Evidence that microsomal triglyceride transfer protein is limiting in the production of apolipoprotein B-containing lipoproteins in hepatic cells

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Abstract **The microsomal triglyceride transfer protein (MTP) is a heterodimeric lipid transfer protein that is required for the assembly and secretion of apolipoprotein B (apoB)-containing lipoproteins. A key unresolved question is whether the MTP-mediated step is rate limiting. To address this, a unique experimental strategy was used that allowed the *in situ* modulation and measurement of MTP triglyceride transfer activity. In order to accomplish this, an irreversible photoaffinity inhibitor, BMS-192951, was designed and synthesized. When incubated with purified MTP and irradiated with UV light at 360 nm, BMS-192951 inhibits triglyceride transfer by covalently binding to the protein. HepG2 cells were treated with either increasing concentrations of BMS-192951 (0–15 μm) with 5 min of ultraviolet irradiation, or 3.0 μm BMS-192951 with various lengths (0–15 min) of ultraviolet irradiation. Microsomal extracts were prepared exhaustively dialyzed to remove unbound inhibitor, and assayed for MTP-mediated triglyceride transfer activity. BMS-192951 was shown to reduce MTP activity in both a dose- and UV exposure time-dependent fashion. Measurement of apoB concentration in the media showed that apoB secretion was reduced in proportion to the in situ inhibition of MTP activity, while no change was observed in apoA-I secretion. Experiments performed in McArdle RH-7777 rat hepatoma cells and primary rat hepatocytes gave nearly identical results; the decrease in apoB secretion was proportional to the decrease in MTP activity. These results indicate that MTP-mediated lipid transfer is limiting in the assembly and secretion of apoB-containing lipoproteins in hepatic cells under the conditions tested.—**Jamil, H., C-H. Chu, J. K. Dickson, Jr., Y. Chen, M. Yan, S. A. Biller, R. E. Gregg, J. R. Wetterau, and D. A. Gordon. **Evidence that microsomal triglyceride transfer protein is limiting in the production of apolipoprotein B-containing lipoproteins in hepatic cells. *J. Lipid Res.* 1998.** 39: **1448–1454.**

Supplementary key words lipid transfer protein • protein disulfide isomerase • photoaffinity • benzophenone • HepG2 cells • McArdle RH-7777 cells • cholesteryl ester • phosphatidylcholine

Apolipoprotein B-100 (apoB-100), a single polypeptide with a molecular mass of 512 kDa, is the primary structural protein of plasma very low density lipoproteins (VLDL). The formation of VLDL is a highly complex process. It requires the addition of four classes of lipid to newly synthesized apolipoprotein B (apoB) within the endoplasmic reticulum (ER) lumen (1–3). This process is further complicated by the physical nature of the apoB polypeptide. Recent computer modeling studies (4) based upon biochemical analyses (5) have shown that large segments of the apoB backbone have a highly amphipathic structure predicted to bind lipid. If these amphipathic β-sheets and α-helices are not folded and associated with lipid in the proper temporal sequence, the structural model predicts that the hydrophobic surfaces would become unstable in the aqueous environment of the ER lumen, leading to improper folding of nascent apoB and eventual degradation.

The regulation of this pathway has been the subject of considerable investigation (6–8). To date, two components uniquely involved in lipoprotein assembly have been identified: apoB and the microsomal triglyceride transfer protein (MTP). The role of apoB gene expression in regulating lipoprotein production has been well studied. For instance, patients with heterozygous defects that inactivate one allele of the apoB gene show decreased levels of apoB lipoproteins in the plasma (9). Similarly, heterozygous knockouts of the apoB gene in mice show a reduction in the concentration of apoB lipoproteins in the circulation (10). Conversely, studies in transgenic mice show that increasing expression of apoB leads to an increase in apoB synthesis and lipoprotein production (11). However, in these cases, the levels of apoB lipoproteins do

Abbreviations: MTP, microsomal triglyceride transfer protein; TG, triglyceride; CE, cholesteryl ester; Chol, cholesterol; PC, phosphatidylcholine; VLDL, very low density lipoproteins; ER, endoplasmic reticulum; SUV, small unilamellar vesicles; apoB, apolipoprotein B; apoA-I, apolipoprotein A-I; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; IC_{50}, 50% inhibitory concentration; UV, ultraviolet.

