

An inhibitor of the microsomal triglyceride transfer protein inhibits apoB secretion from HepG2 cells

(lipoproteins/lipid transfer protein/protein disulfide isomerase/atherosclerosis)

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ABSTRACT The microsomal triglyceride (TG) transfer protein (MTP) is a heterodimeric lipid transfer protein that catalyzes the transport of triglyceride, cholesteryl ester, and phosphatidylcholine between membranes. Previous studies showing that the proximal cause of abetalipoproteinemia is an absence of MTP indicate that MTP function is required for the assembly of the apolipoprotein B (apoB) containing plasma lipoproteins, i.e., very low density lipoproteins and chylomicrons. However, the precise role of MTP in lipoprotein assembly is not known. In this study, the role of MTP in lipoprotein assembly is investigated using an inhibitor of MTP-mediated lipid transport, 2-[1-(3, 3-diphenylpropyl)-4-piperidinyl]-2,3-dihydro-1H-isoindol-1-one (BMS-200150). The similarity of the IC_{50} for inhibition of bovine MTP-mediated TG transfer (0.6 μ M) to the K_d for binding of BMS-200150 to bovine MTP (1.3 μ M) strongly supports that the inhibition of TG transfer is the result of a direct effect of the compound on MTP. BMS-200150 also inhibits the transfer of phosphatidylcholine, however to a lesser extent (30% at a concentration that almost completely inhibits TG and cholesteryl ester transfer). When BMS-200150 is added to cultured HepG2 cells, a human liver-derived cell line that secretes apoB containing lipoproteins, it inhibits apoB secretion in a concentration dependent manner. These results support the hypothesis that transport of lipid, and in particular, the transport of neutral lipid by MTP, plays a critical role in the assembly of apoB containing lipoproteins.

Genetic studies (1-4) have demonstrated that an absence of microsomal triglyceride (TG) transfer protein (MTP) causes abetalipoproteinemia, a disease characterized by a defect in the assembly and secretion of apolipoprotein B (apoB) containing plasma lipoproteins. These studies indicate that MTP is required for the production of the apoB containing lipoproteins, very low density lipoproteins and chylomicrons by the liver and intestine. The requirement for MTP for lipoprotein production is further supported by studies using cell lines that are not of hepatic or intestinal origin. When a truncated form of apoB representing 53% of the full-length apoB-100 is expressed in HeLa cells, virtually no apoB is secreted (5). However, when MTP is coexpressed with apoB, apoB is secreted as a lipoprotein particle. Similar findings have been observed in COS cells (6).

MTP is found in the lumen of microsomes isolated from liver and intestine (7). The protein purified from bovine liver is a heterodimer consisting of the multifunctional enzyme, protein disulfide isomerase, and a unique, large 97-kDa subunit (8-10). *In vitro*, MTP catalyzes the transport of TG, cholesteryl

ester (CE), and phosphatidylcholine (PC) between membranes (8).

Although MTP is required for the assembly of plasma lipoproteins, the precise role of MTP in the assembly process is not known. We have proposed that MTP transports lipid molecules from the endoplasmic reticulum (ER) membrane where they are synthesized, to developing lipoprotein particles in the ER lumen. However, there has not been any direct experimental evidence to support this hypothesis to date. Here we report the identification and characterization of a small molecule inhibitor of MTP-mediated lipid transport. This molecule, BMS-200150, selectively inhibits TG and CE transport. When the inhibitor is added to HepG2 cells, a human liver-derived cell line that assembles and secretes apoB containing lipoproteins, apoB secretion is inhibited. This supports our hypothesis that MTP-mediated lipid transport is required for lipoprotein assembly.

MATERIALS AND METHODS

Radiolabeled 1,2-dipalmitoyl-L-3-phosphatidyl[*N*-methyl-³H]choline and glycerol tri[1-¹⁴C]oleate were obtained from Amersham. All unlabeled lipids were obtained from Sigma. Lipids were stored under N₂ gas in chloroform at -20°C. HepG2 human hepatoblastoma cells were obtained from the American Type Culture Collection, and were maintained as recommended by the supplier under standard cell culture conditions (37°C with a 5% CO₂/95% air atmosphere). Tissue culture media and serum were obtained from GIBCO. Monoclonal and polyclonal antibodies against apoB and apoA1 were obtained from Biodesign International (Kennebunkport, ME).

