Published in final edited form as:
Curr Opin Lipidol. 2008 December ; 19(6): 572–578. doi:10.1097/MOL.0b013e328312707c.

New approaches to target microsomal triglyceride transfer protein

M.M. Hussain and **Ahmed Bakillah**
Departments of Anatomy and Cell Biology, and Pediatrics, SUNY Downstate Medical Center, Brooklyn, New York, USA

Abstract

Purpose of review—Microsomal triglyceride transfer protein (MTP), a chaperone for the biosynthesis of apolipoprotein B lipoproteins and CD1d, is a therapeutic candidate to decrease plasma lipids and to diminish inflammation. MTP inhibition increases plasma transaminases and tissue lipids, and therefore new approaches are needed to avoid them.

Recent findings—Inositol requiring enzyme 1β has been identified as a novel intestine-specific regulator of MTP. A new function of MTP in cholesterol ester biosynthesis has been reported. The importance of the phospholipid transfer activity of MTP in the lipidation of apolipoprotein B and CD1d has been indicated. Diurnal variations in MTP expression and its induction by food availability have been observed. On the basis of these and other findings, we propose that upregulation of inositol requiring enzyme 1β, a combined reduction of cellular free cholesterol or triglyceride or both and MTP activity, specific inhibition of phospholipid or triglyceride transfer activities, and targeting of apolipoprotein B–-MTP protein–protein interactions might be pursued to avoid some of the side effects associated with the inhibition of triglyceride transfer activity of MTP. We further speculate that short-lived MTP antagonists may be useful in controlling plasma and tissue lipids and in avoiding steatosis.

Summary—We have highlighted the importance of addressing the causal relationship between MTP inhibition and aberrant elevations in plasma liver enzymes. The proposed approaches may show that MTP targeting is a viable approach to lower plasma lipids.

Keywords

apolipoprotein B; free cholesterol; lipoproteins; microsomal triglyceride transfer protein; neutral lipids; steatosis; triglyceride

Introduction

Hyperlipidemias are significant risk factors for various cardiovascular, for example, atherosclerosis, and metabolic disorders, such as metabolic syndrome, diabetes, and obesity. Reduced catabolism or increased production of lipoproteins causes hyperlipidemia. Statins enhance lipoprotein catabolism and reduce plasma cholesterol. Despite the success of statins, approximately 60% of statin-treated patients have adverse coronary events. Therefore, there is a need for new drugs that can be used alone or in combination with statins to reduce hyperlipidemias. A possible approach is to inhibit the synthesis of lipoproteins. Lipoproteins are assembled in the endoplasmic reticulum, matured in the Golgi, and secreted by cells. Their biosynthesis depends on two proteins: apolipoprotein B (apoB) and microsomal triglyceride transfer protein (MTP). ApoB is a structural protein. MTP is an essential chaperone for the

Correspondence to M.M. Hussain, Departments of Anatomy and Cell Biology, and Pediatrics SUNY Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203, USA Tel: +1 718 270 4790; fax: +1 718 270 2462; e-mail: mhussain@downstate.edu.

assembly of apoB lipoproteins [1,2,3•]. It transfers several lipids including triacylglycerols, phospholipids, and cholesteryl esters.

Microsomal triglyceride transfer protein defects and steatosis

Abetalipoproteinemia, a rare autosomal recessive disorder with defective MTP activity, is characterized by the absence of plasma apoB lipoproteins [4]. Fat accumulation (steatosis) in the intestine is uniformly observed in all abetalipoproteinemia patients. Hepatic steatosis has also been reported with MTP deficiency. Partin *et al*. [5] described an abetalipoproteinemia infant with substantial hepatomegaly and persistent elevated levels of serum aminotransferases but normal bilirubin levels. Microscopic examination revealed large fat droplets in hepatocytes. They also observed scattered focal accumulation of inflammatory cells. In addition to genetic defects, there is an association between low MTP expression due to a polymorphism in the promoter sequence and liver steatosis in type 2 diabetes patients [6]. Hepatitis C virus type 3 infected patients show reduced MTP activity and mRNA levels as well as high degree of steatosis [7].

Several animal studies also indicate a relationship between decreased MTP expression and steatosis. Chronic alcohol feeding causes fatty liver and is associated with reduced MTP [8]. Transgenic expression of hepatitis C virus core protein inhibits MTP activity, reduces very-low-density lipoprotein (VLDL) secretion, and causes steatosis [9]. We have shown that steatosis induced by carbon tetrachloride involves posttranslational degradation of MTP [10••]. Similarly, inhibition of MTP in cells also results in triglyceride accumulation [11••]. Therefore, significant reduction in MTP activity is usually associated with steatosis.