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not always correlate with apoB gene expression, suggesting modification by additional factors including those that influence lipoprotein production as well as clearance from the plasma. In fact, it has been shown that the amount of apoB secreted can be modified via various metabolic manipulations without changes in apoB mRNA (12 and reviewed in 6–8). Thus, it appears that while the level of apoB gene expression sets a range for lipoprotein production, there are other factors that mediate acute regulation in response to changing metabolic conditions.

While the role of apoB gene expression in determining the level of apoB lipoprotein production has been well characterized, the existing data that address the role of MTP in determining the level of apoB lipoprotein production are few and inconclusive. MTP is a microsomal protein that catalyzes the transport of triglyceride (TG), cholesteryl ester (CE), and phosphatidylcholine (PC) between membranes (13) *in vitro.* MTP is a heterodimer consisting of the multifunctional protein, protein disulfide isomerase, and a unique 97 kDa subunit (14). The 97 kDa subunit has been shown to be responsible for the lipid transfer activity. Identification of MTP as the defective factor in abetalipoproteinemia indicates that this protein is required for apoB lipoprotein assembly (15–17). However, these studies have not been informative with regard to understanding whether MTP is involved in the regulation of the level of apoB lipoprotein production. For instance, abetalipoproteinemia is a recessive disease. Only homozygotes display the phenotype; obligate abetalipoproteinemic heterozygotes show normal concentrations of plasma lipoproteins (18). As MTP is required for apoB lipoprotein secretion, assuming there is little change in lipoprotein turnover rates, then either MTP is not limiting in abetalipoproteinemic heterozygotes or MTP gene expression is up-regulated. If it is the former, then MTP would not be expected to modulate lipoprotein levels except in the case of nearly complete down-regulation. If it is the latter, then MTP may play a significant role in regulating the level of apoB lipoprotein output.

The advent of selective inhibitors of MTP (19–21) has raised the possibility that this question can be answered via a pharmacological approach. Indeed, all three of these inhibitors have been shown to decrease apoB secretion from the human hepatoblastoma HepG2 cell line in a dose-dependent fashion. However, as it is impossible to measure MTP-mediated triglyceride transfer activity in situ, the relationship between a given level of cellular MTP activity and apoB secretion cannot be determined from these types of studies. In order to directly characterize the role of MTP in determining the level of apoB lipoprotein production, a covalent photoaffinity inhibitor of MTP was designed and synthesized. This compound, BMS-192951, was used to decrease MTP-mediated lipid transfer activity in situ in HepG2 human hepatoblastoma cells, in McArdle RH-7777 rat hepatoma cells, and in primary rat hepatocytes. This allowed a direct comparison of the cellular levels of MTP activity with apoB secretion. Under the culture conditions studied, the concentration of apoB in the media decreased in proportion to the decrease in MTP activity, indicating that MTP activity was limiting in the production of apoB lipoproteins in all three cell types.

MATERIALS AND METHODS

Materials

All tissue culture media, serum, and reagents were obtained from GIBCO/Life Technologies (Bethesda, MD). All radiolabeled lipids and Amplify enhancing agent were obtained from Amersham (Arlington Heights, IL). Express [^{35}S]methionine/cysteine labeling mixture was obtained from New England Nuclear (Boston, MA). All lipids for the MTP assay and all other reagents not otherwise listed were obtained from Sigma (St. Louis, MO). Goat anti-human apoB and apolipoprotein A-I (apoA-I) antisera were purchased from Biodesign International (Kennebunk, ME). Rabbit anti-hamster apoA-I antibody has been previously described (22). Rabbit anti-rat apoB antiserum was a generous gift from Drs. Jan Borén and Sven-Olof Olofsson (Göteborg University, Göteborg, Sweden). MTP was purified from bovine liver as previously described (23). The purified protein had an activity of 3.0% TG transfer/min per μg of protein as measured by the previously described assay (19).

