CP-346086: an MTP inhibitor that lowers plasma cholesterol and triglycerides in experimental animals and in humans

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Abstract **A microsomal triglyceride transfer protein (MTP) inhibitor, CP-346086, was identified that inhibited both human and rodent MTP activity [concentration giving half-maximal inhibition (IC_{50}) 2.0 nM]. In Hep-G2 cells, CP-346086 inhibited apolipoprotein B (apoB) and triglyceride secretion (IC_{50} 2.6 nM) without affecting apoA-I secretion or lipid synthesis. When administered orally to rats or mice, CP-346086 lowered plasma triglycerides [dose giving 30% triglyceride lowering (ED_{30}) 1.3 mg/kg] 2 h after a single dose. Coadministration with Tyloxapol demonstrated that triglyceride lowering was due to inhibition of hepatic and intestinal triglyceride secretion. A 2 week treatment with CP-346086 lowered total, VLDL, and LDL cholesterol and triglycerides dose dependently with 23%, 33%, 75%, and 62% reductions at 10 mg/kg/day. In these animals, MTP inhibition resulted in increased liver and intestinal triglycerides when CP-346086 was administered with food. When dosed away from meals, however, only hepatic triglycerides were increased. When administered as a single oral dose to healthy human volunteers, CP-346086 reduced plasma triglycerides and VLDL cholesterol dose dependently with ED_{50}s of 10 mg and 3 mg, and maximal inhibition (100 mg) of 66% and 87% when measured 4 h after treatment. After a 2 week treatment (30 mg/day), CP-346086 reduced total and LDL cholesterol and triglycerides by 47%, 72%, and 75%, relative to either individual baselines or placebo, with little change in HDL cholesterol. Together, these data support further evaluation of CP-346086 in hyperlipidemia.**—Chandler, C. E., D. E. Wilder, J. L. Pettini, Y. E. Savoy, S. F. Petras, G. Chang, J. Vincent, and H. J. Harwood, Jr. **CP-346086: an MTP inhibitor that lowers plasma cholesterol and triglycerides in experimental animals and in humans.** ***J. Lipid Res.*** **2003.** 44: **1887–1901.**

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Cardiovascular disease remains the leading cause of death in industrialized nations and accounted for 950,000, or 41%, of all deaths in the United States in 1998 (1). As a consequence of atherosclerosis, coronary heart disease (CHD) is the most common cause of cardiovascular morbidity and mortality, with an estimated 12 million people suffering from CHD in the United States alone (1). Elevated total and LDL cholesterol are both accepted primary risk factors for atherosclerosis (1–3). An estimated 101 million United States adults have elevated blood cholesterol (>200 mg/dl) and are candidates for LDL cholesterol lowering through dietary intervention (1, 4, 5). Of these, 41 million are considered high risk, having blood cholesterol greater than 240 mg/dl, and drug therapy is recommended (1, 4, 5).

Epidemiological studies have shown that elevated triglycerides and reduced HDL cholesterol are also contributing factors for the development of CHD (2, 3, 6–8). Among the adult United States population, 19% of people have low HDL cholesterol (<40 mg/dl) (3, 9, 10) and 21% have hypertriglyceridemia (>150 mg/dl) (3, 10). Thus, as important as elevated LDL cholesterol is as a risk factor for CHD, it is important to recognize that the most common spectrum of lipid abnormalities is atherogenic dyslipidemia, which is present in 45–50% of men with CHD (11, 12) and includes borderline high-risk LDL cholesterol (e.g., 130–159 mg/dl), elevated triglycerides, small dense LDL particles, and low HDL cholesterol.

The HMG-CoA reductase inhibitors (statins) are very effective in lowering LDL cholesterol and somewhat effective in reducing triglycerides, but they have only minimal effects on HDL cholesterol (2, 5, 13–15). Indeed, although numerous clinical trials have demonstrated that LDL cholesterol reduction can significantly reduce CHD risk, a great number of treated subjects who achieve substantial LDL cholesterol reduction still experience a clinical event (2, 3, 13–18). Therefore, with the goal of develop-

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Abbreviations: CETP, cholesterol ester transfer protein; CHD, coronary heart disease; ER, endoplasmic reticulum; MTP, microsomal triglyceride transfer protein.