Complications associated with microsomal triglyceride transfer protein inhibition

The triglyceride transfer activity of MTP has been exploited to identify several potent antagonists [12–15,16•]. These antagonists (Table 1) [14,16•,17••,18••,19–21] inhibit triglyceride transfer activity *in vitro*, suppress lipoprotein assembly and secretion *in vivo*, and decrease plasma lipid levels in humans and animals. MTP is highly expressed in the intestine and liver; therefore, major side effects of MTP inhibition are obvious in these tissues (Fig. 1). The first side effect is related to the inhibition of chylomicron assembly by enterocytes and manifests as gastrointestinal disturbances such as steatorrhea and diarrhea. The second side effect is related to the inhibition of hepatic lipoprotein assembly and secretion. In about 10–30% of the individuals, MTP inhibitors increase plasma levels of liver enzymes mainly aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [17••,18••] indicating for liver damage. Lower levels of MTP are expressed in several other tissues. Complications associated with its inhibition in other tissues, however, have not yet been appreciated and should be considered for long-term modalities, particularly those related to the inhibition of cardiac MTP.

As MTP primarily transfers neutral lipids *in vitro*, it has been assumed that toxicities associated with MTP inhibition and genetic ablation are due to the accumulation of neutral lipids. We should, however, realize that synthesis of neutral lipids (triglycerides and cholesterol esters) is beneficial in avoiding toxicities associated with excess free fatty acids and free cholesterol. The question then arises why MTP inhibition leads to increases in hepatic enzymes in the plasma. A common explanation provided is that MTP inhibition leads to higher, possibly toxic, amounts of neutral lipids in the liver. However, there are reports indicating that, at least, short-term use of MTP inhibitors does not always lead to an overt accumulation of hepatic lipids [14]. We are not aware of studies describing a clear-cut relationship between hepatic fat accumulation and the appearance of liver enzymes in the plasma. Assuming that the toxicity

due to MTP inhibition is associated with hepatic fat, it can conceivably be avoided by upregulating mitochondrial and peroxisomal oxidation of fatty acids. This possibility has been speculated in the literature but has not yet been reported to be practical. Therefore, there is a need to question the fundamental paradigm that MTP inhibitors increase plasma hepatic enzymes solely by augmenting cellular concentrations of triglycerides and to find new ways to circumvent hepa-tosteatosis.

Microsomal triglyceride transfer protein inhibition and free cholesterol accumulation

Besides triglyceride accumulation, MTP inhibition enhances cellular free cholesterol levels. Iqbal *et al.* [11••] have studied the effects of genetic ablation and chemical inhibition of MTP on tissue lipids. As expected, these treatments enhanced cellular triglyceride. It was anticipated that cholesteryl esters would also increase with triglycerides. Unexpectedly, they found significantly reduced levels of cholesteryl esters in the liver and intestinal cells. More importantly, they observed a significant assimilation of free cholesterol in these cells. Mechanistic studies revealed that MTP plays a novel role in cholesteryl ester biosynthesis [11••]. They reported that enrichment of microsomes with cholesteryl esters reduces cholesteryl ester biosynthesis, and MTP alleviates this inhibition by depositing them into apoB lipoproteins. Chemical inhibition and genetic deletion of MTP, therefore, increase cellular free cholesterol owing to reduction in cholesteryl ester biosynthesis.

Increases in cellular free cholesterol might cause tissue damage and enhance the release of hepatic enzymes into the plasma. It is known that high cellular free cholesterol levels damage extrahepatic tissues. For example, excess amounts of cellular free cholesterol induce apoptosis, especially in the arterial wall macrophages contributing to atherogenesis [22]. Enhanced free cholesterol levels in the endoplasmic reticulum of macrophages induce unfolded protein response and apoptosis [22]. On the contrary, hepatic free cholesterol is not usually considered a problem as liver can either excrete it as such or after its conversion to bile acids. This assumption may not be totally true. The success of statin therapy is due to the inhibition of hepatic 3-hydroxy-3-methyl-glutaryl (HMG) CoA reductase activity present in the endoplasmic reticulum membranes. Therefore, a more plausible explanation is that free cholesterol in the endoplasmic reticulum membrane is critical, and perturbations that lead to increases in microsomal free cholesterol may cause injury. Furthermore, Mari *et al.* [23] have suggested that the progression of hepatosteatosis to steatohepatitis might be related to free cholesterol, not free fatty acid or triglyceride, accumulation in the liver. The relationship between free microsomal cholesterol assimilation, MTP inhibition, and increases in plasma transaminases can be evaluated by lowering cellular free cholesterol.

Avoiding free cholesterol accumulation

A feasible approach to lower cellular cholesterol might be to inhibit HMG CoA reductase. Statins inhibit HMG CoA reductase, increase hepatic LDL receptor expression, and decrease plasma cholesterol. Therefore, it is tempting to suggest that acombined inhibition of MTP and HMG CoA reductase may be useful in lowering plasma cholesterol and avoiding cellular cholesterol accumulation. Similarly, potent inhibitors of squalene synthase can be used with MTP inhibitors to achieve these goals.

