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THE ROLE OF MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN IN LIPOPROTEIN ASSEMBLY: AN UPDATE

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1. ABSTRACT

The discovery of the role of MTP in mediating lipid transfer to apoB has greatly expanded our knowledge of the molecular mechanisms involved in apoB-containing lipoprotein assembly. In this review, advances referring to the structure, regulation and function of MTP are summarized and discussed. In addition to the well-known lipid transfer activity function, MTP has been shown to physically interact with apoB and this association appeared to be critically important in the regulation of lipoprotein production. Recent studies have provided insight into the paradoxical relationship between MTP polymorphism variants and metabolic disease. Genetic variants of MTP and their possible impact in the development of cardiovascular disease are discussed.

2. INTRODUCTION

Microsomal triglyceride transfer protein (MTP) is involved in the assembly of triglycerides-rich chylomicrons in enterocytes and very low density lipoproteins (VLDL) in hepatocytes. Based on its in vitro transfer activity, MTP was shown to play an essential role in the transfer of neutral lipid to apolipoproein B (apoB) during the early stages of lipoprotein assembly (1,2). Lipoprotein biosynthesis is defective in abetalipoproteinemia due to mutations in the MTP gene, which are not linked to known mutations in apoB gene (3). The discovery of this genetic disorder clearly underscored the importance of MTP in lipoprotein biogenesis. Recent findings indicate that variants of MTP gene are associated with changes in plasma cholesterol levels and development of cardiovascular diseases (4). Biochemical, structural and molecular roles of MTP in apoB-containing lipoprotein assembly, has been covered in detailed and comprehensive reviews (1,5-10,103,104). The aim of the present review is to provide an overview of the role of MTP's chaperone-like activity in lipoprotein assembly and to describe recent progress in genetic studies of MTP polymorphism, as well as the importance of MTP regulation in the development of cardiovascular diseases.

3. STRUCTURE, FUNCTION AND REGULATION OF MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN

MTP was originally found in the bovine liver endoplasmic reticulum (ER) to accelerate the transfer of triglycerides (TG), cholesterol ester (CE) and phospatidylcholine (PC) between vesicles (11). MTP activity was subsequently found in microsomal fractions of liver, intestine and heart of other animals (11-14). The purified heterodimeric MTP protein consists of two non-covalently bound polypeptides of 97 (M subunit) and 55 (P subunit) kDa (11). The small 55-kDa "P subunit'', ubiquitous ER resident enzyme disulfide isomerase (PDI), is required to maintain the lipid transfer activity of the M

subunit and to prevent its aggregation in the ER (15-17). The isomerase activity is not essential for its association with the larger M subunit and for the MTP activity, as PDI mutants lacking enzyme activity are normally functional in association with a normal M subunit (18). The human complementary DNA (cDNA) encoding the M subunit was isolated, sequenced and characterized by Sharp *et al.* (19, 20). High homology sequence was found between human MTP (M subunit), bovine (19) and hamster MTP cDNA (21). The crystal structure of MTP is still unsolved. However, based on the known structure of lamprey lipovitellin (LV) (22, 23, 104) a molecular model of MTP was constructed. This model predicts two-domain structure, ßC amphipatic sheet and a domain, in the M subunit with conserved lipid-binding pockets consisting of apolar residues in each domain (2, 24, 25). Mann *et al.* (24) reported in a model that residues 34-263 of amino-terminus domain of MTP have 13 ß strands that correspond to barrel-like domain in lipovitellin. This domain is followed by an a-helical domain between amino acid residues 304-598. Additional information based on X-ray crystal structure of LV provided a structural model for MTP with strong conservation of lipid binding domains ß-strands 2-5 of the A-sheet and helix A and B (46). The predicted ß-sheet structure, which consists of lipid binding cavity with functional polypeptides, was also found in several other lipid modifying and transfer proteins (47-50).

In vitro studies showed that MTP transfers lipids by a shuttle mechanism between membranes (12). This mechanism, involving ping-pong bi-bi kinetics, suggested that MTP molecule is able to interact transiently with a membrane, extract lipid molecules, dissociate from the membrane, bind transiently with another membrane, deliver lipids rapidly to the membrane, and become available for another cycle of lipid transfer. Kinetic analysis of lipid transfer suggests that MTP has two separate lipid binding sites, a fast and a slow one (12). The fast site is implicated in lipid transfer of both TG and CE (12).