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ing a therapy for treating patients with dyslipidemias that extend beyond primary hypercholesterolemia, we targeted inhibition of microsomal triglyceride transfer protein (MTP) as a mechanism for preventing triglyceride-rich lipoprotein assembly in the liver and intestine.

MTP, which is located within the lumen of the endoplasmic reticulum (ER) in hepatocytes and absorptive enterocytes, is a heterodimeric protein consisting of a 97 kDa subunit, which confers all of the lipid transfer activity of the heterodimer, and the 58 kDa multifunctional protein disulfide isomerase (19). MTP plays a pivotal, if not obligatory role, in the assembly and secretion of triglyceride-rich, apolipoprotein B (apoB)-containing lipoproteins (VLDL and chylomicrons) from the liver and intestine and also catalyzes the transport of triglycerides, cholesteryl esters, and phospholipids between membranes (19–21). Although the exact role of MTP in the assembly of apoB-containing lipoproteins is still under investigation (21–23), MTP is proposed to transport lipids from the ER membrane to the growing apoB polypeptide chain in the ER lumen, thereby allowing proper translocation and folding of apoB to occur (19–24). MTP has also been proposed to mediate bulk triglyceride transfer into the ER lumen for incorporation into poorly lipidated apoB-containing lipoprotein particles during the process of VLDL and chylomicron assembly (25, 26). Recent studies have also suggested a role for MTP in the movement of cholesterol ester into the ER lumen for inclusion into nascent apoB-containing lipoprotein particles (27).

The initial suggestion that MTP inhibition could be a viable lipid-lowering therapy came with the discovery that functional MTP is absent in individuals with abetalipoproteinemia, a genetic disorder characterized by low plasma cholesterol and triglycerides due to a defect in the assembly and secretion of apoB-containing lipoproteins (28, 29). A similar phenotype is observed in MTP knockout mice (23, 30). Abetalipoproteinemia, however, represents an extreme example of MTP inhibition and is not without its clinical sequelae, all of which presumably are related directly or indirectly to fat malabsorption (steatorrhea), vitamin malabsorption, and hepatic and intestinal steatosis (29, 31). A less severe, and probably more relevant, example of the consequences of therapeutic MTP inhibition is a related genetic disease, hypobetalipoproteinemia, caused by mutations in apoB (32). Heterozygous individuals with this disease possess half of the normal levels of apoB-containing lipoproteins, lack the clinical signs and symptoms of abetalipoproteinemia, and have a normal lifespan (24).

We devised a two-stage empirical screening protocol for compound evaluation (33) to identify potent MTP inhibitors with the potential to inhibit hepatic and intestinal apoB-containing lipoprotein assembly and consequently lower plasma total, LDL, and VLDL cholesterol and triglycerides in experimental animals and in humans. In the first stage of the protocol, compounds were evaluated for their ability to inhibit apoB, but not apoA-I secretion, from Hep-G2 cells in a high-throughput, 96-well multiplexed format, essentially as described by Haghpassand, Wilder, and Moberly (34). In the second stage of the protocol, confirmed apoB secretion inhibitors were evaluated for their ability to inhibit the MTP-mediated transfer of radiolabeled triolein from synthetic phospholipid donor liposomes to acceptor liposomes (34). Using this two-stage screening protocol, we identified CP-94792, a potent inhibitor of apoB, but not apoA-I, secretion (33). Inhibition of apoB secretion was subsequently determined to be through inhibition of MTP activity (33, 35). However, although CP-94792 inhibited Hep-G2 cell apoB secretion with an half-maximal inhibition (IC_{50}) of 200 nM and inhibited MTP-mediated triglyceride transfer (rat MTP) with an IC_{50} of 250 nM, the compound displayed only weak triglyceride lowering activity when administered orally to rats (33).

