{3}H]leucine (50 μCi, 75 $\mu mol \cdot kg^{-1}$). VLDL was isolated by ultracentrifugation and apoB selectively precipitated with isopropanol. Bars represent mean ± SEM (n = 10/group). Using one-way analysis of variance, $^{**}P < 0.01$ and $^{***}P < 0.001$ for RP 73163-treated groups were compared with appropriate controls.

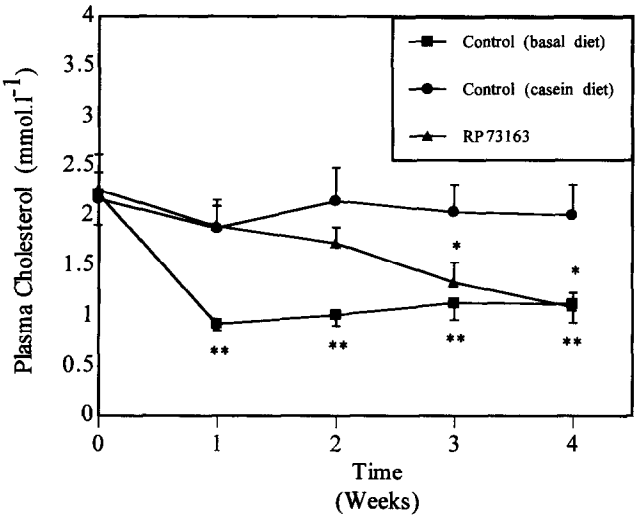

FIG. 5. Reduction of plasma cholesterol levels in casein-fed rabbits by RP 73163. Rabbits were rendered hypercholesterolaemic by feeding a casein-supplemented diet for 21 days. One group was then returned to basal diet, one group received RP 73163 (50 mg · kg^{-1} *b.i.d.*), and one group remained on the casein diet receiving vehicle (0.5% tragacanth mucilage). Each point represents mean ± SEM (n = 9/group). Using one-way analysis of Variance, *$P < 0.05$ and **$P < 0.01$ for cholesterol levels when compared with untreated casein-fed rabbits.

reduces the plasma concentration of VLDL, which becomes relatively rich in cholesterol ester. These latter findings are compatible with those of Davis et al. [35] who showed that hepatocytes from fasted rats have significantly lower concentrations of free cholesterol and triglyceride but more cholesterol ester than hepatocytes from fed animals. In addition, it is known, and confirmed in our studies, that hepatic ACAT activity is increased by fasting [36]. Whether this is due to an increase in mass of the enzyme, an increase in activity, or to an increase in substrate availability is unclear. The studies of Davis *et al.* also demonstrated that fasting reduces the subsequent rates of lipogenesis, apoB synthesis, and VLDL secretion by hepatic tissue *in vitro* [35]. However, in the studies reported here using *in vivo* conditions, fasting did not affect VLDL secretion, implying that an increase in the rate of clearance of VLDL from the blood is important in the hypotriglyceridaemic effect of fasting. This conclusion is compatible with results of studies carried out with perfused livers, which have shown that livers from fasted rats remove triglyceride-rich particles from the medium at a faster rate than livers from fed animals [37]. More importantly, our results also show that, under these conditions in which the availability of triglyceride is limited, ACAT plays an important role in maintaining the rate of VLDL secretion. Because we and others [9] have used HepG2 cells to study the link between ACAT activity and VLDL secretion *in vitro*, it is of interest to note that these cells resemble hepatocytes from fasted rather than fed animals in that they have a low basal rate of triglyceride synthesis [38].

Although RP 73163 reduced plasma triglyceride levels in rats fed *ad libitum*, this action was not associated with a reduction in the rate of VLDL secretion. In complimentary studies, RP 73163 reduced the incorporation of [^{3}H]leucine into plasma VLDL-apoB in both fed and fasted rats. The incorporation of radiolabel reflects the secretion of newly synthesised apoprotein into the plasma pool and its subsequent clearance. Because RP 73163 lowers VLDL levels in fed animals without affecting secretion, the results suggest that the compound increases VLDL clearance under these conditions. In fasted rats, RP 73163 may increase VLDL clearance and reduce secretion. The mechanism by which RP 73163 exerts this effect is unclear. The most marked effect of RP 73163 on VLDL in the fed rat is a reduction in cholesterol ester content. It has been suggested that the concentration and composition of core lipids of VLDL can modify apolipoprotein orientation and hence alter receptor affinity [39]. It is therefore possible to speculate that a reduction in the cholesterol ester content of VLDL might increase the rate of hepatic uptake. Similarly, the composition of VLDL may also affect the activity of lipoprotein lipase. It has previously been suggested that the reduced levels of VLDL observed in cholesterol-fed rabbits treated with the ACAT inhibitor CL 277,082 are due in part to enhanced catabolism of VLDL to LDL [40]. However, the observation that RP 73163 treatment reduces LDL and VLDL levels in fed animals in which there is no change in the rate of VLDL secretion would favour an increase in receptor uptake rather than an increase in catabolism to LDL as the more likely explanation of the increased VLDL