MTP has been reported to play two major roles in apoB-containing lipoprotein assembly: (i) MTP acts as a chaperone that mediates the translocation of newly-synthesized apoB across ER membrane (26-32,102) and (ii) MTP participates in the co-translational lipidation of apoB during its translocation into the ER (33-36,69,70,102). Using several approaches to examine apoB translocation across the ER membrane, Macri et al. (30) demonstrated that MTP activity is not essential for the movement of apoB in the ER lumen. Evidence from studies demonstrating physical interaction between MTP and apoB and the discovery of binding domains extends the notion that MTP chaperone activity participates in the efficient translocation of apoB (24,28,38-43). However, further in vivo investigations are needed to elucidate whether direct chaperone activity of MTP influences newly synthesized apoB translocation across ER membrane. The mechanism by which MTP transfers lipids during the two steps of lipoprotein assembly (27,44,45) remains incomplete and controversial.

Lin *et al*. performed the first in vivo study on the regulation of MTP (21). They showed that high fat diet and high sucrose diet had profoundly enhanced hepatic and intestinal MTP mRNA levels with some differences in response to the duration of exposition. The effect of high fat diet on hepatic MTP mRNA was more chronic, whereas significant up-regulation of intestinal MTP mRNA was observed just after 24h of treatment. In addition, not only the amount of dietary lipids, but also the fatty acid composition in diet plays important role in the regulation of MTP expression. Many studies lack significant correlation between changes in MTP mRNA levels, protein masss, and lipid transfer activity because this protein is not secreted and it has a half –life of 4.4 days as reported in HepG2 cells (74). The mechanism by which dietary fat induces MTP mRNA increase is not known. Recent studies demonstrated that fresh garlic inhibits MTP expression in human liver and intestinal cell lines and in rat intestine (75), but the molecular mechanism of garlic-induced MTP changes and identification of active components are not known. The MTP promoter contains a positive sterol element and a negative insulin response element (76), which appears to be active in vitro and in vivo. Bennett et al. (77) showed that hamsters fed with high cholesterol diet increased their level of hepatic MTP mRNA and this was associated with an elevation of plasma VLDL concentration. In streptozotocin diabetic rats, Wetterau et al. (5) reported a 65% increase in hepatic mRNA levels with no changes in the intestine. This effect was attenuated upon insulin injection. Recent studies revealed that hepatic MTP mRNA expression is increased by 45% in hyperinsulinemic diabetic ob/ob mouse and this was associated with significant effect on MTP activity and triglycerides secretion from the liver (78). In HepG2 cells, insulin negatively regulates MTP gene expression (74). Taghibiglou et al. (79) developed a new golden Syrian hamster model for insulin resistance in which they demonstrated that fructose feeding was associated with a mild hypertriglyceridemia, apoB-VLDL overproduction and increased expression of MTP. Recent studies, from the same group, revealed that amelioration of hepatic insulin resistance with rosiglitazone normalizes MTP expression and ameliorates apoB-VLDL production (80). Research on MTP regulation has recently focused on study of postprandial dyslipidaemia and diabetes. Phillips et al. demonstrated that diabetic rabbits had significant increase in intestinal MTP expression and activity without any change in the liver. This finding suggests that intestinal, rather than hepatic MTP may be the cause of enhanced number of small chylomicron particles observed in postprandial diabetic dyslipidaemia (81). It has been shown that ethanol down-regulates hepatic and intestinal MTP expression in HepG2 cells and rats (82). Similar effect has been observed with endotoxin and cytokines (83). Unlike ethanol treatment, prolonged incubation with cytokines did not alter apoB secretion by HepG2 cells suggesting that MTP mRNA changes do not contribute significantly to cytokines-induced effects on lipoprotein secretion. More investigations are needed, however, to increase our understanding for the molecular mechanisms involved in the MTP regulation and its impact on apoB-lipoprotein assembly and secretion.

4. FUNCTIONAL POLYMORPHISM IN THE MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN GENE

The promoter region of MTP gene is highly conserved between species (76). Human and hamster MTP