Another combinatorial approach to avoid free cholesterol accumulation is to enhance its efflux. In this regards, liver X receptor (LXR) agonists appear promising. LXRs are nuclear hormone receptors that control expression of genes involved in cholesterol efflux in macrophages, hepatic bile acid synthesis, and intestinal cholesterol absorption [24–26]. LXR agonists increase expression of ABCA1 in macrophages, enhance cholesterol efflux, and decrease

atherosclerosis in *apoe*$^{-/-}$ and *ldlr*$^{-/-}$ mice. They increase hepatic bile acid synthesis and reduce hepatic cholesterol levels. These agonists upregulate ABCG5 and ABCG8 in the intestine and reduce cholesterol absorption. Unfortunately, a major side effect of LXR agonist is hypertriglyceridemia [27]. MTP inhibitors reduce hypertriglyceridemia. Therefore, it is worth examining whether LXR agonist and MTP antagonists can be used in combination to prevent hypertriglyceridemia, increases in plasma transaminases, and steatosis.

Reducing cellular triglyceride

Cellular triglyceride buildup is a key feature of MTP inhibition. Triglyceride synthesis involves fatty acid uptake, intracellular transport to microsomes by fatty acid-binding proteins, and acylation with glycerol by several monoacylglycerol and diacylglycerol acyltransferases. Inhibition of these steps will likely reduce cellular triglyceride levels. In this respect, repression of liver fatty acid-binding protein along with MTP inhibition has been shown to lessen steatosis [28]. Several studies have shown that flavonoids inhibit triglyceride transfer activity of MTP [29]. These compounds affect several other biological pathways and have pleiotropic effects. For example, taxifolin, a plant flavonoid, inhibits triglyceride synthesis and MTP activity without increasing cellular lipids [20]. Therefore, joint inhibition of triglyceride synthesis and MTP activity might avoid triglyceride accumulation. This is supported by observations that diacylglycerol acyltransferase (DGAT)1-deficient mice do not develop hepatic steatosis, demonstrate increased energy expenditure, and have significantly lower levels of triglycerides in lipogenic tissues [30]. Thus, DGAT inhibition with MTP antagonism offers an attractive opportunity for therapeutic interventions in obese and diabetic patients.

In theory, cellular triglyceride levels can be reduced by upregulating fatty acid oxidation. Peroxisome proliferator-activated receptors (PPARs) are nuclear hormone receptors that enhance fatty acid oxidation by peroxisomes [31]. Potent PPARα agonists decrease hypercholesterolemia and atherosclerosis in *ldlr*$^{-/-}$ mice [32]. A combined use of MTP inhibitors and PPARα activators has been speculated before. Still, experimental evidence for their beneficial use is lacking.

Selective intestinal microsomal triglyceride transfer protein inhibition

Another possibility is to target intestinal MTP and spare hepatic MTP. For this to be successful, there is a need to understand tissue-specific regulation of MTP as well as the effect of MTP inhibition on intestinal self-renewal and malabsorption of fat-soluble vitamins. Although MTP inhibition leads to intestinal distress, a temporal separation of drug administration and food intake avoids these complications. For example, intestinal disturbances are avoided by administering these inhibitors 4 h after the supper [14]. Furthermore, steatosis associated with intestinal targeting is usually not considered a chronic problem because of its inherent property to self-renew. In fact, preclinical and clinical studies of intestine-specific MTP inhibitors show good efficacy and minimal side effects (Table 1). For example, dirlotapide is a potent and selective intestinal MTP inhibitor used in canines to decrease lipid absorption and reduce weight [19,33]. JTT-130 has been shown to lower plasma triglycerides and LDL cholesterol in guinea pigs without increasing hepatic triglyceride levels [21]. Similar results have been reported for SLx-4090 [16•]. Thus, an intestine-specific inhibition of MTP may be a way for future interventions.

MTP expression is highly regulated at the transcriptional level [3•,34,35]. Thus, knowledge about the upstream regulators that curb tissue-specific MTP expression might be useful. Recently, it has been shown that inositol requiring enzyme 1β (IRE1β) controls intestinal MTP expression and lipoprotein assembly [36••]. IRE1β, expressed mainly in the intestine [37], is a membrane-anchored ribonuclease residing in the endoplasmic reticulum. It has been shown to regulate MTP mRNA involving posttranscriptional degradation. Genetic ablation of

IRE1β leads to hyperlipidemias in mice when subjected to high-fat, high-cholesterol diets. These diets decrease IRE1β mRNA levels and increase MTP activity, protein and mRNA levels in wild type mice. Therefore, it is anticipated that upregulation of IRE1β could be a novel way to lower intestinal MTP expression. In this regard, a comprehensive understanding of IRE1β physiology might be beneficial for future pharmacologic repression of intestinal MTP.