Synthesis of BMS-200150 and [³H]BMS-200150. A mixture of 4-amino-1-benzylpiperidine (101 mmol) and phthalic anhydride (101 mmol) was heated at 125°C for 30 min, cooled to room temperature, and purified by flash chromatography (silica, 30% EtOAc/hexane) to yield 2-[1-(phenylmethyl)-4-piperidinyl]-1H-isoindol-1,3(2H)-dione (compound A) (77% yield) as a white solid [melting point (mp) 151-154°C].

A mixture of compound A (62.5 mmol) and Zn dust (438 mmol) in AcOH (250 ml) was heated at reflux for 18 h, cooled to room temperature, filtered through Celite (Aldrich), and concentrated. The residue was dissolved in CH₂Cl₂ (500 ml), washed with saturated NaHCO₃ (2 × 100 ml) and brine (100 ml), dried over MgSO₄, and concentrated. The crude product was recrystallized from *i*-PrOH to yield 2,3-dihydro-2-[1-

Abbreviations: TG, triglyceride; MTP, microsomal TG transfer protein; CE, cholesteryl ester; PC, phosphatidylcholine; ER, endoplasmic reticulum; SUV, small unilamellar vesicles; apo, apolipoprotein; DMSO, dimethyl sulfoxide.

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(phenylmethyl)-4-piperidinyl]-1H-isoindol-1-one (compound B) (80% yield) as a white solid (mp 130–133°C).

A mixture of compound B (26.4 mmol), AcOH (53 mmol), and 10% Pd/C (0.66 mmol Pd) in EtOH (65 ml) was agitated on a Parr hydrogenator at 45 psi of H₂ for 48 h, filtered through Celite, and concentrated. The residue was dissolved in CHCl₃ (100 ml), washed with 1 M KOH saturated with NaCl (2 × 30 ml), dried over MgSO₄, and concentrated to yield 2-(4-piperidinyl)-2,3-dihydro-1H-isoindol-1-one (compound C) (77% yield) as a white solid (mp 137–140°C).

A mixture of compound C (9.26 mmol), γ -phenylbenzenepropanol, 4-methylbenzenesulfonate ester (11) (9.26 mmol), and K₂CO₃ (14.8 mmol) in *i*-PrOH (25 ml) was heated at reflux for 18 h, cooled to room temperature, filtered, and concentrated. The crude product was purified by flash chromatography (silica, 2.5% MeOH/CH₂Cl₂) to yield the free amine (74% yield) as a colorless oil. A portion of the free amine (2.4 mmol) in MeOH (7.0 ml) was treated with 1 M HCl/Et₂O (4.9 mmol) and concentrated. The residue was recrystallized from EtOH to yield 2-[1-(3, 3-diphenylpropyl)-4-piperidinyl]-2,3-dihydro-1H-isoindol-1-one, monohydrochloride (BMS-200150) (68% yield) as a white solid (mp 237–241°C): ¹H NMR (400 MHz, CD₃OD) δ 7.76 (d, 1H, J = 7.3 Hz), 7.55 (m, 2H), 7.49 (t, 1H, J = 7.3 Hz), 4.50 (s, 2H), 4.39 (m, 1H), 4.05 (t, 1H, J = 7.7 Hz), 3.68 (br d, 2H, J = 12 Hz), 3.12 (m, 4H), 2.59 (m, 2H), 2.20 (m, 2H), 2.08 (br d, 2H, J = 12 Hz).

Radiolabeled BMS-200150 was synthesized as follows. Dimethyl sulfide (14.5 mmol) was added to a solution of *N*-chlorosuccinimide (11.4 mmol) in CH₂Cl₂ (40 ml) at –40°C. The reaction was warmed to room temperature for 30 min and recooled to –40°C, and a solution of 3,3-diphenyl-2-propene-1-ol (12) (10.3 mmol) in CH₂Cl₂ (3 ml) was added dropwise. The reaction was stirred at –40°C for 2 h, diluted with hexane (100 ml), washed with H₂O (50 ml) and brine (2 × 50 ml), dried over Na₂SO₄, and concentrated to yield 1-chloro-3,3-diphenyl-2-propene (compound D) (81% yield) as a colorless oil.