promoter activities were suppressed by cholesterol (76) and similar effect of insulin was observed in HepG2 cells (21). Hypothesis that genetic variation in MTP gene expression might influence plasma lipoprotein levels in humans was based on two important observations: (i) Absence of functional MTP in abetalipoprotenemia, a rare autosomal and recessive disease that causes defect in the assembly and secretion of VLDL and chylomicrons. (ii) MTP plays a critical role in apoB-lipoprotein assembly and secretion in vitro and in vivo. Evidence is emerging to indicate that genetic variations in the MTP promoter are related to coronary atherosclerosis. In 1998, Karpe *et al.* (84) were the first to report that polymorphism of the MTP gene was associated with changes in plasma concentrations of apoB-containing lipoproteins in humans. In fact, they were able to detect a common G/T polymorphism located 493bp upstream from the start of the transcription site in MTP gene. This rare allele with a frequency of 0.25 showed an increase in the transcriptional activity. Elevation of transcriptional activity was confirmed in transfected HepG2 cells using chloramphenicol acetyl transferase reporter gene. Furthermore, healthy human homozygotes had significantly lowered plasma LDL cholesterol. This was associated with significant decrease in apoB content of VLDL and LDL particles as well as an increase in TG/apoB ratio within VLDL particles. The fact that increased expression of MTP was associated with a considerable decrease in plasma LDL cholesterol levels in humans is intriguing. However, authors speculated that in this case, MTP -493T promoter variant could act by shifting the balance between the secretion of large and small VLDLs. Enhanced MTP activity would result then in the formation of fewer and larger VLDL, leading to lower the input of LDL production from VLDL. In addition, the authors arise questions whether high expression of MTP could affect the intracellular cholesterol homeostasis that might result from elevated transfer of cholesterol by MTP and consequently affecting the regulation of LDL receptor activity. In familial hypercholesterolemia, a common autosomal dominant disorder that causes excessive elevation of LDL in plasma, Lundahl *et al*. (85) reported that MTP -493G/T promoter variant shifted the observed LDL-cholesterol lowering effect in healthy subjects to a serum triglyceride-lowering effect in FH, suggesting a possible role for MTP gene expression in modulating clinical phenotype of FH. Recently, Ledmyr *et al.* (4) studied the influence of plasma lipid and lipoprotein levels by three promoter MTP polymorphism (-493G/T, -400A/T, and -164T/C) upstream of transcription start and four common missense polymorphism (Q/H95, I/T128, Q/E244 and H/Q297). This study indicated that two promoter polymorphism (-493G/T and -164T/C) and one missense polymorphism (I/T128) significantly lower plasma total and LDL cholesterol levels and plasma apoB-LDL levels. This was associated with a significant increase in body mass index and waist circumference measurements and plasma insulin levels. The mechanism by which MTP polymorphism induces changes in the degree of obesity such as body mass index and waist circumference remains unanswered.

Results from the coronary artery risk development in young adults (CARDIA) study showed that TT genotype of common functional polymorphism (-493G/T) was associated with significant elevation of total cholesterol, LDL cholesterol, triglycerides and apoB in human plasma (86). The explanation of the opposite finding reported in this study may be due to important differences such as samples size, gender, race, age, BMI, diet and other environmental factors. The functional polymorphism in the promoter region of Mttp gene (-493G/T) has been shown to be associated with liver steatosis in patients with type II diabetes (87) suggesting that the G allele of MTP promoter which encodes a decrease in MTP activity may contribute to intrahepatocyte triglyceride accumulation and, consequently, to a decrease in lipoprotein secretion. It remains to be determined if moderate reduction in MTP activity as a consequence of variation in MTP promoter might, by itself, be sufficient to induce steatosis. This study need to be extended to other metabolic diseases in different populations. Others have not been able to demonstrate a significant change in lipid profiles for the MTP polymorphism gene (88, 89). Herrmann *et al*. (88) identified two polymorphisms in the 5' flanking region of MTP gene, designated -400A/T and -164T/C. The MTP -164T polymorphism lies in a consensus sequence (-174 to -163) that shows homology to the human LDL receptor promoter, sterol regulatory element (SRE) and other physiologically related genes (90). Hagan *et al.* (76) showed that deletion of sequences 5' to -239 bp in transfected HepG2 cells had no effects on the promoter activities whereas, further deletion from -239 to -121 bp enhanced the promoter activity by 2.5 fold.

In the Etude Cas-Témoin de l'infractus du Myocarde (ECTIM) study, none of the -400A/T and -164T/C variants showed any association with angiographically assessed coronary stenosis or plasma lipoprotein profiles (88). Similarly, absence of significant association between genetic variations in the MTP promoter and lipoprotein profiles as well as CHD risk has been reported (89). In the Framingham Offspring Study, Couture *et al.* (89) demonstrated that 493G/T polymorphism, in men and women, has no effect on plasma levels of total cholesterol, LDL cholesterol, apoB and lipoprotein subclasses analyzed by NMR technique.