Inhibition of the phospholipid transfer activity of microsomal triglyceride transfer protein

Besides triglycerides, MTP also transfers phospholipids. Kinetic studies indicate two phospholipid transfer sites in MTP [38]. Evolutionary studies indicate that the phospholipid transfer activity of MTP is the most ancient and sufficient activity for apoB-lipoprotein assembly [39,40,41••]. It is also involved in CD1d biosynthesis [42,43••,44]. Brozovic *et al*. [45] were the first to show a relationship between MTP and CD1d. *Mttp* gene manipulation in hepatocytes was associated with redistribution of CD1d. Moreover, *mttp*-deleted mice were resistant to immunopathologies associated with invariant natural killer T (NKT) cell-mediated hepatitis and colitis [45]. Dougan *et al*. [42,43••] have proposed that MTP acts upstream of saposins and functions as an endoplasmic reticulum chaperone by loading endogenous lipids onto nascent CD1d. On the contrary, Sagiv *et al*. [46•] suggested its role in the lysosomal compartment. Therefore, it is possible that inhibition of the phospholipid transfer activity may reduce lipoprotein production and help avoid inflammatory response of certain immune disorders involving CD1d. Furthermore, specific inhibition of this activity may not interfere with triglyceride transfer and cholesterol ester biosynthesis. On the contrary, toxicities associated with MTP inhibition could be related to inhibition of phospholipid transfer activity because it is important for lipoprotein and CD1d biosynthesis. Thus, there is a critical need to identify specific antagonists of triglyceride and phospholipid transfer activities of MTP.

It was relatively easy to identify inhibitors of triglyceride transfer activity because MTP is the major cellular protein that exhibits robust neutral lipid transfer activity in the presence of synthetic membrane vesicles. In contrast, cells have several proteins that transfer phospholipids. Therefore, use of cellular or tissue homogenates may result in the identification of inhibitors that may or may not inhibit MTP's phospholipid transfer activity. Ideally, purified MTP should be used to identify phospholipid transfer activity inhibitors.

Reducing microsomal triglyceride transfer protein – apolipoprotein B interactions

MTP physically interacts with apoB [1,2,13,35,47–51]. Theoretically, blocking interactions between these proteins without affecting MTP lipid transfer should reduce apoB-lipoprotein biosynthesis. We screened compounds from Atherogenics, Inc. (Alpharetta, Georgia, USA), for their ability to block apoB–MTP protein–protein interactions, identified a compound AGI-S17 that inhibits these interactions without affecting the lipid transfer activity of MTP, and showed that this molecule significantly reduces apoB secretion in liver cells [52]. Although this establishes a proof of concept, more potent inhibitors of apoB–MTP interactions are required to determine the benefits and risks of this approach as a potential new therapy to reach lipid and lipoprotein-lowering goals for cardiovascular and metabolic patients. Like conventional MTP inhibitors, disruption of apoB–MTP interaction can potentially be used in combination therapy with others lipid-lowering agents to achieve acceptable therapeutic efficacy (Table 2).

Short-acting microsomal triglyceride transfer protein inhibitors

A long-term adverse effect of MTP inhibition is hepatic lipid accumulation. Chronic inhibition of intestinal MTP causes steatorrhea and diarrhea. Furthermore, MTP shows diurnal variations, and its expression is induced by food anticipatory activity [53••]. It has been shown that dosing MTP inhibitors away from food intake diminishes intestinal distress [14]. On the basis of these observations, we speculate that short-acting MTP inhibitors with adequate half-lives may be effective. It will be interesting to determine whether tolerable and effective short-acting MTP inhibitors in combination with other lipid-lowering agents will have a greater utility in achieving therapeutic goals of lowering plasma lipids and avoiding hepatic fat accumulation.

Conclusion

Significant new information about MTP biology and regulation has come forth recently. This includes identification of a novel role of MTP in CD1d and cholesteryl ester biosynthesis, MTP regulation by IRE1β, and identification of intestine-specific antagonists. Because of the enhanced interest in MTP research from different disciplines, we anticipate resurgence in research to inhibit MTP alone or in combination with other targets. As genetic ablation and chemical inhibition of MTP are associated with steatosis, a careful evaluation of MTP inhibitors for their safety and efficacy is required (Fig. 1). Novel approaches to inhibit MTP with simultaneous avoidance of associated side effects are needed. Such developments may be useful in the treatment of various lipidemias, such as familial hypercholesterolemia, familial combined hyperlipidemia, and metabolic disorders such as obesity and metabolic syndrome.

Acknowledgements

This work was supported in part by grants from the National Institutes of Health (DK-46900) and American Heart Association.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest

•• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

1. Hussain MM, Shi J, Dreizen P. Microsomal triglyceride transfer protein and its role in apolipoprotein B-lipoprotein assembly. J Lipid Res 200;44:22–32. [PubMed: 12518019]
2. Hussain MM, Iqbal J, Anwar K, et al. Microsomal triglyceride transfer protein: a multifunctional protein. Front Biosci 2003;8:S500–S506. [PubMed: 12700048]
3. Hussain MM, Rava P, Pan X, et al. Microsomal triglyceride transfer protein in plasma and cellular lipid metabolism. Curr Opin Lipidol 2008;19:277–284. [PubMed: 18460919] A recent review that summarizes studies related toMTP activity and its regulation in plasma and cellular lipid homeostasis.
4. Berriot-Varoqueaux N, Aggerbeck LP, Samson-Bouma M, Wetterau JR. The role of the microsomal triglyceride transfer protein in abetalipoproteinemia. Annu Rev Nutr 2000;20:663–697. [PubMed: 10940349]
5. Partin JS, Partin JC, Schubert WK, McAdams AJ. Liver ultrastructure in abetalipoproteinemia: evolution of micronodular cirrhosis. Gastroenterology 1974;67:107–118. [PubMed: 4135110]
6. Phillips C, Mullan K, Owens D, Tomkin GH. Microsomal triglyceride transfer protein polymorphisms and lipoprotein levels in type 2 diabetes. QJM 2004;97:211. [PubMed: 15028851]