The potent and orally efficacious MTP inhibitor, CP-346086 (4′-trifluoromethyl-biphenyl-2-carboxylic acid [2-(2H-[1,2,4]triazol-3-ylmethyl)-1,2,3,4-tetrahydro-isoquinolin-6-yl] amide); **Fig. 1**, inset), was ultimately identified (33, 35–37) by *1*) employing a robotics-assisted parallel synthesis strategy as a means of developing structure-activity relationships and improving in vitro potency, and *2*) using in vitro hepatic microsomal clearance and in vivo triglyceride lowering as guides for improving pharmacokinetic properties. In this report, we describe the biochemical

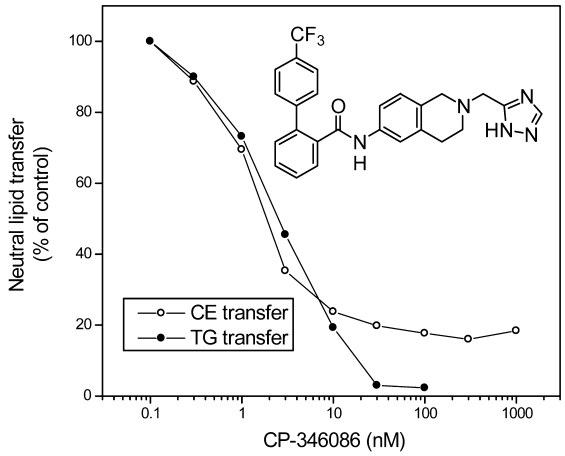

Fig. 1. Inhibition of human microsomal triglyceride transfer protein (MTP)-mediated neutral lipid transfer by CP-346086. Aliquots of solubilized human liver MTP, 150 μl, were incubated at 37°C for 45 min with 50 μl donor liposomes, 100 μl acceptor liposomes, and 200 μl assay buffer containing either 5% BSA (control) or 5% BSA plus sufficient CP-346086 to produce the indicated final concentrations of CP-346086, as described in Experimental Procedures. After incubation, triglyceride transfer was terminated by addition of 300 μl of a 50% (w/v) DEAE cellulose suspension in assay buffer. After a 4 min agitation, the donor liposomes, bound to DEAE cellulose, were selectively sedimented by low speed centrifugation (3,000 *g*, 5 min). An aliquot of the supernatant containing the acceptor liposomes was assessed for radioactivity as outlined in Experimental Procedures. Shown is the percentage of control [^{14}C]triolein or [^{14}C]cholesterol oleate transfer as a function of CP-346086 concentration. Control triolein and cholesterol oleate transfer during the 45 min assay averaged 42% and 13%, respectively. Inset: The structure of CP-346086 (4′-trifluoromethyl-biphenyl-2-carboxylic acid [2-(2H-[1,2,4]triazol-3-ylmethyl)-1,2,3,4-tetrahydro-isoquinolin-6-yl]amide).

mechanism of action of CP-346086 that leads to its LDL cholesterol-, VLDL cholesterol-, and triglyceride-lowering efficacy in experimental animals and in humans.