Synthesis of photoaffinity inhibitor of MTP, BMS-192951

A mixture of 3.2 g (15.0 mmol) of 2-(4-piperidinyl)-2,3-dihydro-1H-isoindol-1-one (19), 3.0 g (15.0 mmol) of 4-fluorobenzophenone (Aldrich Chemical Co., Milwaukee, WI), and 2.3 g (16.5 mmol) of K_2CO_3 in 30 ml of N,N-dimethylacetamide was heated at reflux for 15 h, and then cooled to room temperature. Diethyl ether (300 ml) was added and the mixture was washed twice with 70 ml water and 70 ml brine, dried over $MgSO_4$, and concentrated to give a solid. The crude product was purified by flash chromatography on silica gel (300 g) eluting with 4% acetone/CH_2Cl_2 to afford 2-[1-(4-benzoylphenyl)-4-piperidinyl]-2,3-dihydro-1H-isoindol-1-one (BMS-192951, 3.5 g, 59% yield) as a yellow solid (m.p. 173–175°C).

Lipid transfer assay

The assay to measure MTP-mediated [^{14}C]TG transfer was performed as previously described (19).

Inhibition of MTP activity by BMS-192951 in vitro

Pure bovine liver MTP (10 μg) was incubated with various concentrations of BMS-192951 for 1 h at room temperature in a final volume of 100 μl of assay buffer (15 mm Tris-HCl, pH 7.5, 40 mm NaCl, 1 mm sodium EDTA, and 0.02% sodium azide) in Opticlear glass vials (Kimble). The samples were irradiated with ultraviolet (UV) light by placing them on the top of an ultraviolet transluminator box (360 nm, UVP, San Gabriel, CA, Model TL-33E, 115 V, 60 Hz, 1.8 Amps) for 5 min at 4°C. The temperature of the sample was less than 30°C after the UV exposure. The samples were then dialyzed overnight in assay buffer at 4°C to remove unbound inhibitor and TG transfer activity was determined.

Measurement of in situ inhibition of MTP activity by BMS-192951 in HepG2 cells, McArdle RH-7777 cells, and primary hepatocytes

Cells were maintained in 60 × 100 mm dishes containing 2.0 ml medium. HepG2 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum. McArdle RH-7777 rat hepatoma cells were cultured in minimal essential medium (MEM) supplemented with 20% fetal bovine serum, non-essential amino acids, sodium pyruvate, and MEM-vitamins (growth me-

dium). All supplements were added according to the recommendations of the manufacturer. Rat hepatocytes were isolated by a collagenase perfusion technique (24) and were plated (8.0×10^6 cells/dish) in DMEM/F12 medium containing 20% fetal bovine serum. More than 90% of the cells excluded trypan blue. Fresh medium was added after 3 h, and the hepatocytes were incubated for 24 h prior to use.

Experiments were initiated by adding fresh medium to culture dishes containing 0.5% DMSO and the indicated concentrations of BMS-192951. After 1 h of incubation, cells were exposed to UV light for various lengths of time by placing the culture dishes (with cover) on top of the ultraviolet transluminator box (360 nm, UVP, San Gabriel, CA, Model TL-33E, 115 V, 60 Hz, 1.8 Amps) at 4°C. The temperature of the medium was less than 37°C after the UV exposure. Subsequently, cells were washed with PBS, homogenized in 0.25 ml of homogenization buffer (50 mm Tris-HCl, pH 7.4, 5.0 mm EDTA, 50 mm KCl, 1.0 mm phenylmethylsulfonyl fluoride and 0.5 μg/ml leupeptin) using a Polytron PT3000 (Brinkmann Instruments, Inc., Westbury, NY) for 20 sec at half maximal speed. Cell homogenates were diluted with homogenization buffer to a protein concentration of 1.75 mg/ml. To release microsomal lumenal protein, one part of sodium deoxycholate (0.54%, pH 7.5) was added to 10 parts of diluted homogenate and incubated on ice for 30 min prior to centrifugation at 412,000 *g* for 30 min at 4°C in a Beckman TL-100 ultracentrifuge. The supernatant was removed and dialyzed exhaustively overnight against the assay buffer at 4°C to remove unbound inhibitor. TG transfer activity was measured as described. In a separate experiment using radiolabeled BMS-192951, it was observed that under identical conditions, BMS-192951 that was not covalently bound to MTP partitioned out of HepG2 cells within 30 min, and thus did not contribute to the inhibition of MTP activity (data not shown).