A mixture of compound C (7.56 mmol), compound D (8.32 mmol), and K₂CO₃ (7.94 mmol) in dimethylformamide (35 ml) was heated at 50°C overnight, cooled to room temperature, and concentrated. The residue was dissolved in CH₂Cl₂ (150 ml), washed with H₂O (2 × 50 ml) and brine (2 × 50 ml), dried over MgSO₄, and concentrated. The crude solid was purified by flash chromatography (silica, 3% MeOH/CH₂Cl₂) to yield 2-[1-(3, 3-diphenyl-2-propenyl)-4-piperidinyl]-2,3-dihydro-1H-isoindol-1-one (compound E) (63% yield) as a white solid (mp 164–167°C).

Olefin E was reduced by catalytic tritiation performed by NEN to yield [³H]BMS-200150 with a specific activity of 44.0 Ci/mmol (1 Ci = 37 GBq) and a radiochemical purity of 99%.

Isolation and Purification of MTP. Bovine MTP was purified from bovine liver as described previously (13). The purified protein had an activity of 3.0% TG transfer/min/ μ g of protein in our assay (see below), and showed only two bands with apparent molecular masses of 88 and 58 kDa on SDS/8% polyacrylamide gels.

Human MTP was partially purified from 10/150 cm² flasks of HepG2 cells (3 × 10⁸ cells per flask) grown to confluency in RPMI 1640 medium containing 10% fetal bovine serum. Cells were homogenized in 50 ml of 50 mM Tris-HCl, pH 7.4/5.0 mM EDTA/50 mM KCl containing 1.0 mM phenylmethylsulfonyl fluoride and 0.5 μ g/ml leupeptin (homogenization buffer) using a Polytron PT3000 (Brinkmann) for 1 min at the half maximal setting. Cell homogenate was diluted with homogenization buffer to a protein concentration of 1.75 mg/ml. To release protein from the microsomal lumen, one part of sodium deoxycholate (0.54%, pH 7.5) was added to 10 parts of diluted homogenate while vortexing, and then it was left to stand on ice for 30 min. Cell membranes were subsequently removed by centrifugation at 100,000 × *g* for 1 h. The

mM EDTA/0.02% sodium azide and protease inhibitors (1.0 mM benzamidine/1.0 mM phenylmethylsulfonyl fluoride/0.01 mM L-tosylamido-2-phenylethyl chloromethyl ketone/0.4 μ g/ml each leupeptin, antipain, and pepstatin), loaded onto a DEAE-Sephacel (Sigma) column (20 ml) and eluted with a linear gradient of 0 to 500 mM NaCl. MTP eluted between 200 and 300 mM NaCl.

Inhibition of Lipid Transport. The inhibition of lipid transfer activity in the presence of BMS-200150 was measured in an assay similar to one that has been previously described (1, 14). Donor and acceptor small unilamellar vesicles (SUVs) were prepared by bath sonication in 15 mM Tris-HCl, pH 7.5/1 mM EDTA acid/40 mM NaCl/0.02% sodium azide (assay buffer). The lipid transfer assay mixture contained donor membranes (40 nmol egg PC/7.5 mol % cardiolipin/0.25 mol % radiolabeled substrate), acceptor membranes (240 nmol egg PC), 5.0 mg BSA, and various concentrations of BMS-200150 in a total volume of 0.68 ml assay buffer. BMS-200150 was dissolved in dimethyl sulfoxide (DMSO) and added to the reaction mixture. The final concentration of DMSO was 0.5%. The reaction was started by the addition of MTP in 20 μ l assay buffer. After a 60-min incubation at 37°C, the reaction was terminated by the addition of 0.5 ml of DE-52 cellulose (Whatman) pre-equilibrated in 15 mM Tris-HCl, pH 7.4/1.0 mM EDTA/0.02% sodium azide (1:1, vol/vol). The mixture was agitated for 5 min and centrifuged at maximum speed in a Biofuge B centrifuge (Baxter Scientific Products, McGaw Park, IL) for 3 min to pellet the DE-52 bound donor vesicles. First order kinetics were used to calculate the lipid transfer rate using the equation $[S] = [S]_0 e^{-kt}$ (8), where $[S]_0$ and $[S]$ are the fraction of the available labeled lipid in the donor membrane at times 0 and *t*, respectively, and *k* is the fraction of the available labeled lipid transferred per unit time. This calculation corrects for the depletion of labeled lipid in donor vesicles that occurs with time.

