

A Small-Molecule Inhibitor of Enterocytic Microsomal Triglyceride Transfer Protein, SLx-4090: Biochemical, Pharmacodynamic, Pharmacokinetic, and Safety Profile

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ABSTRACT

First-generation microsomal triglyceride transfer protein (MTP) inhibitors were designed to inhibit hepatic MTP and provide a novel treatment of dyslipidemia. Effective at lowering low-density lipoprotein-cholesterol (LDL-C), these inhibitors also elevate liver enzymes and induce hepatic steatosis in animals and humans. MTP is highly expressed in the enterocytes, lining the lumen of the jejunum, and is critical in the production of chylomicrons assembled from lipid/cholesterol and their transfer into systemic circulation. 6-(4'-Trifluoromethyl-6-methoxy-biphenyl-2-ylcarboxamido)-1,2,3,4-tetrahydroisoquinoline-2-carboxylic acid phenyl ester (SLx-4090) (IC_{50} value ~ 8 nM) was designed to inhibit only MTP localized to enterocytes. In Caco-2 cells SLx-4090 inhibited apolipoprotein B (IC_{50} value ~ 9.6 nM) but not apolipoprotein A1 secretion. Administered orally to rats SLx-4090 reduced postpran-

dial lipids by $>50\%$ with an ED_{50} value ~ 7 mg/kg. SLx-4090 was not detected in the systemic or portal vein serum of the animals (lower limit of quantitation ~ 5 ng/ml) after single or multiple oral doses in fasted rodents. When coadministered with tyloxapol, SLx-4090 did not inhibit the secretion of hepatic triglycerides (TG), consistent with the absence of systemic exposure. Chronic treatment with SLx-4090 in mice maintained on a high-fat diet decreased LDL-C and TG and resulted in weight loss without the elevation of liver enzymes or an increase in hepatic fat. The compound did not result in toxicity when administered to rats for 90 days at a dose of 1000 mg/kg per day. These data support the concept that the inhibition of enterocytic MTP could serve as a useful strategy in the treatment of metabolic disorders.

Introduction

Impaired lipid metabolism (dyslipidemia) is considered a major contributor to the etiology of metabolic and cardiovascular disease (Cannon, 2008; Ginsberg and MacCallum, 2009). Dyslipidemia, broadly characterized by elevated circulating levels of low-density lipoprotein-cholesterol (LDL-C) and triglycerides (TG), is commonly treated by therapeutic intervention at the level of hepatic function (Reiner, 2010).

Therapeutic response is determined by the measurement of serum lipid levels while a patient is in a "fasting" state

having not eaten for at least an overnight period. This measure, while useful because of its simplicity, is not representative of the changes in plasma lipid levels that most individuals experience throughout the day. In fact, the consumption of multiple meals during the day creates a condition of metabolic flux commonly referred to as the postprandial (PP) state. The PP state is characterized by relatively prolonged spiking of TG-rich lipoproteins that occurs after the ingestion of each meal, and it is this state that is truly representative of the human metabolic condition. Increases in serum TG-lipoprotein levels vary according to an individual's metabolism and the content of their meals (Mattes, 2009). Therefore, individuals have distinct basal PP profiles differing in the rate and amount of lipids that are absorbed. It has also been suggested that certain PP lipid patterns can not only exacerbate metabolic diseases but play a fundamental role in

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ABBREVIATIONS: LDL, low-density lipoprotein; LDL-C, LDL-cholesterol; MTP, microsomal triglyceride transfer protein; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; ApoE(-/-), apolipoprotein E-deficient; HDL-C, high-density lipoprotein-cholesterol; HFD, high-fat diet; LC-MS/MS, liquid chromatography with tandem mass spectroscopy; LFD, low-fat diet; LOQ, lower limit of quantitation; MC, methylcellulose; PK, pharmacokinetic; PP, postprandial; TG, triglycerides; VLDL-C, very low-density lipoprotein cholesterol; CP-346086, 4'-trifluoromethyl-biphenyl-2-carboxylic acid[2-(2H-[1,2,4]triazol-3-yl-methyl)-1,2,3,4-tetrahydro-isoquinolin-6-yl]amide; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; LD, low dose; MD, mid dose; HD, high dose; SLx-4090, 6-(4'-trifluoromethyl-6-methoxy-biphenyl-2-ylcarboxamido)-1,2,3,4-tetrahydroisoquinoline-2-carboxylic acid phenyl ester.

the development of the disease itself (Phillips et al., 2006; Tushuizen et al., 2010).