Inhibition of apoB secretion by photoaffinity inhibitor in HepG2, McArdle cells, and primary hepatocytes

HepG2 cells. To determine the effect of inhibition of MTP activity on apoB and apoA-I secretion, cells were incubated in the presence or absence of BMS-192951 as described above. After 1 h, the cells were exposed to UV light for various lengths of time to covalently bind BMS-192951 to MTP and permanently inactivate it. The cultures were washed twice and then incubated with RPMI 1640 medium supplemented with 3% BSA, 0.8 mm oleic acid, and 1.0 mm glycerol under standard cell culture conditions (37°C, 5% CO_2). After 4 h of incubation, the media were harvested and assayed for apoB and apoA-I by enzyme-linked immunosorbent assay (ELISA) as previously described (25). To bring the concentration of the apolipoproteins into the linear range of the assay, media were diluted 30-fold and 60-fold for apoB and apoA-I, respectively. Each treatment condition was tested in duplicate cultures, and apoB and apoA-I were measured in triplicate.

McArdle RH-7777 cells. The effect of BMS-192951 on apoB and apoA-I secretion from McArdle RH-7777 cells was determined using a pulse-chase analysis as previously described (26), except the cells were chased for 2 h with medium supplemented with 3% BSA, 0.8 mM oleic acid, and 1.0 mm glycerol.

Primary rat hepatocytes. After treatment with inhibitor, the hepatocytes were incubated for 1 h with methionine and cysteine-free RPMI 1640 medium supplemented with 20% dialyzed fetal bovine serum. Subsequently, cells were pulsed with 200 μCi/ml [^{35}S]methionine/cysteine in the same medium for 30 min and then chased for 2 h with DMEM/F12 medium containing 20% undialyzed fetal bovine serum, 3% bovine serum albumin, 0.8 mm oleic acid, and 1 mm glycerol. The media were collected, adjusted to 1.0 mm PMSF, 1.0 mm benzamidine, 5.0 mm EDTA, and 50 μg/ml aprotinin, and centrifuged at 1,500 *g* for 5 min at 4°C to remove loose cells. ApoB-100 and apoB-48 were immunoprecipitated as previously described (26) using anti-rat apoB antiserum and were separated on a 4–20% SDS-PAGE gel. The gel was dried, exposed to X-ray film, and apoB-100 and apoB-48 were quantitated on a Personal Densitometer SI (Molecular Dynamics, Inc., Sunnyvale, CA).

RESULTS

BMS-192951 inhibits MTP lipid transfer activity by a covalent interaction

We previously described a compound, BMS-200150, that inhibited MTP-mediated TG transfer in vitro and selectively inhibited apoB secretion from HepG2 cells. BMS-192951 (**Fig. 1**) is a related compound containing a benzophenone photophore that reacts with closely placed C–H bonds when excited by UV light (27), resulting in covalent binding of the benzophenone moiety to the protein. Similar to its parent compound, BMS-192951 reversibly inhibited TG transport mediated by MTP without activation by ultraviolet light in a dose-dependent manner with an IC_{50} of 4.6 μm (data not shown). To test the ability of BMS-192951 to covalently inactivate MTP, the purified bovine liver protein was incubated with various concentrations of BMS-192951 and irradiated with UV light for 5 min. After exhaustive dialysis of the samples against assay buffer to remove non-covalently bound inhibitor, MTP-mediated TG transfer was measured. **Figure 2** shows that BMS-192951 inhibited TG transport in a dose-dependent manner when exposed to UV light. There was no inhibition of lipid transfer activity when MTP was incubated with the inhibitor without UV irradiation and then dialyzed, or when MTP was exposed to UV light in the absence of the inhibitor (results not shown). The IC_{50} obtained after 5.0 min of UV irradiation was 4.2 μm, a value similar to the IC_{50} obtained in the presence of inhibitor without UV irradiation (4.6 μm).