Equilibrium Binding Assay. Fifty or one hundred micrograms of bovine MTP in 200 μ l of assay buffer was pipetted into a 12–14 kDa cut-off dialysis bag (Spectra/Por) and dialyzed against various concentrations of [³H]BMS-200150 (0.1 to 100 μ M) in 1.0 ml of assay buffer in 1.5 ml Eppendorf tubes for 24 h. The concentration of bound and free inhibitor was quantitated by measuring the protein inside the dialysis bag and radioactivity both inside and outside the dialysis bag.

Inhibition of Apolipoprotein Secretion from HepG2 Cells. HepG2 cells were seeded at a density of 50% confluency in 48-well plates and allowed to grow for 48 h before treatment. At this time, the medium was replaced with fresh medium containing 0.5% DMSO and the indicated concentrations of BMS-200150. After a 16 h incubation under standard cell culture conditions, the medium was diluted with fresh tissue culture medium 30-fold for an apoB ELISA, and 60-fold for an apoA1 ELISA. A sandwich ELISA was used to measure apoB in the media as has been reported (15). A similar assay was used to quantitate apoA1. For the apoA1 assay, the primary and secondary antibodies were a monoclonal anti-human apoA1 (1:500 dilution) and a goat anti-human apoA1 polyclonal antibody (1:500). The concentration of the respective proteins was measured against a 2-fold dilution standard curve from 1.25–40 ng/ml of the purified proteins. In this range of concentrations, both assays show a linear response. Each drug concentration was tested in duplicate cultures, and apoB and apoA1 were measured by ELISA in each culture in triplicate.

Analyses of ApoB Secretion from HepG2 Cells by [³⁵S]Methionine Labeling and Immunoprecipitation. Duplicate 100-mm culture dishes containing confluent monolayers of HepG2 cells were incubated for 30 min in 10 ml of methionine-free RPMI 1640 medium containing 16.5 mg/ml BSA and 0.8 mM sodium oleate. This was followed by a 30-min incubation

(time = 0 min) a 1-ml aliquot of medium was removed and saved for immunoprecipitation analysis. One milliliter of fresh medium containing 100 $\mu\text{Ci/ml}$ of [^{35}S]methionine and 50 μM unlabeled L-methionine was added. Immediately thereafter, one culture was brought to 10 μM BMS-200150 + 0.5% DMSO, whereas the other was brought to 0.5% DMSO only. At the indicated times, 1-ml aliquots were removed and replaced with fresh labeling medium containing the appropriate concentrations of radiolabeled methionine, drug, and/or DMSO. The concentration of radiolabeled apoB in each media sample was measured by immunoprecipitation of 250- μl aliquots with 5 μl of goat anti-human apoB polyclonal antiserum using a protocol previously described (15). Preimmune serum was used to determine background levels of radioactivity immunoprecipitated for each media sample. The data are expressed as the counts per minute immunoprecipitated, following correction for background and dilution of the media over the course of the experiment.

[^3H]Thymidine Incorporation into DNA. HepG2 cells were grown to 90% confluency in 24-well plates. To investigate the effect of BMS-200150 on HepG2 cell growth, the cells were incubated for an additional 16 h in the absence or presence of various concentrations of BMS-200150. Each concentration of compound was tested in triplicate. The cells were then pulsed with [^3H]thymidine (0.5 $\mu\text{Ci/ml}$) for 1 h at 37°C. After washing the monolayers two times with PBS, 0.5 ml ice cold 10% trichloroacetic acid was added, and the plates were incubated at 4°C for 5 min. This step was repeated, and then the plates were washed briefly with cold PBS. The precipitate was solubilized in 0.5 ml of 0.1 M NaOH for 15 min at room temperature, and the radioactivity was quantitated by liquid scintillation counting.