EXPERIMENTAL PROCEDURES

Materials

Sodium [2-^{14}C]acetate (56 mCi/mmol), [^{14}C]triolein (110 mCi/mmol), cholesteryl [1-^{14}C]oleate (55 mCi/mmol), [^{3}H]triolein (25 Ci/mmol), [^{3}H]egg phosphatidylcholine (50 mCi/mmol), and Aquasol-2 were from New England Nuclear (Boston, MA). [^{3}H]glycerol (20 Ci/mmol) was from American Radiochemicals (St. Louis, MO). Ready Safe™ liquid scintillation cocktail was from Beckman Instruments (Fullerton, CA). Dulbecco's modified Eagle's medium (DMEM), L-glutamine, and gentamicin were from GIBCO Laboratories (Grand Island, NY). Heat-inactivated fetal bovine serum was from HyClone Laboratories (Logan, UT). DEAE cellulose was from Whatman International (Maidstone, England). Silica gel 60C TLC plates were from Eastman Kodak (Rochester, NY). BCA protein assay reagent was from Pierce (Rockford, IL). Cholesterol/HP reagent (Cat. 1127578), TG/GPO reagent (Cat. 1128027), Preciset Cholesterol Calibrator Set (Cat. 125512), and Precitrol-N serum (Cat. 620212) were from Boehringer Mannheim (Indianapolis, IN). Cholesterol CII reagent kit (Cat. 276-64909), Triglyceride E reagent kit (Cat. 432-40201), Standard Cholesterol CII Solution (Cat. 277-64939), and Standard Triglyceride G Solution (Cat. 998-69831) were from Waco Chemicals USA (Richmond, VA). Hep-G2 cells were from the American Type Culture Collection (Rockville, MD). Mouse anti-human apoB monoclonal antibodies (MoAB-012), goat anti-human apoB polyclonal antibodies (AB-742), mouse anti-human apoA-I monoclonal antibodies (MAB-011), goat anti-human apoA-I polyclonal antibodies (AB-740), and human apoA-I purified standard (ALP10) were from Chemicon (Temecula, CA). B6CBAF1J mice were from Jackson Laboratory (Bar Harbor, ME). Transgenic huA1/CIII/cholesteryl ester transfer protein (CETP) mice, originally obtained from Charles River (Boston, MA), were bred on site. Sprague Dawley rats were from Charles River. RMH 3200 laboratory meal was from Agway, Inc. (Syracuse, NY). AIN76A semipurified diet was from US Biochemicals (Cleveland, OH). F0739 liquid diet powder was from Bio-Serve, Inc. (Frenchtown, NJ). Fast protein liquid chromatography (FPLC) instrumentation was from Gilson, Inc. (Middletown, WI). Superose-6 gel filtration columns were from Pharmacia (Piscataway, NJ). Postcolumn reaction (PCX) instrumentation was from Pickering Laboratories (Mountain View, CA). All other chemicals and reagents were from previously listed sources (38, 39).

Preparation of human MTP

The procedure for isolating and solubilizing human MTP from frozen hepatic tissue is based on the method of Wetterau and Zilversmit (40) and was conducted essentially as described by Haghpassand, Wilder, and Moberly (34). Solubilized human liver MTP was stored at 4°C and was diluted 1:5 with assay buffer just before use. MTP preparations showed no notable loss of transfer activity with storage up to 30 days. Rat and mouse MTP were prepared similarly, except that freshly isolated liver tissue was used.

Preparation of phospholipid vesicles for use in lipid transfer assays

Donor and acceptor liposomes were prepared essentially as described by Wetterau et al. (28). Donor liposomes were prepared under nitrogen by room temperature bath sonication of a dispersion containing 447 μM egg phosphatidylcholine, 83 μM bovine heart cardiolipin, and 0.91 μM [^{14}C]triolein (110 Ci/mol). Acceptor liposomes were prepared under nitrogen by room temperature bath sonication of a dispersion containing 1.3 mM egg phosphatidylcholine, 2.6 μM triolein, and 0.5 nM [^{3}H]egg phosphatidylcholine in assay buffer. The donor and acceptor liposomes were centrifuged at 160,000 *g* for 2 h at 7°C. The upper 80% of the supernatant, containing small unilamellar liposomes, was carefully removed and stored at 4°C until use.

Measurement of MTP inhibitory activity

MTP inhibitory activity as outlined by Haghpassand, Wilder, and Moberly (34) was determined by adding 200 μl of a buffer containing either 5% BSA (control) or 5% BSA plus CP-346086 to a mixture containing 50 μl donor liposomes, 100 μl acceptor liposomes, and 150 μl of solubilized, dialyzed MTP protein diluted in assay buffer as outlined above. After incubation at 37°C for 45 min, triglyceride transfer was terminated by addition of 300 μl of a 50% (w/v) DEAE cellulose suspension in assay buffer. After 4 min of agitation, the donor liposomes, bound to DEAE cellulose, were selectively sedimented by low speed centrifugation (3,000 *g*; 5 min). An aliquot of the supernatant containing the acceptor liposomes was assessed for radioactivity, and the ^{14}C and ^{3}H counts obtained were used to calculate the percent recovery of acceptor liposomes and the percent triglyceride transfer using first order kinetics.