Effect of in situ MTP inhibition on apoB secretion in HepG2, McArdle RH-7777, and primary hepatocytes

The ability of BMS-192951 to block MTP activity by a covalent bond to the protein upon UV irradiation provided

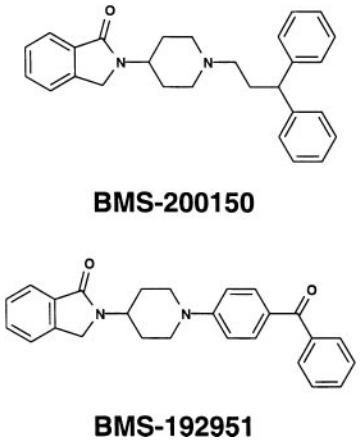

Fig. 1. The structures of BMS-200150 and its photoaffinity analog BMS-192951.

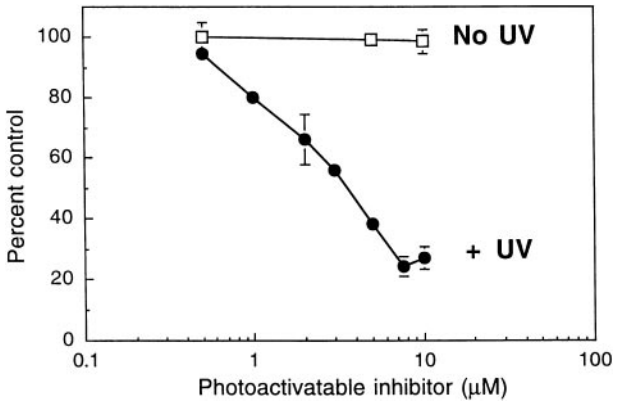

Fig. 2. Covalent inhibition of MTP activity by BMS-192951. Bovine liver MTP was incubated with various concentrations (0–10 μm) of BMS-192951 in a total volume of 100 μl and irradiated under UV light for 5 min. In addition, a series of tubes containing MTP and various concentrations of BMS-192951 were not exposed to UV light (No UV). A sample not containing BMS-192951 without any exposure to UV light was used as a control (12% transfer/100 ng protein) against which the percent inhibition was calculated. TG transfer activity of MTP was measured by SUV assay after exhaustive dialysis of MTP against assay buffer to remove unbound inhibitor. Results represent the mean ± SE of three measurements (n = 3). This experiment was repeated twice with similar results.

the means to decrease, in a step-wise fashion, MTP activity in situ, in order to investigate the relationship between cellular MTP activity and apoB secretion. MTP activity was inhibited to various levels in HepG2 cells by two different approaches. In one, HepG2 cells were incubated with 0–15 μm of BMS-192951 (**Fig. 3**, panel A) and the cells were exposed to UV light for 5 min. After inhibitor treatment, the cells were washed, fresh medium was added, and apoB and apoA-I secretion were quantitated after a 4-h incubation. In addition, the cells were harvested and TG transfer activity was measured in the microsomal extracts. The results demonstrated that BMS-192951 inhibited apoB secretion in proportion to the inhibition of MTP transfer activity. Little or no inhibition of apoA-I secretion was observed up to a concentration of approximately 8.0 μm. Concentrations of BMS-192951 higher than 8.0 μm were observed to be toxic in this cell line based upon inhibition of [^{3}H]thymidine incorporation into cellular DNA (data not shown). In order to achieve greater inhibition of MTP without damaging the cells, a second strategy to inhibit MTP activity was used. BMS-192951 (3.0 μm) was added to the cells and the length of exposure to ultraviolet light was varied from 0–15 min. Figure 3, panel B shows that MTP activity was inhibited approximately 70% after 15 min of exposure. Similar to the results shown in Fig. 3, panel A, apoB secretion decreased in proportion to decreasing MTP activity. No effect was observed on apoA-I secretion. Furthermore, no effect was observed on MTP activity or apoB secretion when cells were exposed to UV light in the absence of BMS-192951 (Fig. 3, panel C).