RESULTS

Identification and Characterization of BMS-200150. High-throughput screening of the Bristol-Myers Squibb compound collection identified BMS-200150 (Fig. 1) as a potent inhibitor of bovine MTP-mediated transport of TG between SUVs (Fig. 2 Left). The IC_{50} for inhibition of TG transfer was 0.6 μM . BMS-200150 also inhibits human MTP-mediated TG transfer (Fig. 3) with a similar IC_{50} of 2.2 μM . In a control experiment, BMS-200150 had no effect on human plasma cholesterol ester transfer protein-mediated CE transfer (data not shown).

MTP binds and shuttles lipid molecules between SUVs (16). There are two possible mechanisms by which a small molecule could inhibit MTP-mediated lipid transport. First, it could partition into the SUVs in the assay and disrupt the ability of MTP to bind to the vesicle surfaces or disrupt the ability of MTP to extract lipid molecules from or deposit lipid molecule into the membrane. Alternatively, it could bind directly to MTP and render it unable to transport lipid. To investigate the means by which BMS-200150 inhibits MTP-mediated lipid transport, [^3H]BMS-200150 was synthesized to study the interaction of BMS-200150 with bovine MTP in an equilibrium dialysis experiment. Scatchard analysis of the binding revealed a single binding site on MTP with a K_d value of 1.3 μM (Fig. 2 Right). The K_d is very similar to the IC_{50} for inhibition of lipid transfer (0.6 μM) (Fig. 2 Left), suggesting that the direct

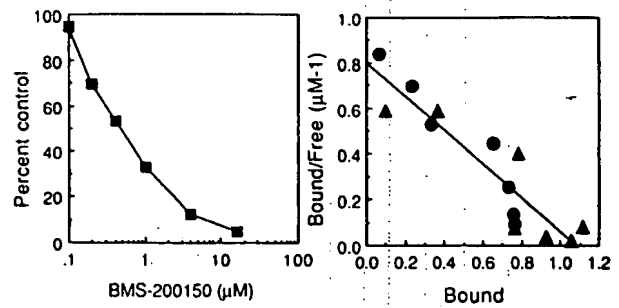
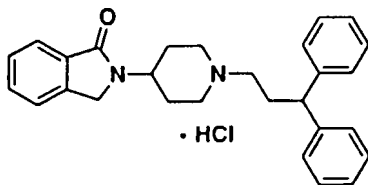


Fig. 2. BMS-200150 binds to and inhibits bovine MTP. (Left) The lipid transfer activity of bovine liver MTP was measured in the presence of 0–15 μM BMS-200150. The assay measures the rate of [^{14}C]TG transfer from donor SUVs to acceptor SUVs as described in *Materials and Methods*. TG transfer in the absence of inhibitor was 15% of the labeled TG in the donor vesicles. (Right) The binding of [^3H]BMS-200150 to bovine MTP was determined by equilibrium dialysis at various concentrations (0.1 to 100 μM) of the compound using 50 μg (\blacktriangle) and 100 μg (\bullet) of MTP. A Scatchard plot derived from the equation $[L]_b/[L]_f[P]_t = (-1/K_d)[L]_b/[P]_t + n/K_d$ is shown, where $[L]_b/[P]_t$ is moles BMS-200150 bound per mole of MTP (Bound), $[L]_f$ is free BMS-200150 (Free), $[P]_t$ is the total MTP concentration, n is the number of BMS-200150 binding sites per molecule of MTP, and K_d is the BMS-200150 dissociation constant of a site.

binding of BMS-200150 to MTP is responsible for inhibition of MTP-mediated lipid transport.

MTP transports a wide variety of both neutral lipids and phospholipids (8, 14). The ability of BMS-200150 to inhibit the human MTP-mediated transport of different lipid substrates was investigated using donor vesicles containing 0.25 mol % of different ^{14}C -labeled substrates. The IC_{50} for the inhibition of CE transfer was 1.7 μM , a value similar to the IC_{50} for TG transfer (Fig. 3). BMS-200150 also inhibited PC transport over a similar concentration range, but to a lower extent. The inhibition of PC transport was only 30%.