There is a clear inconsistency in the reported association between MTP polymorphism and variations in lipid profiles and this may be due to differences in environmental factors and/or differences in genetic background of populations studied. However, caution is required in interpreting these results and further investigations are required to determine mechanisms that could link MTP polymorphism to variations of lipoprotein profiles in high-risk population. In addition, functional analysis is needed to investigate whether the effect on lipid abnormalities accounted by one or more polymorphism variants that shows linkage disequilibrium with other unidentified variants within a gene encoding a protein regulating MTP expression. Screening for more functional MTP promoter variants in high-risk population might significantly help in the choice of lipid lowering strategies for the prevention and treatment of cardiovascular diseases.

5. ROLE OF MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN IN THE ASSEMBLY OF PLASMA LIPOPROTEIN

5.1. Lipid transfer and chaperone activities of MTP

As mentioned earlier, intracellular assembly of apoB with lipids in hepatocytes and enterocytes requires MTP. From evidence based upon in vitro studies, apoB has been demonstrated to be lipidated through two discrete steps (45,51,52,103,104). Whether or not the second step requires MTP or not is still debated (34,45,53,54). When lipid synthesis or MTP activity are limited, ApoB100 is not secreted but rapidly degraded by the proteasomal pathway and/or by non proteasomal mechanisms (31,33,55-59). Bakillah *et al*. first showed that MTP contains two independent, apoB binding and lipid transfer, domains (10,27). Liang and Ginsberg showed that lipid transfer and apoB binding activities of MTP are independent of each other but both are required for efficient secretion of apoB-containing lipoproteins (29).

The importance of MTP in lipoprotein assembly has been demonstrated by the findings of previous and recent studies using MTP inhibitor molecules (6,9,60-63). The apoB-lipid assembly and the function of MTP have been well established in vitro and in vivo (see reviews (1,5-10,103,104) for more details). In vivo studies demonstrated that specific inhibitors for MTP activity were able to lower plasma cholesterol and triglycerides levels by > 80% in rabbits, hamsters and rats (63). Homozygous Mttp knockout mice are not viable, but heterozygous knockout mice developed normally and showed 50% reduction in MTP activity levels in both the liver and intestine. This was accompanied with significant reduction of plasma apoB100 but less effect on ApoB48 (37,64). Liver-specific inactivation of Mttp gene, using Cre/LoxP strategy, abrogated the plasma VLDL/LDL response to high cholesterol diet (65). Interestingly, Raabe *et al.* (37) demonstrated that absence of MTP prevents the formation of TG droplets necessary for the fusion with nascent apoB in the ER lumen, suggesting a potential role of MTP in the formation of lipid droplets. Additional investigations are needed to clarify the role of MTP in this process. In addition, adenoviral overexpression of MTP in mouse liver has been shown to increase hepatic secretion of triglyceride-rich apoB-containing lipoproteins (66,67).

Two independent studies have provided the first evidence for in vitro physical interaction between apoB and MTP (38,39). In order to explore these interactions, Hussain *et al.* developed an independent solid-liquid inter-phase binding assay (40). Protein-protein interactions between MTP and apoB were found to be of high affinity and ionic in nature. Baculoviral expression studies have confirmed that apoB17 and the M subunit of MTP interact with each other with high affinity (24). Using chemical modification method, we have shown that lysine and arginine residues in apoB are critical for MTP binding, but are different from those involved in heparin and LDL receptor binding (41). Most likely, positively charged amino-acids in apoB interact with negatively charged residues in MTP. In addition, we have demonstrated that the binding of apoB to MTP decreases with increases in the length and degree of lipidation in apoB polypeptide (40). We also have proposed that MTP binding and proper disulfide bonds formation in apoB are mutually exclusive and independent events in the biosynthesis of apoB-containing lipoproteins. Most likely, MTP binding precedes disulfide bond formation (68). Expression of flag-apoB chimeras in COS cells indicated that amino acids 430-570 in apoB are critical for MTP binding (42). Bradbury *et al.* (43) have used yeast two-hybrid system and coimmunoprecipitation techniques to map the MTP binding site in apoB. They discovered an 'overlapping' second apoB binding site between residues 512 and 721, which interacts with residues 517-603 of MTP. An additional binding site was also identified in the N-terminal region of apoB (residues 1-264), which interacts with residues 22-303 of MTP (24). Du *et al.* (32) have provided compelling evidence, from abetalipoproteinemia patients, that MTP plays essential role in apoB translocation. More recently, Liang and Ginsberg (29) performed a series of studies with deleted apoB constructs and provided direct evidence for the role of MTP-apoB binding in lipoprotein secretion by liver cells.