In order to test whether MTP is limiting in other cell culture models for lipoprotein assembly and secretion, the relationship between MTP activity and apoB secretion was investigated in McArdle RH-7777 cells and primary rat hepatocytes. Similar to HepG2 cells, a concentration of BMS-192951 (6.0 μm), previously determined to be nontoxic to the cells (data not shown), was used to progressively decrease MTP activity by exposing the cells to UV light for increasing lengths of time. In the absence of BMS-192951, there was no effect of UV exposure (15 min) on MTP activity in McArdle RH-7777 cells (91 ± 12% of control) or rat hepatocytes (104 ± 5% of control). The effect of MTP inhibition on apoB secretion was measured in both cell types by pulse-chase analysis. As was the case for the HepG2 cells, apoB secretion decreased in proportion to the decrease in MTP activity in both McArdle RH-7777

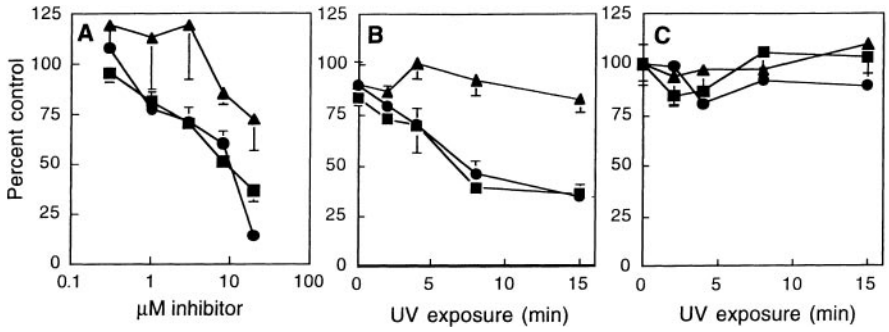

Fig. 3. Effect of inhibition of MTP activity on apoB and apoA-I secretion from HepG2 cells. Panel A: HepG2 cells were treated with various concentrations of BMS-192951 for 1 h and exposed to UV light for 5 min. Panel B: HepG2 cells were treated with 3.0 μm of BMS-192951 and exposed to UV light for various lengths of time. The amount of apoB (■) and apoA-I (▲) accumulated in the media over 4 h was determined by ELISA. The inhibition of MTP activity (●) was measured by the standard in vitro assay as described in Methods. At time = 0 min, MTP activity was 0.3% transfer/μg of cellular protein, apoB was 200 ng/mg of cellular protein per 4 h and apoA-1 was 6.0 ng/mg cellular protein per 4 h. Panel C: HepG2 cells were exposed to ultraviolet light (360 nm) for the indicated times without BMS-192951. The amount of apoB and apoA-I was measured by ELISA. MTP-mediated triglyceride transfer activity was measured by the standard assay. For each panel, the values shown are the mean ± SE of samples in triplicate from two separate experiments (n = 6), assayed twice. This experiment was repeated four times and similar results were obtained.