TABLE 5. Effects of RP 73163 on the concentrations of cholesterol in plasma lipoprotein fractions of casein-fed rabbits

	Lipoprotein cholesterol (mmol · L^{-1} plasma)		
Lipoprotein fraction	**Standard diet**	**Casein diet**	**Casein + RP 73163**
VLDL	0.17 ± 0.08	0.14 ± 0.07	0.08 ± 0.03
LDL	0.49 ± 0.04*	1.32 ± 0.06	0.43 ± 0.04*
HDL	0.30 ± 0.03	0.37 ± 0.03	0.39 ± 0.03
VHDL	0.11 ± 0.02	0.17 ± 0.03	0.10 ± 0.03

Rabbits were treated according to the description in the caption of Fig. 5. Results are expressed as mean ± SEM (n = 9/group). One-way analysis of variance: *$P < 0.01$ compared with untreated casein-fed rabbits.

TABLE 6. Effects of RP 73163 on LDL mass and composition in casein-fed rabbits

LDL components	Concentration ($mg \cdot dL^{-1}$)		
	Standard diet	Casein diet	Casein + RP 73163
Free cholesterol	6.0 ± 1.8*	13.4 ± 2.5	5.5 ± 1.4*
Cholesterol ester	21.6 ± 6.7**	63.6 ± 12.1	19.1 ± 5.4**
Triglyceride	17.6 ± 5.0	8.8 ± 1.3	12.8 ± 2.0
Phospholipid	13.3 ± 4.2	21.7 ± 3.7	10.0 ± 2.1*
Protein	22.0 ± 4.5	37.0 ± 5.9	21.8 ± 3.2*
Lipoprotein (mass)	80.5 ± 21.7*	144.5 ± 23.1	69.2 ± 13.8**

Rabbits were treated according to the description in the caption to Fig. 5. Results are expressed as mean ± SEM (n = 9/group). One-way analysis of variance: *$P < 0.05$ and **$P < 0.01$ compared with untreated casein-fed rabbits.

clearance. Our data do not support the suggestion that ACAT inhibition will decrease the fractional turnover rate of VLDL by downregulating hepatic receptors subsequent to the accumulation of free cholesterol within the cell [11].

The hypolipidaemic actions of RP 73163 in casein-fed rabbits are also consistent with the suggestion that ACAT has a role in the control of hepatic lipoprotein secretion. Rabbits fed a casein-supplemented diet develop a marked hypercholesterolaemia that is characterised by an increase in the plasma concentration of LDL [41] and an increase in the ratio of cholesterol ester:triglyceride in VLDL and LDL [42]. These changes are secondary to an increase in the secretion of cholesterol-ester-rich VLDL and LDL by the liver [42] and to a reduction in the receptor dependent hepatic clearance of these lipoproteins [43]. The fatty acid composition of cholesterol esters in VLDL and LDL of casein-fed rabbits are rich in oleate indicating that the esters are synthesised through the actions of ACAT [42]. The marked effect of RP 73163 on LDL levels observed in this study is consistent with the idea that ACAT inhibition can reduce hepatic secretion of LDL particles in the casein-fed rabbit and possibly increase their clearance from the circulation.

In summary, RP 73163 has been shown to be a relatively potent and systemically bioavailable inhibitor of ACAT. The data support the hypothesis that ACAT is important in regulating the hepatic secretion of apoB containing lipoproteins *in vivo* and *in vitro* but also suggests that ACAT may affect the clearance of these lipoproteins from the bloodstream.