Measurement of Hep-G2 cell apoB and apoA-I secretion inhibition

HepG2 cells were grown in DMEM containing 10% fetal bovine serum in 96-well plates in a humidified, 5% CO_2 atmosphere at 37°C until ~70% confluent. CP-346086 was dissolved in DMSO, diluted to 1 μM in growth medium, serially diluted in growth medium to the desired concentration range, and added in 100 μl aliquots to separate wells of 96-well culture plates containing HepG2 cells. Twenty-four hours later, growth medium was collected and assessed for apoB and apoA-I concentrations as described by Haghpassand and Moberly (41).

Measurement of Hep-G2 cell cholesterol and fatty acid synthesis

Cholesterol and fatty acid synthesis were evaluated in Hep-G2 cells by measuring incorporation of [2-^{14}C]acetate into cellular lipids as previously described (38, 39), with the following modifications to allow simultaneous assessment of both cholesterol and fatty acid synthesis. After collection and assessment of the hexane fraction containing cholesterol and nonsaponifiable lipids as previously described (38, 39), the remaining aqueous phase (containing fatty acid sodium salts) was acidified to pH <2 by addition of 0.5 ml of 12 M HCl. The resulting mixtures were then transferred to glass conical tubes and extracted three times with 4.5 ml hexane. The pooled organic fractions were dried under nitrogen, resuspended in 50 μl of chloroform-methanol (1:1; v/v), and applied to 1 × 20 cm channels of Silica Gel 60C TLC plates. Channels containing nonradioactive fatty acids were included on selected TLC plates as separation markers. TLC plates were developed in hexane-diethyl ether-acetic acid (70:30:2; v/v/v) and air dried, and the region of chromatograms corresponding to fatty acid mobility was removed and assessed for radioactivity in Aquasol-2 using a Beckmann LS6500 liquid scintillation counter. Cholesterol and fatty acid synthesis are expressed as dpm [2-^{14}C]acetate incorporated into either cholesterol or saponifiable lipids during the 6 h incubation at 37°C per mg cellular protein.

Measurement of HepG2 cell triglyceride synthesis and secretion

HepG2 cells, grown in T-75 flasks as previously described (38, 39), were seeded into 24-well plates at 4–6 ×10^{5} cells/well and

maintained in a humidified, 5% CO_2 atmosphere at 37°C for 48 h prior to use. At the beginning of each experiment, media were removed and replaced with fresh media containing 0.2% DMSO ± CP-346086. One hour after compound addition, 25 μl of media containing 50 μCi of [^{3}H]glycerol was added to each incubation well. Plates were then sealed with Parafilm to avoid evaporation, and cells were incubated at 37°C for 6 h with gentle shaking. After incubation, the media were removed, and the cells were washed three times with PBS and scraped from wells into PBS. Lipids were extracted from the media and the cell lysate with chloroform-methanol (2:1; v/v) and applied to 1 × 20 cm channels of Silica Gel 60C TLC plates. Channels containing nonradioactive triglycerides were included on selected TLC plates as separation markers. TLC plates were developed in petroleum ether-diethyl ether-acetic acid (75:25:1; v/v/v) and air dried. The region of chromatograms corresponding to triglyceride mobility was removed and assessed for radioactivity in Aquasol-2 using a Beckman LS6500 liquid scintillation counter. Triglyceride synthesis and triglyceride secretion are expressed as dpm [^{3}H]glycerol incorporated into cellular triglycerides or secreted into the culture medium during the 6 h incubation at 37°C per mg cellular protein.

Measurement of plasma cholesterol and triglyceride levels

Plasma triglyceride and total cholesterol concentrations were determined using Cholesterol CII and Triglyceride E reagent kits and employing Standard Cholesterol CII Solution and Standard Triglyceride G Solution as calibration standards.