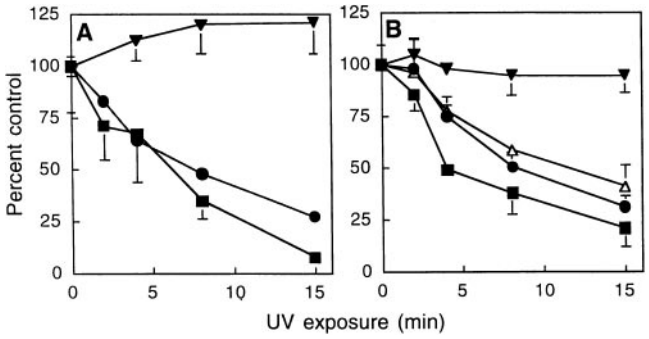

Fig. 4. Inhibition of MTP activity and apoB secretion in McArdle RH-7777 cells and primary hepatocytes by photoaffinity inhibitor. McArdle RH-7777 cells (panel A) and cultured primary hepatocytes (panel B) were treated with 6.0 μm BMS-192951 for 1 h and then exposed to UV light for various lengths of time. The effects of inhibitor treatment on the secretion of apoB (■) and apoA-I (▼) from McArdle RH-7777 cells and apoB-100 (■), apoB-48 (△), and apoA-I (▼) from primary hepatocytes were followed by a pulse-chase analysis. The inhibition of MTP activity (●) in microsomal extracts of McArdle RH-7777 cells and primary hepatocytes was measured by the standard in vitro assay. MTP activity was 0.23% and 0.31% transfer per mg of cellular protein in McArdle RH-7777 and rat hepatocytes, respectively. Each value is the mean ± SE of samples in duplicate from two separate experiments (n = 4), assayed twice.

cells (**Fig. 4**, panel A) and primary rat hepatocytes (Fig. 4, panel B). In addition, there was roughly equivalent inhibition of secretion of apoB in McArdle RH-7777 cells and apoB-48- and apoB-100-containing lipoproteins in rat hepatocytes. No effect was observed on apoA-I secretion from McArdle RH-7777 cells and primary hepatocytes.

DISCUSSION

In a previous study (19) we showed that MTP inhibitor BMS-200150 blocks apoB secretion from HepG2 cells with an IC_{50} of 1.8 μm, a value similar to that for inhibition of TG transfer activity measured in vitro and the K_d of binding at a single site. Similar observations have been reported for two other unrelated compounds (20, 21). These results are consistent with the studies of abetalipoproteinemia (15–17) and MTP and apoB expression studies in non-hepatic and non-intestinal cell lines (28–30) that showed that in the absence of functional MTP, assembly and secretion of apoB-containing lipoproteins does not occur. While these studies clearly show that MTP is required for apoB lipoprotein assembly and secretion, they do not address the role MTP plays in regulating the number of particles produced.

In order to investigate the relationship between MTP levels and apoB secretion, a method is required that allows MTP activity levels to be modified in situ, such that they can be measured. One approach would be to change MTP protein levels via regulation of expression of the MTP gene. However, the level of expression of the MTP gene is not highly regulated by external stimuli (25, 31). Furthermore, even if mRNA levels could be substantially changed, the long half-life of the protein (4.4 days) would require unacceptably long periods of treatment to achieve new steady-state levels of MTP activity. Alternatively, MTP expression levels may be modified by genetic means such as stable transfection. However, this introduces additional complications such as clonal differences between cell lines and genetic compensation. Furthermore, our attempts to date to increase MTP expression in hepatoma cells via transient transfection have been unsuccessful. Finally, MTP activity may be modified using a selective inhibitor. However, the currently available inhibitors are all non-covalent in nature. Therefore, the standard assay for measuring MTP activity which utilizes both dialysis and dilution of the sample would cause the compounds to dissociate from MTP. This makes it impossible to measure the level of MTP activity present in the intact cell and therefore determine the relationship between MTP activity and the level of apoB secretion. In order to circumvent this problem, the previously reported MTP inhibitor, BMS-200150, was modified to produce the photoaffinity compound BMS-192951 (Fig. 1). This novel compound retained the ability to inhibit MTP-mediated triglyceride transfer in vitro in the absence of ultraviolet light (IC_{50} = 4.6 μm, data not shown). As shown in Fig. 2, incubation of purified MTP with BMS-192951 followed by exposure to ultraviolet light at 360 nm permanently inactivated MTP. With this unique chemical reagent, it was possible to variably reduce MTP levels in situ, in a covalent manner that allowed measurement of MTP-mediated triglyceride transfer activity. Analyses of the relationship between in situ MTP activity and apoB secretion were carried out in HepG2 cells, McArdle RH-7777 rat hepatoma cells, and primary rat hepatocytes.