7. Mirandola S, Realdon S, Iqbal J, et al. Liver microsomal triglyceride transfer protein is involved in hepatitis C liver steatosis. Gastroenterology 2006;130:1661–1669. [PubMed: 16697730]
8. Sugimoto T, Yamashita S, Ishigami M, et al. Decreased microsomal triglyceride transfer protein activity contributes to initiation of alcoholic liver steatosis in rats. J Hepatol 2002;36:157–162. [PubMed: 11830326]
9. Perlemuter G, Sabile A, Letteron P, et al. Hepatitis C virus core protein inhibits microsomal triglyceride transfer protein activity and very low density lipoprotein secretion: a model of viral-related steatosis. FASEB J 2002;16:185–194. [PubMed: 11818366]
10. Pan X, Hussain FN, Iqbal J, et al. Inhibiting proteasomal degradation of microsomal triglyceride transfer protein prevents CCl_4 induced steatosis. J Biol Chem 2007;282:17078–17089. [PubMed: 17405876] This study shows that CCl_4 covalently modifies MTP and induces its degradation involving ubiquitinylation and proteosomes.
11. Iqbal J, Rudel LL, Hussain MM. Microsomal triglyceride transfer protein modulates cellular cholesterol esterification by relieving product inhibition. J Biol Chem 2008;283:19967–19980. [PubMed: 18502767] This study provides evidence for a regulatory role of MTP in cholesteryl ester biosynthesis.
12. Wetterau JR, Gregg RE, Harrity TW, et al. An MTP inhibitor that normalizes atherogenic lipoprotein levels in WHHL rabbits. Science 1998;282:751–754. [PubMed: 9784135]
13. Bakillah A, El Abbouyi A. The role of microsomal triglyceride transfer protein in lipoprotein assembly: an update. Front Biosci 2003;8:D294–D305. [PubMed: 12456294]
14. Chandler CE, Wilder DE, Pettini JL, et al. CP-346086: an MTP inhibitor that lowers plasma cholesterol and triglycerides in experimental animals and in humans. J Lipid Res 2003;44:1887–1901. [PubMed: 12837854]
15. Chang G, Ruggeri RB, Harwood HJ Jr. Microsomal triglyceride transfer protein (MTP) inhibitors: discovery of clinically active inhibitors using high-throughput screening and parallel synthesis paradigms. Curr Opin Drug Discov Devel 2002;5:562–570.
16. Burnett JR, Watts GF. MTP inhibition as a treatment for dyslipidaemias: time to deliver or empty promises? Expert Opin Ther Targets 2007;11:181–189. [PubMed: 17227233] A recent review that summarizes different MTP inhibitors and their clinical out-comes.
17. Samaha FF, McKenney J, Bloedon LT, et al. Inhibition of microsomal triglyceride transfer protein alone or with ezetimibe in patients with moderate hypercholesterolemia. Nat Clin Pract Cardiovasc Med 2008;5:497–505. [PubMed: 18506154] This study shows that low doses of MTP antagonist alone or in combination with cholesterol absorption inhibitors decrease plasma lipids.
18. Cuchel M, Bloedon LT, Szapary PO, et al. Inhibition of microsomal triglyceride transfer protein in familial hypercholesterolemia. N Engl J Med 2007;356:148–156. [PubMed: 17215532] This clinical study in homozygous familial hypercholesterolemia patients highlights potential value of inhibiting MTP in lowering plasma LDL cholesterol.
19. Wren JA, Ramudo AA, Campbell SL, et al. Efficacy and safety of dirlotapide in the management of obese dogs evaluated in two placebo-controlled, masked clinical studies in North America. J Vet Pharmacol Ther 2007;30:81–89. [PubMed: 17567518]
20. Casaschi A, Rubio BK, Maiyoh GK, Theriault AG. Inhibitory activity of diacylglycerol acyltransferase (DGAT) and microsomal triglyceride transfer protein (MTP) by the flavonoid, taxifolin, in HepG2 cells: potential role in the regulation of apolipoprotein B secretion. Atherosclerosis 2004;176:247–253. [PubMed: 15380446]
21. Aggarwal D, West KL, Zern TL, et al. JTT-130, a microsomal triglyceride transfer protein (MTP) inhibitor lowers plasma triglycerides and LDL cholesterol concentrations without increasing hepatic triglycerides in guinea pigs. BMC Cardiovasc Disord 2005;5:30. [PubMed: 16188040]
22. Tabas I. Consequences of cellular cholesterol accumulation: basic concepts and physiological implications. J Clin Invest 2002;110:905–911. [PubMed: 12370266]
23. Mari M, Caballero F, Colell A, et al. Mitochondrial free cholesterol loading sensitizes to TNF- and Fas-mediated steatohepatitis. Cell Metab 2006;4:185–198. [PubMed: 16950136]
24. Zelcer N, Tontonoz P. Liver X receptors as integrators of metabolic and inflammatory signaling. J Clin Invest 2006;116:607–614. [PubMed: 16511593]