References

1. Zemel PC and Sowers JR, Relation between lipids and atherosclerosis: epidemiologic evidence and clinical implications. *Am J Cardiol* **66:** 7I–12I, 1990.
2. Lipid Research Clinics Program, The Lipid Research Clinics Coronary Primary Prevention Trial Results II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. *JAMA* **251:** 365–374, 1984.
3. Mack WJ, Hodis HN and Blankenhorn DH, Regression of atherosclerosis: a report from two clinical trials. In: *Atherosclerosis Reviews* (Eds. Weber PC and Leaf A), Vol. 25, pp. 301–309. Raven Press, New York, 1993.
4. Kesaniemi YA and Miettinen TA, Cholesterol absorption efficiency regulates plasma cholesterol level in the Finnish population. *Eur J Clin Invest* **17:** 391–395, 1987.
5. Sniderman A, Brown BG, Stewart BF and Cianflone K, From familial combined hyperlipidemia to hyperapoB: unravelling the over production of hepatic apolipoprotein B. *Curr Opin Lipidol* **3:** 137–142, 1992.
6. Bennett Clark S and Tercyak AM, Reduced cholesterol transmucosal transport in rats with inhibited mucosal acyl CoA: cholesterol acyltransferase and normal pancreatic function. *J Lipid Res* **25:** 148–159, 1984.
7. Carr TP and Rudel LL, Partial inhibition of ACAT decreases apo B secretion by the liver of African green monkeys. *Arteriosclerosis* **10:** 823A, 1990.
8. Field FJ, Albright E and Mathur S, Inhibition of acyl CoA: cholesterol acyltransferase activity by PD 128042: effect on cholesterol metabolism and secretion in CaCo2 cells. *Lipids* **26:** 1–8, 1991.
9. Cianflone KM, Yasruel Z, Rodriguez MA, Vas D and Sniderman AD, Regulation of apoB secretion from HepG2 cells: evidence for a critical role of cholesteryl ester synthesis in the response to a fatty acid challenge. *J Lipid Res* **31:** 2045–2055, 1990.
10. Suckling KE and Stange EF, Role of acyl-CoA:cholesterol acyltransferase in cellular cholesterol metabolism. *J Lipid Res* **26:** 647–671, 1985.
11. Sliskovic DR and White AD, Therapeutic potential of ACAT inhibitors as lipid lowering and anti-atherosclerotic agents. *TiPS* **12:** 194–199, 1991.
12. Matsuda K, ACAT inhibitors as antiatherosclerotic agents: compounds and mechanisms. *Med Res Rev* **14:** 271–305, 1994.
13. Gallo LL, Bennett Clark S, Myers S and Vahouny GV, Cholesterol absorption in rat intestine: role of cholesterol esterase and acyl coenzyme A:cholesterol acyltransferase. *J Lipid Res* **25:** 604–612, 1984.
14. Krause BR, Anderson M, Bisgaier CL, Bocan T, Bousley R, DeHart P, Essenburg A, Hamelehle K, Homan R, Kieft K, McNally W, Stanfield R and Newton RS, In vivo evidence that the lipid-regulating activity of the ACAT inhibitor CI-976 in rats is due to inhibition of both intestinal and liver ACAT. *J Lipid Res* **34:** 279–294, 1993.
15. Krause BR, Pape ME, Kieft K, Auerbach B, Bisgaier CL, Homan R and Newton RS, ACAT inhibition decreases LDL cholesterol in rabbits fed a cholesterol-free diet: Marked changes in LDL cholesterol without changes in LDL receptor mRNA abundance. *Arterioscler Thromb* **14:** 598–604, 1994.
16. Harris NV, Smith C, Ashton MJ, Bridge AW, Bush RC, Coffee ECJ, Dron DI, Harper MF, Lythgoe DJ, Newton CG