5.2. Physiological significance of MTP-apoB binding in lipoprotein assembly

To address potential importance of MTP-apoB interactions during lipoprotein assembly, we used an MTP-apoB antagonist (AGI-S17) in our invitro binding assay, as well as in invitro cell culture system. AGI-S17 abolished MTP-apoB binding by 60-70% at 40 μM, but had no effect on MTP's lipid transfer activity (27). In human and rat liver cells, AGI-S17 inhibited significantly the intracellular MTP-apoB association without affecting lipid-transfer activity. In addition, nascent apoB was inhibited by 70-85%, suggesting that inhibition of apoB secretion by AGI-S17 is a direct consequence of the inhibition of intracellular MTP-apoB binding. These findings provide strong evidence that protein-protein interactions between MTP and apoB may be important for lipoprotein assembly and secretion. Multiple lines of evidence from different laboratories support our finding. Bradbury *et al.* (43) demonstrated that Arg-531 mutation of the apoB buried salt bridge residues 512-592, which disrupted interactions between MTP and apoB, had a marked effect on apoB-containing lipoprotein secretion. Additional support was reported recently by Liang and Ginsberg (29) demonstrating that deletion of the first 210 amino acids that contains the first MTP binding site significantly decreased the secretion of apoB34 but had no effect on the secretion of apoB16. The authors concluded that MTP binding and transfer activities are independent of each other, but both are essential for the secretion of apoB-containing lipoproteins by liver cells (29). These observations strengthen the hypothesis that MTP-apoB binding is critical for proper lipoprotein assembly.

Extensive studies using MTP inhibitors to elucidate the role of MTP in lipoprotein assembly contributed to configuration of several models proposing mechanisms by which neutral lipid core may be added to nascent apoB (6,9,25,27,34,103,104). In the light of

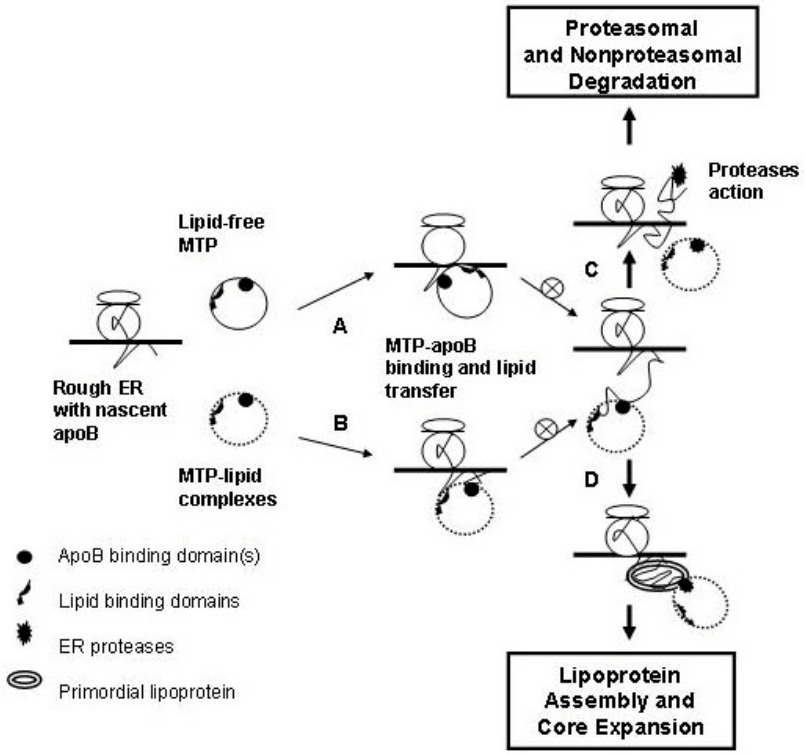

Figure 1. Proposed roles for MTP chaperone activity in lipoprotein assembly.The nascent apoB polypeptide is shown associated with the inner leaflet of the ER membrane, and MTP has been depicted to contain two independent, lipid transfer and apoB binding, domains. When the nascent polypeptide containing the MTP binding site emerges toward the lumenal side of the ER, it interacts with lipid-free MTP or MTP-lipid complexes. A successful binding between these proteins will result in complete translation, translocation, proper lipidation and assembly of apoB into primordial lipoprotein precursor particles (A, B). MTP-lipid complexes may play an important role in the fusion of triglyceride droplets with primordial lipoproteins to form mature lipoproteins during the 'core expansion' in the two-step process (D). If the binding between MTP and apoB is inhibited (represented by "X") by the use of inhibitors, such as AGI-S17, nascent apoB polypeptide will then be exposed to intracellular degradation involving proteasomal and non proteasomal mechanisms (C) depending on specific cell type and metabolic state as described in (59). Details for the two-step lipidation process are not mentioned in this simplified model (see 6,8-10,103,104).