Effect of BMS-200150 On ApoB Secretion by HepG2 Cells. HepG2 cells are a human liver-derived cell line that assemble and secrete apoB containing lipoproteins. To test the role of MTP-mediated lipid transport in lipoprotein production, the effect of BMS-200150 on apoB secretion from HepG2 cells was investigated. HepG2 cells were incubated for 16 h with various concentrations of BMS-200150. The amount of apoB secreted

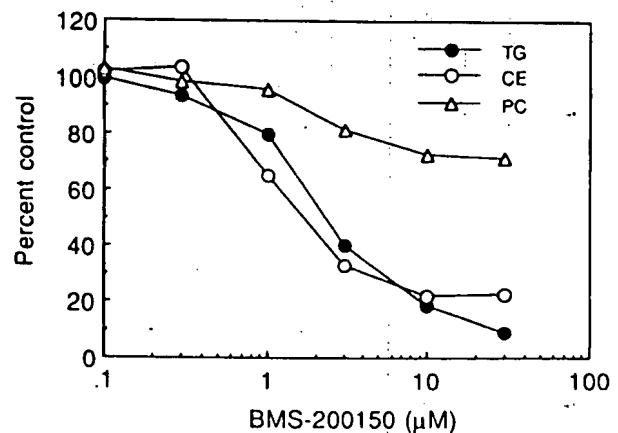


Fig. 3. Inhibition of human MTP by BMS-200150. The lipid transfer activity of human MTP was measured in the presence of 0–30 μM BMS-200150 using donor vesicles containing 0.25 mol % [^{14}C]TG, CE, or PC. The assay measures the rate of lipid transfer from donor SUVs to acceptor SUVs as described in *Materials and Methods*. The

into the medium was quantitated by an ELISA assay. ApoB secretion was inhibited in a concentration-dependent manner with an IC_{50} of 1.8 μ M (Fig. 4). The selectivity of the effect was confirmed by measuring apoAI secretion. At 30 μ M BMS-200150, a concentration that is more than 15 times the IC_{50} for inhibition of apoB secretion and that inhibited apoB secretion 90%, there was no effect on apoAI secretion (Fig. 4).

The nonspecific toxicity of BMS-200150 to HepG2 cells was tested by measuring [3 H]thymidine incorporation into DNA after incubation with varying concentrations of the compound for 16 h. At 2 μ M BMS-200150, the IC_{50} for inhibition of apoB secretion, the thymidine incorporation was 100% that of control cells. At 20 μ M BMS-200150, a concentration that inhibits apoB secretion over 90%, thymidine incorporation was 80% that of control (data not shown). This indicates that BMS-200150 is not toxic to HepG2 cells at concentrations that selectively inhibit apoB secretion.

The time of onset of the effect of BMS-200150 on the secretion of apoB by HepG2 cells was investigated by measuring the inhibition of [35 S]methionine-labeled apoB secretion into the medium (Fig. 5). After a lag period of \sim 30 min, the rate of apoB secretion in the media of drug treated cells was dramatically inhibited. No effect of BMS-200150 was seen on incorporation of [35 S]methionine into total cellular protein.

DISCUSSION

Although genetic studies have clearly demonstrated a requirement for MTP in lipoprotein assembly, they have not revealed its precise role in the process. Here we report that BMS-200150 inhibits MTP-mediated transfer of TG and CE between membranes. Binding studies further demonstrated that BMS-200150 binds to MTP with a K_d similar to the IC_{50} for inhibition of lipid transfer. This strongly supports that the inhibition of lipid transport results from a direct effect of the inhibitor on MTP, rather than an indirect effect due to its partitioning into the substrate membranes. In addition, BMS-200150 selectively inhibits apoB secretion from HepG2 cells with an IC_{50} similar to the IC_{50} for inhibition of human MTP TG transport (1.8 μ M compared with 2.2 μ M).