One strategy for the controlling circulating levels of LDL-C and TG is the inhibition of hepatic MTP. MTP plays a crucial role in the assembly of TG, cholesterol esters, and phospholipids into ApoB-containing lipoproteins and is integral in the assembly of very low-density lipoprotein-cholesterol (VLDL-C) in the liver (Tietge et al., 1999). Initial discovery of small-molecule inhibitors of MTP was thought to provide an alternative treatment for patients not at goal on statin therapy by reducing the hepatic production of VLDL-C. These efforts led to the discovery of molecules that were potent inhibitors of hepatic MTP and proved very effective in reducing the ability of the liver to export VLDL-C (Wetterau et al., 1998; Roevens et al., 1999; Gruetzmann et al., 2000; Chandler et al., 2003; Aggarwal et al., 2005; Li et al., 2007). Unfortunately, blockade of hepatic MTP was quickly associated with a significant decrease in the ability of the liver to rid itself of fat, and administration of MTP inhibitors in clinical trials universally resulted in unacceptable rises in liver transaminases and increased hepatic fat disposition consistent with hepatic steatosis or "fatty liver" (Lammens et al., 1999; Lilly and Rader, 2007; Miyazaki et al., 2007; Hussain and Bakillah, 2008). The clinical development of molecules that inhibited hepatic MTP was essentially discontinued with respect to use across broad indications. The advancement of a traditional hepatic MTP inhibitor into clinical trials to assess the effectiveness of such treatment in a small genetic population of type IV familial hyperlipoproteinemia has confirmed the alarming increases in liver transaminases at extremely low doses (Samaha et al., 2008).

MTP is also found in the intestine and is critical in the transfer of TG onto ApoB₄₈ for subsequent transfer to the systemic circulation (Gordon and Jamil, 2000; Hussain and Bakillah, 2008). The development of agents that affect PP lipid levels by inhibiting only intestinal MTP may serve as

the next generation of agents for the treatment of metabolic disorders.

Given the proven ability of small molecules to effectively inhibit MTP, our approach was to design a drug that would inhibit the MTP found in the intestinal lining but would have no impact on the liver. This strategy required the design of a small molecule that interacted preferentially with the lipids that comprises the apical membrane of the enterocyte. Understanding the interaction of 6-(4'-trifluoromethyl-6-methoxy-biphenyl-2-ylcarboxamido)-1,2,3,4-tetrahydroisoquinoline-2-carboxylic acid phenyl ester (SLx-4090) with the plasma membrane lipids that comprise the extensive apical surface area of the enterocyte enabled controlled access and delivery to MTP found within this cell type.

SLx-4090 demonstrates all of the pharmacological and physiological properties of a potent inhibitor of MTP. Unlike earlier MTP inhibitors, the compound is not systemically available and is not associated with hepatic toxicities. The ability to inhibit enterocytic, but not hepatic MTP, will make it possible to pharmacologically isolate the intestine to better understand the PP state and its impact on the etiology of metabolic disease.

Materials and Methods

SLx-4090 and CP-346086 Synthesis. The synthesis of SLx-4090 (Fig. 1) is described fully in European patent EP1877369 (Bartolozzi et al., 2008). 4'-Trifluoromethyl-biphenyl-2-carboxylic acid[2-(2H-[1,2,4]triazol-3-yl-methyl)-1,2,3,4-tetrahydro-isoquinolin-6-yl]amide (CP-346086), a bioavailable MTP inhibitor first reported by Chandler et al. (2003), was synthesized at Surface Logix according to published methods (Chandler et al., 2003).

Materials. Caco-2 cells were purchased from the American Type Culture Collection (Manassas, VA). Pooled mixtures of human male microsomes purchased from BD Biosciences (San Jose, CA) and InVitro Technologies (Baltimore, MD) were used as sources of MTP