25. Rader DJ. Liver X receptor and farnesoid X receptor as therapeutic targets. Am J Cardiol 2007;100:n15–n19. [PubMed: 18047847]
26. Rigamonti E, Chinetti-Gbaguidi G, Staels B. Regulation of macrophage functions by PPAR-alpha, PPAR-gamma, and LXRs in mice and men. Arterioscler Thromb Vasc Biol 2008;28:1050–1059. [PubMed: 18323516]
27. Zadelaar S, Kleemann R, Verschuren L, et al. Mouse models for atherosclerosis and pharmaceutical modifiers. Arterioscler Thromb Vasc Biol 2007;27:1706–1721. [PubMed: 17541027]
28. Spann NJ, Kang S, Li AC, et al. Coordinate transcriptional repression of liver fatty acid-binding protein and microsomal triglyceride transfer protein blocks hepatic very low density lipoprotein secretion without hepatosteatosis. J Biol Chem 2006;281:33066–33077. [PubMed: 16950764]
29. Allister EM, Borradaile NM, Edwards JY, Huff MW. Inhibition of microsomal triglyceride transfer protein expression and apolipoprotein b100 secretion by the citrus flavonoid naringenin and by insulin involves activation of the mitogen-activated protein kinase pathway in hepatocytes. Diabetes 2005;54:1676–1683. [PubMed: 15919788]
30. Chen HC, Farese RV Jr. Inhibition of triglyceride synthesis as a treatment strategy for obesity: lessons from DGAT1-deficient mice. Arterioscler Thromb Vasc Biol 2005;25:482–486. [PubMed: 15569818]
31. Lefebvre P, Chinetti G, Fruchart JC, Staels B. Sorting out the roles of PPAR alpha in energy metabolism and vascular homeostasis. J Clin Invest 2006;116:571–580. [PubMed: 16511589]
32. Getz GS, Reardon CA. Diet and murine atherosclerosis. Arterioscler Thromb Vasc Biol 2006;26:242–249. [PubMed: 16373607]
33. Li J, Bertinato P, Cheng H, et al. Discovery of potent and orally active MTP inhibitors as potential antiobesity agents. Bioorg Med Chem Lett 2006;16:3039–3042. [PubMed: 16529928]
34. Hagan DL, Kienzle B, Jamil H, Hariharan N. Transcriptional regulation of human and hamster microsomal triglyceride transfer protein genes. Cell type-specific expression and response to metabolic regulators. J Biol Chem 1994;269:28737–28744. [PubMed: 7961826]
35. Shoulders CC, Shelness GS. Current biology of MTP: implications for selective inhibition. Curr Top Med Chem 2005;5:283–300. [PubMed: 15857311]
36. Iqbal J, Dai K, Seimon T, et al. IRE1β inhibits chylomicron production by selectively degrading MTP mRNA. Cell Metab 2008;7:445–455. [PubMed: 18460335] This study suggests intestine-specific IRE1β regulates MTP mRNA involving posttranscriptional mechanisms. Its deficiency results in hyperlipidemia when challenged with high-cholesterol and high-fat diets.
37. Bertolotti A, Wang X, Novoa I, et al. Increased sensitivity to dextran sodium sulfate colitis in IRE1beta-deficient mice. J Clin Invest 2001;107:585–593. [PubMed: 11238559]
38. Atzel A, Wetterau JR. Identification of two classes of lipid molecule binding sites on the microsomal triglyceride transfer protein. Biochemistry 1994;33:15382–15388. [PubMed: 7803401]
39. Sellers JA, Hou L, Athar H, et al. A drosophila microsomal triglyceride transfer protein homolog promotes the assembly and secretion of human apolipoprotein B: implications for human and insect lipid transport and metabolism. J Biol Chem 2003;278:20367–20373. [PubMed: 12657646]
40. Rava P, Ojakian GK, Shelness GS, Hussain MM. Phospholipid transfer activity of microsomal triacylglycerol transfer protein is sufficient for the assembly and secretion of apolipoprotein B lipoproteins. J Biol Chem 2006;281:11019–11027. [PubMed: 16478722]
41. Rava P, Hussain MM. Acquisition of triacylglycerol transfer activity by micro-somal triglyceride transfer protein during evolution. Biochemistry 2007;46:12263–12274. [PubMed: 17924655] This study suggests that MTP was evolved as a phospholipid transfer protein and acquired triglyceride transfer activity during a transition from invertebrates to vertebrates.
42. Dougan SK, Salas A, Rava P, et al. Microsomal triglyceride transfer protein: lipidation and control of CD1d on antigen presenting cells. J Exp Med 2005;202:529–539. [PubMed: 16087713]
43. Dougan SK, Rava P, Hussain MM, Blumberg RS. MTP regulated by an alternate promoter is essential for NKT cell development. J Exp Med 2007;204:533–545. [PubMed: 17312007] This report shows that mice express two forms of MTP with identical biochemical activities. The major difference is the presence of additional two N-terminal amino acids due to the utilization of an alternate promoter and exon 1 in the synthesis of the variant form of MTP.