progress towards the importance of MTP chaperone activity in lipoprotein assembly, we propose the following model (Figure 1): As soon as apoB nascent polypeptide containing the MTP binding site emerges toward the lumenal side of the ER, it interacts with either lipid-free MTP molecules or MTP-lipid complexes. MTP associated with lipids has a high affinity for apoB (10) and thus may readily associate with apoB. We emphasize that the binding process may precede lipidation of apoB because the MTP binding site (42) is translated prior to the lipid binding domains (71-73) of apoB. Lipid-free MTP bound to apoB may also extract lipids from the ER membrane and lipidate apoB. MTP-lipid complexes may play an important role in the fusion of triglyceride droplets with primordial lipoproteins to form mature lipoproteins during the core expansion process. MTP has been proposed to contain sequences that may have fusogenic properties (46), but the fusion mechanism is unknown. We speculate that protein-protein interactions between MTP and apoB may induce conformational changes in apoB which juxtapose lipids domains in MTP with exposed lipid-binding domains of apoB and consequently, facilitate fusion process and core expansion. Increases in the length and lipidation of apoB would decrease interactions between these proteins (40) and result in the formation of primordial lipoproteins that are secretion-competent (10,27,53). Thus, the two independent MTP activities and lipid availability are essential for efficient apoB secretion.

As discussed before, MTP by interacting with nascent apoB may act first as a chaperone before the lipidation process starts. Inhibition of these interactions may lead to decreased translocation of nascent apoB peptides into the lumen, decreased lipoprotein assembly, and increased intracellular degradation most likely involving ubiquitin-proteasomal and/or nonproteasomal mechanisms (Figure 1& Figure 2). We speculate that under such condition, in which only chaperone activity is affected but not lipid transfer activity, fewer and larger lipid-rich apoB-VLDL species will be formed leading to efficient triglyceride transporting capacity and to less pronounced fatty liver development (Figure 2). It is well established that large VLDL particles are not direct precursors of LDL, and consequently their input for the net circulating LDL fraction will be decreased. It is difficult to speculate about potential attempt for the liver to limit triglycerides accumulation, but

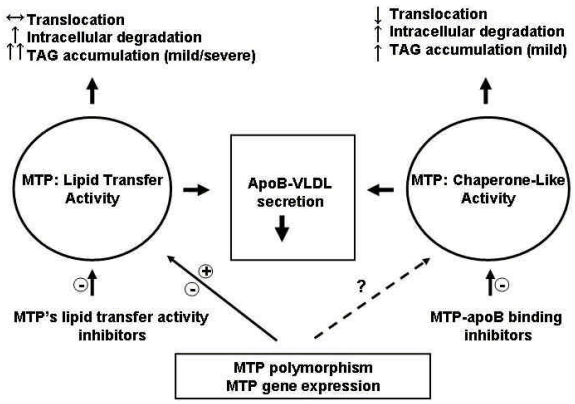

Figure 2. Postulated actions for partial inhibition of MTP lipid activity and MTP chaperone activity. Exogenous and genetic factors increase (+) or decrease (-) MTP responsive elements of MTP gene through various mechanisms, thereby modifying MTP lipid transfer activity or MTP chaperone-like activity. MTP lipid activity drugs inhibit apoB lipidation without affecting its translocation. Non lipidated apoB undergoes intracellular degradation involving ubiquitin-proteasomal and/or nonproteasomal mechanisms. This may result in a significant accumulation of intracellular triglyceride content (fatty liver). In contrast, MTP binding inhibitors, such as AGI-S17, may affect specifically apoB translocation without altering MTP lipid transfer activity resulting, probably, in less pronounced accumulation of triglycerides within the ER. The residual MTP activity might probably promote secretion of fewer and larger apoB-particles. Specific drugs targeting either MTP lipid activity or its chaperone activity will result, in both cases, in significant decrease of apoB-containing lipoprotein production.

in the situation of reducing hepatic lipoprotein production by MTP binding inhibitors this issue may not be totally free of nonalcoholic steatohepatitis. Another question is whether modest changes in MTP chaperone activity could have significant impact on the amount and composition of secreted apoB-lipoproteins.

New potent and specific inhibitors targeting independently MTP activities can now be explored in this proposed hypothetical MTP-assisted lipoprotein assembly.