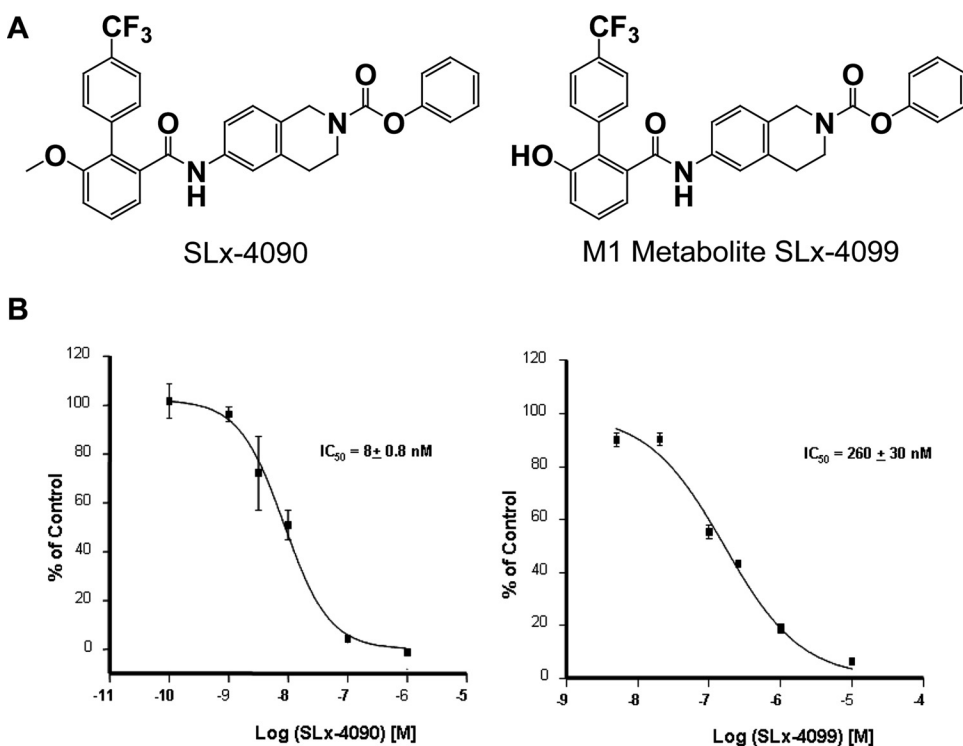


Fig. 1. Structure and activity of SLx-4090 and its principal metabolite, SLx-4099. A, SLx-4090 (left) is a free base. SLx-4099 (right) is the desmethyl product of SLx-4090. B, inhibition of MTP-mediated lipid transfer by SLx-4090 and SLx-4099. MTP isolated from human liver microsomes was incubated with commercially available small unilamellar donor vesicles containing quenched fluorescent lipids and acceptor vesicles as described under *Materials and Methods*. The data are the mean \pm S.E.M. of six replicates.

and for stability studies. Primary hepatocytes were purchased from CellzDirect (Carlsbad, CA) or InVitro Technologies and were prepared freshly from livers of male donors. Where tissues were donor human tissues all appropriate requirements concerning the use of human tissues were observed. DMEM, minimal essential medium, and fetal bovine serum (FBS) were purchased from American Type Culture Collection. ApoB and ApoA1 enzyme-linked immunosorbent assay kits (AlerChek, Portland, ME) were used according to the manufacturer's instructions. Reagent-grade chemicals were sourced from Sigma-Aldrich (St. Louis, MO) and used as received. Sprague-Dawley rats were from Hilltop Laboratory Animals, Inc. (Scottsdale, PA). ApoE(-/-) animals were from Taconic Farms (Rockville, MD). All animals were fed standard rodent chow [low-fat diet (LFD); 15.7% calories from fat; 5.3% fat by weight; Harlan (Indianapolis, IN)] ad libitum unless otherwise indicated. Where animals were maintained on a high-fat diet (HFD) the chow was Teklad TD.88137 (Harlan), with 42% calories from fat (21% by weight).

LFD and HFD chows containing SLx-4090 as an admix were manufactured under contract by Harlan.

Inhibition of MTP Activity In Vitro. The TG transfer assay for MTP activity was based on the protocol described previously (Chandler et al., 2003) using human liver microsomes containing active MTP. Microsomes from male donors were pooled before use. The potency of compounds as inhibitors of MTP was determined by fluorometry using a kit from Chylos (Woodbury, NY) that contained preassembled donor and acceptor lipid-vesicles.