44. Barral DC, Brenner MB. CD1 antigen presentation: how it works. Nat Rev Immunol 2007;7:929–941. [PubMed: 18037897]
45. Brozovic S, Nagaishi T, Yoshida M, et al. CD1d function is regulated by microsomal triglyceride transfer protein. Nat Med 2004;10:535–539. [PubMed: 15107843]
46. Sagiv Y, Bai L, Wei DG, et al. A distal effect of microsomal triglyceride transfer protein deficiency on the lysosomal recycling of CD1d. J Exp Med 2007;204:921–928. [PubMed: 17403933] This study suggests that MTP defect affects CD1d recycling in the lysosomes.
47. Hussain MM, Bakillah A, Nayak N, Shelness GS. Amino acids 430–570 in apolipoprotein B are critical for its binding to microsomal triglyceride transfer protein. J Biol Chem 1998;273:25612–25615. [PubMed: 9748226]
48. Nicodeme E, Benoist F, McLeod R, et al. Identification of domains in apolipoprotein B100 that confer a high requirement for the microsomal triglyceride transfer protein. J Biol Chem 1999;274:1986–1993. [PubMed: 9890955]
49. Bradbury P, Mann CJ, Köchl S, et al. A common binding site on the microsomal triglyceride transfer protein for apolipoprotein B and protein disulfide isomerase. J Biol Chem 1999;274:3159–3164. [PubMed: 9915855]
50. Liang J, Ginsberg HN. Microsomal triglyceride transfer protein binding and lipid transfer activities are independent of each other, but both are required for secretion of apolipoprotein B lipoproteins from liver cells. J Biol Chem 2001;276:28606–28612. [PubMed: 11358959]
51. Wu XJ, Zhou MY, Huang LS, et al. Demonstration of a physical interaction between microsomal triglyceride transfer protein and apolipoprotein B during the assembly of ApoB-containing lipoproteins. J Biol Chem 1996;271:10277–10281. [PubMed: 8626595]
52. Bakillah A, Nayak N, Saxena U, et al. Decreased secretion of apoB follows inhibition of apoB-MTP binding by a novel antagonist. Biochemistry 2000;39:4892–4899. [PubMed: 10769147]
53. Pan X, Hussain MM. Diurnal regulation of microsomal triglyceride transfer protein and plasma lipid levels. J Biol Chem 2007;282:24707–24719. [PubMed: 17575276] This report describes diurnal variations in plasma triglyceride and intestinal and hepatic MTP levels.