5.3. In vitro modulation of of MTP-apoB binding

In HepG2 cells, Wu *et al.* (38) showed that oleic acid treatment, which protects apoB from intracellular degradation, increased both the degree and the duration of binding between MTP and apoB. In addition, inhibition of TG synthesis by triacsin D inhibited MTP-apoB association suggesting that lipids availability may play essential role in the modulation of interaction between these two proteins (38). Hussain *et al.* (40) showed that MTP binding was maximal for apoB17 and increasing the length of apoB from 17% to 42% results in substantial decrease in MTP-apoB association. The reasons for this decrease in MTP binding with increase in apoB length are not known and need further investigation. We also observed that addition of exogenous zwitterionic phospholipids, enhanced by 2-4 fold MTP-apoB binding, whereas negatively charged phospholipids, decreased MTP-apoB binding (10). In addition, we demonstrated that incubation of MTP with lipid vesicles resulted in a stable association of MTP with vesicles, and MTP-lipid complexes bound better (5 fold) to apoB than did lipid-free MTP. These studies clearly indicate that the association of MTP with phospholipid vesicles results in increased affinity for apoB (10). At the present, we do not know how MTP-lipids complexes increase its affinity for apoB, but we were able to provide evidence that such stable MTP-lipid complexes exist in the lumen of ER from liver cells (10). Biochemical and molecular characterization of these MTP-lipid complexes is needed to better understand their role in lipoprotein assembly. Also, the potential role of the network ER-resident chaperones in this process remains to be determined.

6. MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN INHIBITORS AND THE ROAD AHEAD

MTP is a key factor in the assembly of VLDL, the direct precursor of LDL. Interest in MTP has been stimulated by the large reports describing the effects of MTP inhibitors on lipoprotein production. The liver-specific knockout mice (37, 65) and MTP overexpression studies (66, 67) have demonstrated that MTP is rate-limiting for apoB-VLDL secretion. These findings suggested that MTP may play essential role in modulating lipoprotein assembly and secretion. In liver (60) and intestinal (91) cells inhibition of MTP activity has been shown to significantly decrease the rate of apoB-containing lipoprotein production in a dose dependent manner. Interestingly, Wetterau *et al.* (63) reported an MTP inhibitor that was able to normalize atherogenic lipoprotein levels in the Watanabe-heritable hyperlipidemic rabbit, a model for homozygous familial hypercholesterolemia.

Complete absence of MTP in abetalipoprotenemia leads to the accumulation of triglycerides in the small intestine and liver. In contrast, heterozygous carriers are unaffected suggesting that partial deficiency of MTP has no functional consequences. In addition, liver-specific Mttp knockout mice had only moderate hepatic steatosis on a low-fat chow diet compared to the apoB mutation that appeared to induce much greater liver triglyceride stores (37,79,92). This interesting finding increased efforts from investigators in pharmaceutical industry that are seeking new therapeutic strategies for treating hyperlipidaemia. As shown in (Table 1), a number of MTP inhibitors have been developed but unfortunately many of them have been discontinued. Safety profile of such compounds remains the major concern and unfortunately, many of them were discontinued at early stages of development probably because of sign of severe hepatic lipid accumulation.

A study of fatty liver disease and plasma lipoproteins in kindred familial hypobetalipoproteinemia with apoB-54.5 truncation revealed that four of eight carriers developed fatty liver disease which appears to be more severe in the presence of high alcohol intake (93).

Table 1. MTP inhibitors (adapted from Pharmaprojects database)

Compound	Company	Indication	Development status
BMS-200150	Bristol-Myyers Squibb	Atherosclerosis	Preclinical/ Active
BMS-212122	(USA)	Hyperlipidaemia	Preclinical/ Active
BMS201038			Phase I/ Ceased
R-103757	Johnson & Johnson	Atherosclerosis	Preclinical/ Ceased
	(USA)		
CP-467688	Pfizer	Atherosclerosis	Preclinical/ Ceased
CP-319340	(USA)		
GW-328713	GlaxoSmithKline	Atherosclerosis	Phase I/ Ceased
	(UK)	Hyperlipidaemia	
BAY-139952	Bayer	Mixed Dyslipidaemia	Phase II/ Active
	(Germany)		
MTP-1403	Maruko Seiyaku	Antidiabetic	Preclinical/ Ceased
MTP-1307	(Japan)		Preclinical/ Ceased
MTP-3115			Preclinical/ Ceased
MTP-3631			Preclinical/ Ceased