Caco-2 Cell Drug Permeability Assay. Caco-2 cells were cultured for 21 days on polyethylene terephthalate membranes with 1.0- μm pores using BD Biosciences' 96-MultiWell Insert System. Under these culture conditions Caco-2 cells form a confluent barrier of polarized human intestinal enterocyte-like cells (Hidalgo et al., 1989). Compounds were dissolved in neat DMSO and diluted in the culture medium to a final concentration of 20 μM , maintaining a 0.1% DMSO concentration. Apparent compound permeability ($P_{\text{app}} \times 10^{-6}$ cm/s) was evaluated by exposing the apical or basal surface of the cells and incubating them in 10% FBS/DMEM for 2 h at 37°C and 0.5% CO₂. Samples were collected and analyzed by LC-MS/MS.

Inhibition of ApoB and ApoA1 Secretion in Hepatocytes and Caco-2 Cells. The cellular assays used to determine MTP inhibition (ApoB and ApoA1 secretion from primary hepatocytes and Caco-2 cells) were described by Chandler et al. (2003). Human hepatocytes were freshly harvested from male donors, pooled, plated in 96-well plates, cultured in collagen and Matrigel sandwich (CellzDirect or InVitroTech), and maintained in the vendor's specified culture media. Caco-2 cells were prepared as described under *Caco-2 Cell Drug Permeability Assay*.

Compounds were dissolved in DMSO and serially diluted in the culture medium, maintaining a 0.1% DMSO concentration. Compounds were evaluated in both cell types (Caco-2 assay test compounds were evaluated by exposing the apical surface) by incubating them in 10% FBS/DMEM for 24 h at 37°C and 0.5% CO₂. Concentrations of ApoB and ApoA1 were determined from aliquots of culture media obtained from the basal sides of Caco-2 monolayers, using an enzyme-linked immunosorbent assay kit (AlerChek).

Studies Using Animals. The Institutional Animal Care and Use Committee approved all procedures using experimental animals. Sprague-Dawley rats and ApoE(-/-) mice were provided the indicated diets ad libitum unless otherwise stated, and water was available ad libitum. Rats were treated by oral gavage at a volume of 5 ml/kg body weight with an aqueous solution containing 0.4% methylcellulose (MC) or an aqueous solution containing 0.4% MC plus MTP inhibitors. Mice were administered SLx-4090 by gavage (1 ml/kg body weight) or in HFD or standard rodent chow/LFD. Animals on a HFD were acclimated to the diet at least 1 week before administration of SLx-4090 was initiated as a dietary supplement in the chow.

Serum Chemistries. For TG studies using rats, blood was collected via retro-orbital bleeds at an approximate volume of 0.5 ml. No

animal was bled on more than three occasions. For terminal studies, blood was collected via cardiac puncture in CO₂-anesthetized animals and placed into serum-separating tubes to obtain serum. The serum was frozen on dry ice before shipment to IDEXX Laboratories (Sacramento, CA) for analyses of LDL-C, HDL-C, and TG.

Analysis of serum free fatty acids was performed using a commercially available kit (Zen-Bio, Inc., Research Triangle Park, NC).

Postprandial Lipids after Acute Lipid Challenge in Rats. Male Sprague-Dawley (200–250 g) were fasted overnight (~14–18 h), with water available ad libitum. The next morning, blood samples were drawn for baseline TG determinations. The animals were dosed orally with SLx-4090 or reference compounds followed 20 min later by an oral gavage of a 1-ml corn oil/sucrose mixture (320 mg of corn oil and 430 mg of sucrose per 1 ml). Control animals received vehicle and the corn oil/sucrose mixture. Blood samples were collected via retro-orbital sinus at various time points for TG and PK measures. Approximately 0.5 ml of blood was drawn per time point per rat in a tube containing citric acid. The blood samples were stored on ice until they were spun via centrifugation to separate the plasma, which was stored at -80°C until analyzed.

Duration of Action on Postprandial Lipid Response. Male Sprague-Dawley (200–250 g) were treated as described immediately above with the exception that administration of the corn oil/sucrose mixture was delayed to 0, 2, 4, 6, 8, 20, or 24 h after the administration of SLx-4090 (30 mg/kg) or vehicle.