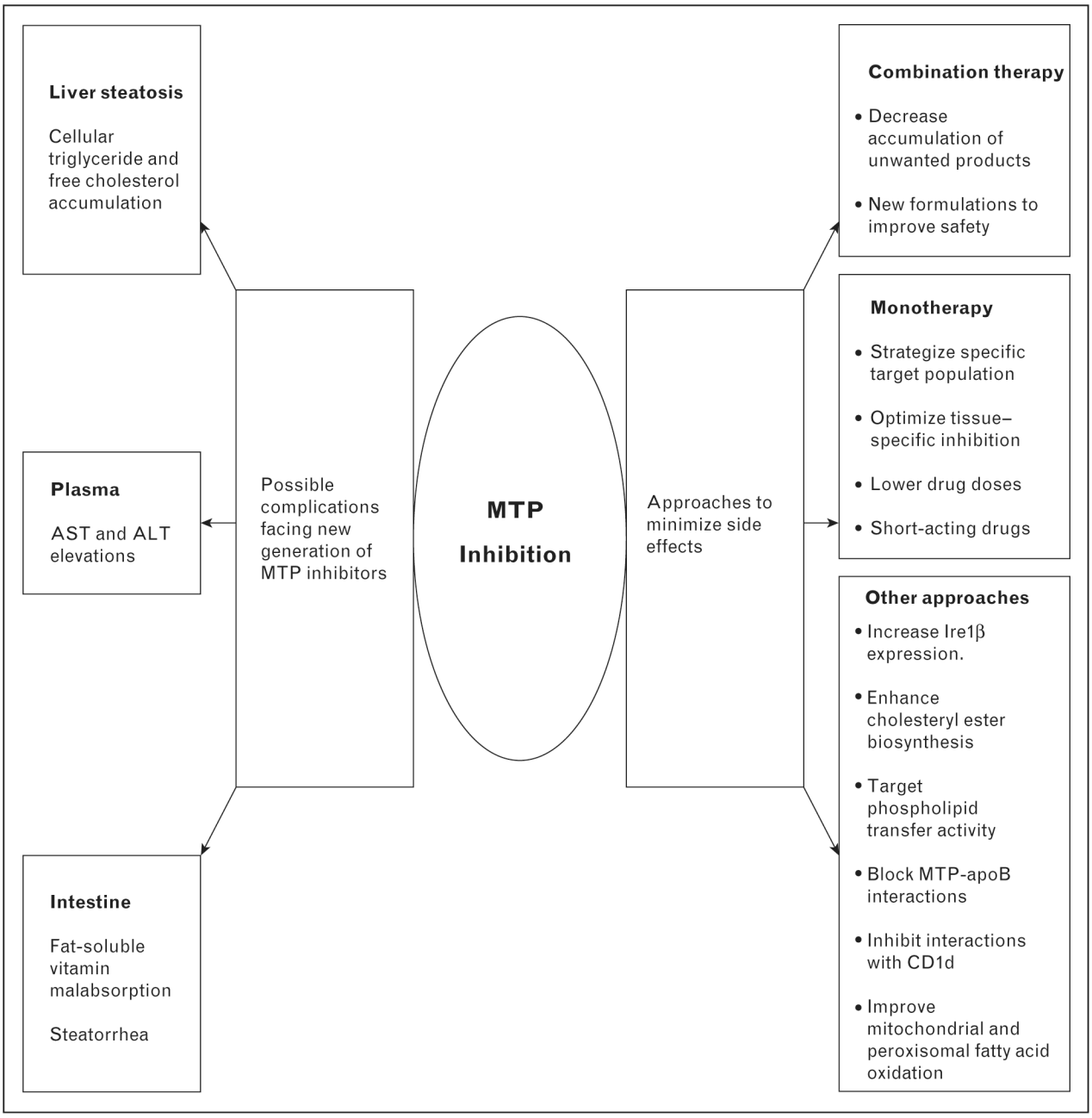

Figure 1. Avoiding toxicities associated with microsomal triglyceride transfer protein inhibition

ALT, alanine aminotransferase; apoB, apolipoprotein B; AST, aspartate aminotransferase; MTP, microsomal triglyceride transfer protein.

Table 1

Few examples of emerging microsomal triglyceride transfer protein inhibitors

Inhibitors	Company	Salient features	Potential side effects	References
AEGR-733	Aegerion	Reduce total cholesterol, apoB, and triglyceride	Mild steatosis	[17••,18••]
		Reduce weight	Increases in liver transaminases	
CP-346086	Pfizer	Reduce total cholesterol, apoB, and triglyceride	Mild/severe steatosis	[14]
			Increases in liver transaminases	
Dirlotapide	Pfizer	Reduce lipid absorption	Mild steatosis	[19]
		Reduce weight	Gastrointestinal complications	
Flavonoids (taxifolin)	Ametis	Antioxidant and anti-inflammatory properties	Mild/severe steatosis	[20]
		Reduce triglyceride and apoB	Gastrointestinal complications	
JTT-130	Japan Tobacco	Decrease triglyceride and LDL cholesterol	Mild steatosis	[21]
		No hepatic triglyceride accumulation	Gastrointestinal complications	
SLx-4090	Surface Logix	Reduce triglyceride and LDL cholesterol	Mild steatosis	[16•]
			Gastrointestinal complications	

apoB, apolipoprotein B; LDL, low-density lipoprotein.

Table 2

Potential targets for combined therapy with microsomal triglyceride transfer protein inhibition

Targets	Mode of action	Plasma changes	Tissues	Possible complications
HMG CoA reductase	Inhibit cholesterol synthesis	Lower plasma LDL cholesterol, apoB, and triglyceride	Liver	Elevation of creatine kinase and transminases
	Upregulate LDL receptors			Possible mild fatty liver and aggravation of adverse affects associated with HMG CoA reductase inhibition
	Decrease cellular free cholesterol			
Liver X receptors	Increase cholesterol efflux	Lower plasma LDL cholesterol, apoB, and triglyceride	Liver, macrophages, and intestine	May cause undesirable triglyceride effect
	Decrease hepatic free cholesterol			
	Increase bile acid synthesis			
Fatty acid-binding protein	Modulate cellular fatty acid transport and utilization	Lower plasma LDL and VLDL	Liver, adipose, macrophages, muscle, and intestine	Enterocyte toxicity
		Reduce triglyceride and apoB		Gastrointestinal complications and liver enzyme elevations
Triglyceride-synthesizing enzymes	Reduce triglyceride synthesis	Lower plasma apoB lipoproteins	Liver, adipose, and intestine	Gastrointestinal complications and liver enzyme elevation
PPARs	Enhance fatty acid oxidation	Reduce triglyceride and VLDL	Liver, macrophages, adipose, and muscle	May raise LDL cholesterol
				Increase creatinine and homocysteine levels
Cholesterol absorption inhibitors and bile acid sequestrants	Inhibit absorption of dietary and biliary cholesterol	Lower LDL cholesterol	Intestine and liver	May elevate triglyceride
				Gastrointestinal complications and liver enzyme elevations

apoB, apolipoprotein B; HMG, 3-hydroxy-3-methyl-glutaryl; PPAR, peroxisome proliferator-activated receptors; VLDL, very-low-density lipoprotein.