Measurement of hepatic and intestinal cholesterol and triglyceride levels

Hepatic and intestinal cholesterol and triglyceride levels were measured as previously described (42) with the following modifications. Sections, 0.5 g, of hepatic or intestinal tissue were homogenized at 4°C in 3.0 ml PBS using a Polytron tissue homogenizer. Aliquots, 200 μl, of the homogenates were transferred to 15 ml screw-capped glass tubes containing 7.5 ml of a mixture of $CHCl_3$-MeOH (2:1; v/v) and mixed vigorously for 20 s. One milliliter of ddH_2O was then added, and the resulting suspension was mixed vigorously for 15 s then centrifuged at 3,000 rpm for 5 min at room temperature in a Sorvall RT-6000 bench-top centrifuge. The chloroform-methanol layer was removed, placed in a 13 × 100 mm test tube, and evaporated to dryness under nitrogen at 60°C. The lipid residue was resuspended in 200 μl 1% Triton X-100 in absolute EtOH, and the cholesterol and triglyceride concentrations were determined using Cholesterol CII and Triglyceride E reagent kits, adapted for colorimetric analysis in 96-well plate format.

Plasma lipoprotein subfractionation and visualization by FPLC and PCX

Plasma lipoproteins were separated on a single Superose-6 column by FPLC, and the effluent was split into two equal streams. One stream was reacted postcolumn with cholesterol enzymatic assay reagents for the determination of lipoprotein cholesterol. The second stream was reacted postcolumn with triglyceride enzymatic assay reagents for the determination of lipoprotein triglyceride. Lipoprotein FPLC utilized a single Gilson autoinjector and dual PCX setups, each consisting of a Pickering nitrogen pressurized reagent delivery system, a Gilson reagent pump, a Pickering CRX 400 PCX with a custom 2.8 ml knitted reaction coil, and a Gilson spectrophotometer set at 500 nm. The stream splitting method used one Gilson mobile phase pump, one Pharmacia Superose-6 10 × 300 mm column, and multiple switching valves for controlling effluent stream splitting and PCX reagent flow. The entire system was computer controlled by Gilson model 715-system controller software running under Microsoft Windows. The mobile phase was 154 mM NaCl, 1 mM EDTA, 0.02% sodium azide (pH 8.1) at a flow rate of 0.36 ml/min. The cholesterol-PCX reagent, Cholesterol/HP, was prepared at 2× concentration by adding deionized water. The triglyceride-PCX reagent, TG/GPO, was prepared at 2× concentration by adding two bottles of enzyme to one bottle of buffer. The sterile filtered PCX reagents were stored and used under nitrogen in the dark at 4°C at a flow rate of 0.06 ml/min. Preciset Cholesterol Calibrators, Standard Cholesterol CII, Standard Triglyceride G, Precitrol-N serum, fresh human plasma (EDTA), and unknown samples were serially diluted in mobile phase and placed in a 2°C refrigerated rack for FPLC sampling. Aliquots of 50–500 μl were injected automatically. Plasma standards were included to compare peak areas obtained via direct PCX injection (e.g., Superose-6 column bypassed), with peak areas obtained after column fractionation via FPLC-PCX and as an intra/inter-assay control sample, which was used before, interdispersed with, and after the unknown samples. The split-column eluent was combined with the cholesterol or triglyceride reagent in the PCX module forming a reaction product that was measured spectrophotometrically at 500 nm after an 11 min pass through the 37°C reaction coil. The Gilson 715 software performed the analysis of the spectrophotometer output. The peak start, peak end, and baselines were checked visually and adjusted as necessary for each standard, control plasma, and sample prior to integrating the peak areas. In the case of unresolved peaks, a perpendicular was drawn from each peak valley to the horizontal baseline for determining the peak area. A plot of peak area versus quantity of cholesterol injected was made for the cholesterol and triglyceride standards, and a least squares regression analysis was performed and then used to convert chromatographic peaks from unknown samples into cholesterol and triglyceride concentrations in units of mg/dl.