and Riddell D, Acyl-CoA:cholesterol O-acyl transferase (ACAT) inhibitors. 1. 2-(alkylthio)-4,5-diphenyl-1H-imidazoles as potent inhibitors of ACAT. *J Med Chem* **35:** 4384–4392, 1992.
17. Jones GD, Hoff TA, Gunraj PE, Facchini V and Chauhan PB, Assay and single dose pharmacokinetics of a novel systemic acyl coenzyme A:cholesterol O-acyltransferase inhibitor, RP 73163, in rat plasma, using automated solid-phase extraction with high performance liquid chromatography. *J Chromatogr B* **661:** 119–131, 1994.
18. Bello AA, Bridge AW, Bright C, Burton BJ, Bush RC, Casey J, Dron DI, Fachinni V, Joannou PP, Parrott DP, Riddell D, Roberts S and Williams RJ, RP 64477: An inhibitor of acyl-coenzyme A:cholesterol O-acyltransferase with low systemic bioavailability. *Biochem Pharmacol* **51:** 413–421, 1996.
19. Folch J, Lees M and Sloan-Stanley GH, A simple method for the isolation and purification of total lipides from animal tissue. *J Biol Chem* **226:** 497–509, 1957.
20. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
21. Vahouny GV and Treadwell CR, Pancreatic sterol ester hydrolase. *Methods Enzymol* **15:** 537–543, 1969.
22. Stokke KT and Norum KR, Determination of lecithin:cholesterol acyltransferase in human blood plasma. *Scand J Clin Lab Invest* **27:** 21–27, 1971.
23. Havel RJ, Eder HA and Bragdon JH, The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J Clin Invest* **34:** 1345–1353, 1955.
24. Hamilton JG and Comai K, Rapid separation of neutral lipids, free fatty acids and polar lipids using prepacked silica Sep-Pak columns. *Lipids* **23:** 1146–1149, 1988.
25. Otway S and Robinson DS, The use of a non-ionic detergent (Triton WR 1339) to determine rates of triglyceride entry into the circulation of the rat under different physiological conditions. *J Physiol* **190:** 321–332, 1967.
26. Egusa G, Brady DW, Grundy SM and Howard BV, Isopropanol precipitation method for the determination of apolipoprotein B specific activity and plasma concentrations during metabolic studies of very low density lipoprotein and low density lipoprotein apolipoprotein B. *J Lipid Res* **24:** 1261–1267, 1983.
27. Largis EE, Wang CH, DeVries VG and Schaffer SA, CL 277,082: a novel inhibitor of ACAT-catalysed cholesterol esterification and cholesterol absorption. *J Lipid Res* **30:** 681–690, 1989.
28. Chapman MJ. Comparative analysis of mammalian lipoproteins. *Methods Enzymol* **128:** 70–143, 1986.
29. Krause BR, Black A, Bousley R, Essenburg A, Cornicelli J, Holmes A, Homan R, Kieft K, Sekerke C, Shaw-Hes MK, Stanfield R, Trivedi B and Woolf T, Divergent pharmacologic activities of PD 132301-2 and CL 277082 urea inhibitors of acyl-CoA: cholesterol acyltransferase. *J Pharm Exp Ther* **267:** 734–743, 1993.
30. Bell FP, Gammill RB and St. John LC, U-73482: A novel ACAT inhibitor that elevates HDL-cholesterol, lowers plasma triglyceride and facilitates hepatic cholesterol mobilization in the rat. *Atherosclerosis* **92:** 115–122, 1992.
31. Cianflone K, Dahan S, Monge JC and Sniderman AD, Pathogenesis of carbohydrate-induced hypertriglyceridemia using HepG2 cells as a model system. *Arterioscler Thromb* **12:** 271–277, 1992.
32. Dashti N. The effect of low density lipoproteins, cholesterol, and 25-hydroxycholesterol on apolipoprotein B gene expression in HepG2 cells. *J Biol Chem* **267:** 7160–7169, 1992.
33. Khan B, Wilcox HG and Heimberg M, Cholesterol is required for secretion of very-low-density lipoprotein by rat liver. *Biochem J* **259:** 807–816, 1989.
34. Fungwe TV, Cagen L, Wilcox HG and Heimberg M, Regulation of hepatic secretion of very low density lipoprotein by dietary cholesterol. *J Lipid Res* **33:** 179–191, 1992.
35. Davis RA, Boogaerts JR, Borchardt RA, Malone-McNeal M and Archambault-Schexnayder J, Intrahepatic assembly of very low density lipoproteins: varied synthetic response of individual apolipoproteins to fasting. *J Biol Chem* **260:** 14137–14144, 1985.
36. Erickson SK, Shrewsbury MA, Brooks C and Meyer DJ, Rat liver acyl-coenzyme A: cholesterol acyltransferase: its regulation in vivo and some of its properties in vitro. *J Lipid Res* **21:** 930–941, 1980.
37. Quarfordt SH, Hanks J, Shelburne F and Schirmer B, Differing uptake of emulsion triglyceride by the fed and fasted rat liver. *J Clin Invest* **69:** 1092–1098, 1981.
38. Dixon JL and Ginsberg HN, Hepatic synthesis of lipoproteins and apolipoproteins. *Sem Liver Dis* **12:** 364–372, 1992.
39. Sehayek E, Friedman G and Eisenberg S, An apo-E (arginine 158) specific catabolic site in Hep-G2 cells. In: *Atherosclerosis IX* (Eds. Stein O, Eisenberg S and Stein Y), pp. 157–159. R&L Creative Communications, Tel Aviv, 1992.
40. Kelley JL, Suenram CA, Rozek MM, Schaffer SA and Schwartz CJ, Influence of the acyl-CoA:cholesterol O-acyltransferase inhibitor, CL 277082, on cholesteryl ester accumulation in rabbit macrophage-rich granulomas and hepatic tissue. *Biochim Biophys Acta* **960:** 83–90, 1988.
41. Hrabek-Smith JM, Kurowska EM and Carroll KK, Effects of cholesterol-free, semipurified diets containing different levels of casein or soy protein on distribution of cholesterol and protein among serum lipoproteins of rabbits. *Atherosclerosis* **76:** 125–130, 1989.
42. Chao YS, Yamin TT, Seidenberg J and Kroon PA, Secretion of cholesteryl ester-rich lipoproteins by the perfused livers of rabbits fed a wheat-starch-casein diet. *Biochim Biophys Acta* **876:** 392–398, 1986.
43. Samman S, Khosla P and Carroll KK, Effects of dietary casein and soy protein on metabolism of radiolabelled low density apolipoprotein B in rabbits. *Lipids* **24:** 169–172, 1989.