The level of MTP activity in cultured cells was reduced in situ in a step-wise fashion by covalent inactivation with BMS-192951. The concentration of apoB in the media decreased in proportion to the decrease in MTP activity. No effect was observed on apoA-I under these conditions in these cell culture models. These results indicate that MTP is rate limiting for the assembly and secretion of apoB-containing lipoproteins. Therefore decreases in the amount of cellular MTP activity, either at the level of gene expression or post-translationally, will decrease apoB lipoprotein assembly and secretion in these cell culture models.

This conclusion, however, does not explain the observation that obligate abetalipoproteinemic heterozygotes have normal levels of plasma apoB lipoproteins. One possible explanation for this discrepancy is that the normal allele is up-regulated in heterozygotes providing normal levels of MTP. Unfortunately, the levels of MTP in lipoprotein-producing tissues from obligate heterozygotes have never been measured. Even though previous studies have shown that the levels of MTP activity are not acutely regulated in response to external stimuli, a longer term response to dietary and hormonal regulation has been demonstrated (25, 31). Thus, it is possible that a 2-fold increase in expression of the functional allele occurs during cellular differentiation in heterozygotes. It is also formally possible that a compensatory decrease in lipoprotein clearance

along with a decrease in production could explain the lack of a decrease in lipoprotein levels in obligate abetalipoproteinemia heterozygotes. However, there is currently no evidence to support this hypothesis. Therefore, given that a defect in MTP is the only known perturbation in abetalipoproteinemia, any compensation most likely occurs at the level of production.

Evidence has accumulated that TG, cholesterol, CE, and PC synthesis have an impact on the formation and secretion of apoB-containing lipoproteins (reviewed in 6–8). For example, feeding fatty acids to HepG2 and McArdle RH-7777 cells increases TG synthesis and apoB secretion. Similar effects of lipid supplementation have been observed in non-hepatic and non-intestinal cells co-transfected with MTP and apoB. The total amount of apoB secreted was increased 2- to 2.5-fold in both HeLa cells and COS cells after addition of lipid to the growth media (28, 29). Thus, although the current study shows that MTP is limiting in these cell lines, an increase in lipid synthesis can also increase apoB secretion, and this has been shown to occur without an increase in MTP levels (25). This can be explained based upon the lipid transfer characteristics of MTP. Atzel and Wetterau (32) showed that there is a positive correlation between the TG concentration in donor membranes and the rate of MTP-mediated lipid transfer. As a result, MTP-mediated TG transport increases with increasing TG concentrations in the donor membranes. Thus, by feeding oleic acid to a cell, the rate of lipid synthesis increases as does the availability of TG in the endoplasmic reticulum membrane. This stimulates MTP-mediated lipid transport allowing recruitment of additional apoB polypeptides into the assembly and secretory pathway. Thus, both MTP and lipid levels can influence the assembly and secretion of lipoproteins, indicating that MTP-mediated lipid transport to nascent apoB polypeptides is limiting.

In summary, a photoaffinity inhibitor of MTP, BMS-192951, was used to show that covalent inactivation of lipid transfer activity in situ blocks apoB secretion from HepG2 cells, McArdle RH-7777 rat hepatoma cells, and primary rat hepatocytes. The ability of this compound to variably reduce MTP activity was utilized to demonstrate that apoB secretion decreased in parallel to the decrease in MTP activity in all three cell systems. We conclude from these studies that MTP-mediated lipid transport is limiting in the assembly and secretion of apoB-containing lipoproteins in these hepatocyte cell models. Thus, the level of MTP activity plays a significant role in determining the level of apoB lipoprotein production.

Manuscript received 20 August 1997 and in revised form 12 February 1998.

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