Studies using experimental animals

The Institutional Animal Care and Use Procedures Review Board approved all procedures using experimental animals. B6CBAF1J mice, mice hemizygous for the human genes encoding apoA-I, apoC-III, and CETP (huA1/CIII/CETP mice), or Sprague Dawley rats were given food (RMH3200 laboratory meal or AIN76A semipurified diet) and water ad libitum and treated orally at a volume of 1.0 ml/200 g body weight (rats) or 0.25 ml/25 g body weight (mice) with either an aqueous solution containing 0.5% methyl cellulose (vehicle) or an aqueous solution containing 0.5% methyl cellulose plus CP-346086. In experiments in which CP-346086 was administered in the feed, animals were fed powdered RMH3200 laboratory meal for 1 week prior to commencing administration of CP-346086 as a dietary supplement to powdered chow. In studies in which rates of hepatic and intestinal triglyceride secretion were determined after Tyloxapol treatment, Tyloxapol (1.0 ml; 125 mg/ml) was administered intravenously 60 min after oral administration of CP-346086. In experiments in which rates of intestinal triglyceride absorption were determined in the absence of Tyloxapol, [^{14}C]triolein mixed in Bioserve F0739 liquid diet [17.7% (w/w) fat] at a concentration of 0.5 μCi/ml was administered orally to fasted mice as a 0.5 ml aliquot.

Human subjects and study design

Protocols involving human subjects were approved by the Institutional Protocol Review Board of Pfizer, Inc., and by the Protocol Review Board of the Food and Drug Administration. Forty-eight healthy male adults, aged 18–45 years, participated in the randomized, double-blind, placebo-controlled, escalating single-dose study, and 30 healthy male adults, aged 18–45 years, partici-

pated in the 2 week randomized, double-blind, placebo-controlled, parallel-group multidose study. Inclusion criteria for both studies were body weight within 10% of ideal based on 1983 Metropolitan Life Insurance Height and Weight Tables (43), fasting plasma cholesterol levels in the upper 50% of the normal range for the American population based on age, sex, and race (e.g., 180–250 mg/dl) (1), fasting plasma triglyceride levels >150 mg/dl and <400 mg/dl, no evidence of clinically significant disease based on complete medical history, full physical examination, 12-lead ECG and clinical laboratory testing, and no prescription, OTC, or other drug usage for at least 2 weeks prior to beginning the study. Subjects were confined to the Clinical Research Unit and fed standardized meals (30% of daily calories from fat).

In the single-dose study, after randomization (six subjects/group; eight groups) each group was assigned a CP-346086 dose ranging in half-log intervals from 0.1 mg to 300 mg. At 7 AM on the day of study, after fasting for at least 8 h, four subjects from each group received their respective doses of CP-346086, and two subjects received placebo. Plasma samples were obtained at various times over the next 72 h for use in determining total and lipoprotein cholesterol and triglyceride levels by FPLC (see above).

In the multidose study, subjects were randomized with eight receiving 30 mg CP-346086 and six receiving placebo at 10 PM (bedtime) for 14 days. Plasma samples were obtained daily predose, frozen at −20°C, and forwarded to Medical Research Labs (39 Excelsiorlaan Zaventem, Brussels, Belgium) for determination of total and lipoprotein cholesterol and triglyceride levels using standardized autoanalyzer technology.

RESULTS

Inhibition of MTP-mediated lipid transfer by CP-346086

When donor and acceptor liposomes, prepared as described in Experimental Procedures, were incubated with varying concentrations of inhibitor, CP-346086 dose dependently inhibited human MTP-mediated triglyceride transfer between vesicles with a concentration giving an IC_{50} equal to 2.0 nM (Fig. 1). Similarly, when the radiolabeled triolein of donor vesicles was replaced by radiolabeled cholesteryl oleate, CP-346086 inhibited transfer of cholesteryl oleate between vesicles with an IC_{50} of 1.9 nM (Fig. 1), demonstrating the compound's ability to equally inhibit transfer of both neutral lipids. Similar IC_{50} values were also noted for inhibition of rat (1.7 nM) and mouse (6.7 nM) MTP activity.

Inhibition of neutral lipid transfer by CP-346086 was specific to MTP-mediated lipid transfer, however, as in an identical assay in which MTP was replaced by CETP, no inhibition of CETP-mediated lipid transfer was observed at concentrations of CP-346086 up to 10 μM (data not shown), indicating the compound's specificity for inhibition of MTP-mediated lipid transfer and demonstrating the lack of an effect of CP-346086 on the physicochemical properties of the donor and acceptor vesicles.