This finding suggests that additional environmental, hormonal or genetic factors may contribute to lipid accumulation (94). Recent reports showed that decreased MTP activity contributes to initiation of alcoholic liver steatosis in rats (95). In addition, Hepatitis C virus core protein inhibits MTP activity and VLDL secretion in a model of viral-related steatosis (96). Even though the mechanism of fatty liver development is not clearly known, the increase efforts in better understanding VLDL assembly will provide a good strategy for a tissue-specific MTP targets. There is substantial evidence that MTP inhibitors acts differently in liver and intestine, however, there has been a tendency to extrapolate findings from studies of liver cells to intestinal cells. Inhibition of MTP activity in human liver hepatoma cell lines, HepG2, decreased the net synthesis of apoB100 probably by delaying its translation and promoting co-translational proteasomal degradation (33). In contrast, inhibition of MTP activity in rat hepatoma cell lines, McA-RH7777, did not affect significantly apoB100 synthesis (59). Kulinski *et al.* (97) showed that MTP inhibitor reduced TG secretion from murine hepatocytes by 85% and decreased the amount of apoB100 in the ER lumen, whereas the secretion of apoB48 was slightly decreased and the amount of apoB48 in the ER lumen was unaffected. This is in accordance with Lin's *et al.* study in which rats fed with fresh garlic extract had significantly lower intestinal MTP mRNA levels compared to the control rats, whereas hepatic MTP mRNA levels were unchanged (75). Using a cholesterol-fed alloxan diabetic rabbit as a model for diabetes and atherosclerosis, Phillips *et al.* (81) demonstrated that intestinal rather than hepatic MTP may be the cause of postprandial dyslipidaemia in diabetes. New approaches for lowering intestinal MTP only or hepatic MTP remain attractive issues for development of novel classes of tissue-specific MTP inhibitors.

Novel series of highly potent MTP inhibitors with acceptable pharmacokinetic and safety profile has been developed (98-100). These compounds offer the potential for great efficacy and plasma lipid control in hypercholesterolemia, hypertriglyceridemia and mixed hyperlipidaemia. Ongoing synergetic drug combination with known cholesterol lowering agents or fat soluble vitamin such as vitamin E, vitamin A and vitamin K are under investigations.

We and other have demonstrated that MTP acts as a chaperone in binding to apoB during early stages of lipoprotein assembly (10,27,29,40,42,43,57,103,104) & (Figure 1). This chaperone function of MTP is an attractive target for development of new inhibitors and partial inhibition of MTP-apoB association may therefore be therapeutically beneficial. But the main question is whether these new MTP-apoB binding inhibitors can decrease lipoprotein secretion without inducing severe fatty liver and, if not, whether moderate effect is an acceptable price to pay.

Recently, Biessen *et al.* (101) reported an elegant design and synthesis of antisense peptide nucleic acid glycoconjugate prodrug which was able to reduce MTP mRNA levels in HepG2 cells by 35-40% at 100nM. Further investigations are needed with the combination of emerging genomic and proteomic technologies and availability of new potent molecules that inhibits MTP-apoB binding to help in evaluating new strategy for developing powerful and safe anti-atherogenic drugs.

7. CONCLUDING REMARKS

It seems clear that depending upon the specific cell context, liver or intestine, MTP inhibitors might act differently in reducing the amount of apoB100 or apoB48. Although clarifications of exact mechanisms behind this phenomenon are still needed, significant progress has been made recently, particularly with the use of animal models in studies of fasting and postprandial dyslipidemia that may be the major cause of atherosclerosis in diabetes.

Recent studies on MTP gene promoter polymorphism showed a potential link to the development of cardiovascular diseases . However, the mechanism is poorly understood and evaluation of great number of patients in various risk-populations is still needed.

MTP chaperone activity studies, which are just emerging, have produced equivocal result regarding the

physiological significance of MTP binding in lipoprotein assembly and secretion. Many laboratories are now focusing on clarification of this issue and forthcoming reports will be issued in the near future. This will help to answer many of remaining questions and, perhaps, might help in design and choice of new powerful lipid lowering therapy for cardiovascular diseases.

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Abbreviations: ApoB, apolipoprotein B, CE, cholesterol ester, ER, endoplasmic reticulum, FH, Familial hypercholesterolemia, LV, lamprey lipovitellin, LDL, low density lipoproteins, MTP, microsomal triglyceride transfer protein, M subunit, 97-kDa subunit of the MTP complex, PC, phospatidylcholine, P subunit, the 55-kDa PDI subunit of the MTP complex, PDI, protein disulfide isomerase, TG, triglycerides, VLDL, very low density lipoproteins

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