MTP appears to possess two to three lipid molecule binding sites that can be divided into two classes (17). The primary fast site or sites appear to be involved in the transport of both neutral lipid and phospholipid. A second slow site appears to

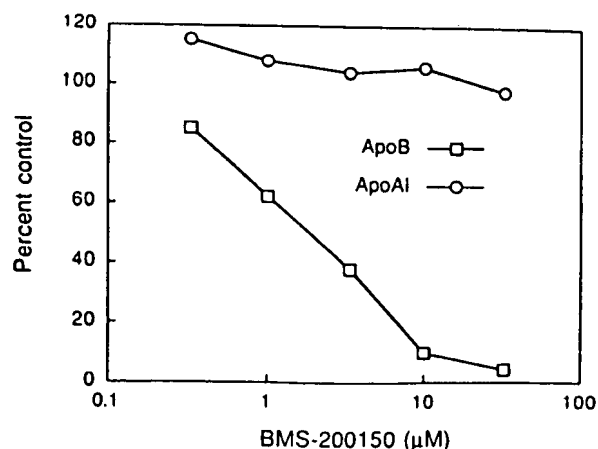


FIG. 4. Effect of BMS-200150 on apoB and apoAI secretion from HepG2 cells. HepG2 cells were treated with various concentrations of BMS-200150 for 16 h. The amount of apoB and apoAI accumulated in the media over 16 h was determined by ELISA. The data are representative of four independent experiments. Control values for apoB and apoAI secretion were 270 and 410 ng/ml, respectively.

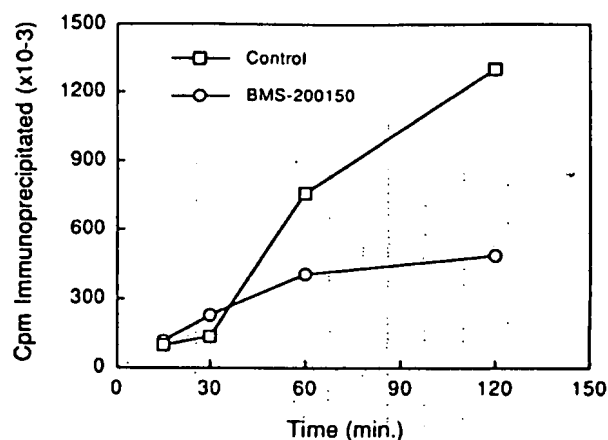


FIG. 5. Two-hour time course of apoB secretion from HepG2 cells following the administration of BMS-200150. HepG2 cells were labeled with [35 S]methionine in the presence or absence of 10 μ M BMS-200150. At the indicated time, aliquots of medium were removed and radiolabeled apoB was quantitated by immunoprecipitation.

specifically bind phospholipid. BMS-200150 binds to MTP in a fashion that completely disrupts the transport of neutral lipid, but only partially disrupts phospholipid transfer. The simplest explanation of these results would be that BMS-200150 binds to MTP at a location that disrupts a binding site on MTP that is responsible for TG, CE, and PC transport. To determine if this is the result of BMS-200150 binding directly in this site or to an allosteric site will require further investigation.

Newly synthesized apoB follows a sequence of steps that leads either to its degradation within the cell or the formation and secretion of a mature lipoprotein particle. The lipoprotein assembly process is initiated as apoB is translocated into the lumen of the ER (18). Small apoB particles with a density of low density lipoproteins can be secreted from liver derived cell lines including HepG2 cells (reviewed in ref. 19). Similar particles are not detected in abetalipoproteinemic subjects, suggesting that the assembly defect resulting from an absence of MTP lies early in the assembly process prior to the formation of a small, secretion competent, lipoprotein particle. The results of this study indicate that a crucial step in this early process is MTP-mediated transport of lipid. TG or CE transport appears particularly important early in the assembly process. These studies, however, do not address the extent that MTP mediates the transport of all the lipid incorporated into the mature lipoprotein particles.

Elevated plasma lipid levels cause premature atherosclerosis (20). Restriction of dietary fat and drug therapy are currently used to lower elevated plasma lipoprotein levels. However, in many cases these treatments are not capable of attaining the required decrease in plasma lipid levels. This has led to a search for better ways to control plasma lipid levels. The studies of the role of MTP in abetalipoproteinemia demonstrated that MTP is required for both hepatic and intestinal apoB containing lipoprotein production (very low density lipoprotein and chylomicrons), and suggested that inhibition of MTP function may be an effective strategy to prevent very low density lipoprotein and chylomicron assembly and to lower plasma lipid levels. The results of this work support this proposal, and demonstrate that inhibition of its lipid transfer activity is a viable approach to inhibit lipoprotein production. If effective *in vivo*, this approach should have a profound effect on the ability to lower plasma lipid levels therapeutically.

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