Inhibition of apoB and triglyceride secretion from Hep-G2 cells by CP-346086

When Hep-G2 cells were incubated with various concentrations of CP-346086 for 24 h at 37°C, and the culture media was evaluated for human apoB, apoA-I, and apoC-III concentrations by ELISA, CP-346086 inhibited Hep-G2 cell apoB secretion with an IC_{50} of 2.0 nM (**Fig. 2A**). Under these conditions, neither apoA-I secretion (Fig. 2A) nor apoC-III secretion (not shown) were inhibited by CP-

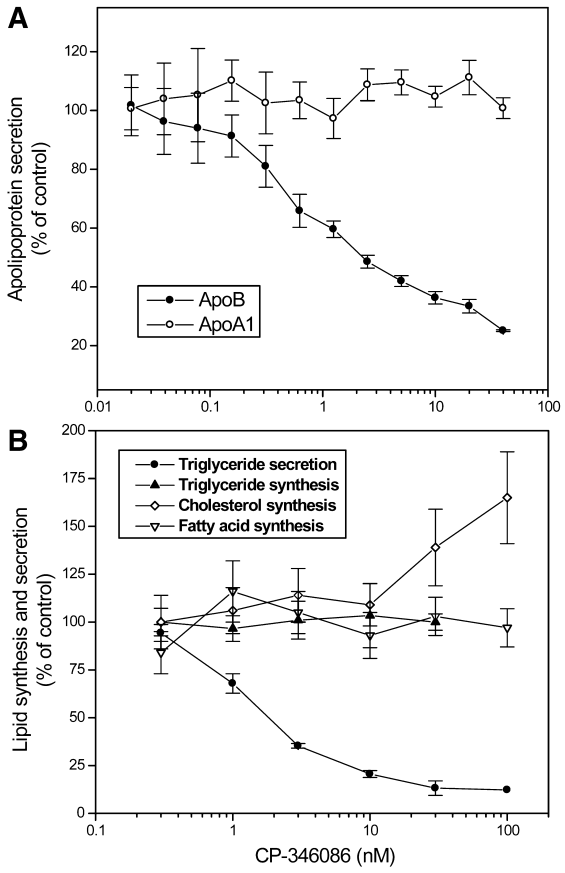

Fig. 2. Inhibition of Hep-G2 cell apolipoprotein B (apoB) and triglyceride secretion by CP-346086. A: Hep-G2 cells seeded and maintained in culture as described in Experimental Procedures were incubated for 24 h at 37°C in supplemented DMEM plus the indicated concentrations of CP-346086. After incubation, the medium was removed and assessed for apoB and apoA-I concentrations by apolipoprotein-specific ELISAs as outlined in Experimental Procedures. Data for apoB and apoA-I secretion are the mean of triplicate determinations ± SD and are expressed as a percentage of control apolipoprotein secretion as a function of CP-346086 concentration. B: Hep-G2 cells seeded and maintained in culture as described in Experimental Procedures were incubated for 6 h at 37°C in supplemented DMEM containing either 1% DMSO (control) or 1% DMSO containing CP-346086 sufficient to produce the indicated final inhibitor concentrations. For measurement of triglyceride synthesis and secretion, cells also received 50 μCi of [^{3}H]glycerol (closed symbols). After incubation, the media was removed, the cells were washed three times with PBS, and the secreted (media) and cellular triglycerides were quantitated as described in Experimental Procedures. For measurement of cholesterol and fatty acid synthesis, cells also received 4 μCi of [2-^{14}C]acetate (open symbols). After incubation, newly synthesized cholesterol and fatty acids were separated and quantitated as described in Experimental Procedures. Data for triglyceride secretion (closed circles), triglyceride synthesis (closed triangles), cholesterol synthesis (open diamonds), and fatty acid synthesis (open triangles) are the mean of triplicate determinations ± SD and are expressed as a percentage of control synthesis and secretion as a function of CP-346086 concentration.

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