Determination of the Rates of Hepatic and Intestinal TG Secretion. Tyloxapol (Triton WR1339) prevents the catabolism of TG-rich lipoproteins by serum lipases and allows a time-dependent and linear increase in plasma TG levels. In the fasted state, where there is little to no intestinal chylomicron production, hepatic VLDL production is the predominant source of nascent plasma TG (Chandler et al., 2003). It is therefore possible to determine whether SLx-4090 inhibits the production of liver VLDL by evaluating plasma TG accumulation over time. Male Sprague-Dawley (200–250 g) were fasted overnight (~14–18 h), but allowed access to water ad libitum. Tyloxapol (1.0 ml; 125 mg/ml) was administered intravenously 60 min after oral administration of MTP inhibitors. Approximately 0.5 ml of blood (retro-orbital) was drawn per indicated time point per rat in a tube containing citric acid. The blood samples were stored on ice until they were spun down via centrifugation to separate the plasma, which was stored at -80°C before analysis.

ApoE(-/-) Mice. Three- to 4-week-old male apolipoprotein E-deficient [ApoE(-/-); B6.129-ApoEtm1UncN11] mice were purchased from Taconic Farms. Mice received LFD and water ad libitum and were acclimated to the environmental- and lighting-controlled (12-h light/dark phases) facility for 1 to 2 weeks. At 5 weeks of age, the mice were placed into one of six groups ($n = 7$ –8/group). Two groups of mice received either the LFD diet ($n = 8$) or the LFD diet containing 0.06% SLx-4090 ($n = 8$; LFD-4090). The remaining mice received either the HFD diet ($n = 7$; HFD, TD.88137, Harlan) or a HFD diet containing a low dose ($n = 7$; HFD-LD, 0.006% SLx-4090), a middle dose ($n = 8$; HFD-MD, 0.019% SLx-4090), or a high dose ($n = 8$; HFD-HD, 0.06% SLx-4090) of the test article, ad libitum, for 10 weeks. Body weight and food consumption were recorded daily throughout the study. Estimated SLx-4090 dose was calculated based on food consumption per period, multiplied by the appropriate SLx-4090 concentration, divided by body weight. After the 10-week treatment period, all mice were terminated via CO₂ inhalation in the nonfasted state, and liver and visceral (omental and epididymal) fat pads were excised and weighed.

Toxicological Evaluation of SLx-4090. The effects of SLx-4090 administered once daily for 90 days was evaluated in male and female Sprague-Dawley rats under contract to Bridge Pharmaceuticals (Gaithersburg, MD) as part of the Good Laboratory Practice evaluation of the compound. Bridge Pharmaceuticals is a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. In brief, animals were housed in single suspended cages under standard vivarium conditions and adminis-

tered SLx-4090 at 40, 200, or 1000 mg/kg per day. At periodic intervals throughout the study body weight and food consumption was recorded and animals were observed for clinical signs of morbidity. Blood samples were collected from a separate cohort of animals for clinical chemistry panels and PK analysis. After 90 days of drug administration, the animals were euthanized using CO₂ inhalation and exsanguination. A full battery of organs was obtained for histological evaluation by hematoxylin and eosin staining.

Effect of SLx-4090 on Serum Levels of Vitamins E, A, and C. Adult male Sprague-Dawley rats were dosed for 4 or 14 consecutive days with SLx-4090 or CP-346086 (30 mg/kg p.o., 0.4% MC). Animals were allowed access to standard laboratory chow and water throughout the dosing period and were euthanized without an overnight fast. On the morning of day 5 or 15, the animals were euthanized with CO₂, and approximately 5 ml of blood was collected via cardiac puncture into K₃EDTA-containing vacutainer tubes. The samples were reduced to plasma, and the levels of endogenous fat-soluble vitamins E and A and water-soluble vitamin C were determined via HPLC (Paulo et al., 1999).

In Vitro Metabolism of SLx-4090. SLx-4090 (5 μM) was incubated at 37°C with human liver microsomes (0.4 mg/ml protein) in the presence and absence of reductase NADPH (8 mM). After 60 min, the reaction was stopped by adding a 0.3% solution of acetic acid in acetonitrile containing internal standard (haloperidol; 1.67 μM). The samples were diluted with water and centrifuged, and the resulting supernatants were analyzed for SLx-4090 and metabolites according to the LC-MS/MS method described under *LC-MS/MS Analysis*.

Pharmacokinetics (Systemic). Male Sprague-Dawley rats previously implanted with jugular vein cannula were dosed orally by gavage (10 or 30 mg/kg; 0.4% MC; 10 ml/kg) or intravenously [1 mg/kg; 5% *N*-methyl-pyrrolidinone, 45% PEG400, 50% β-(2-hydroxypropyl)-cyclodextrin (20% in water); 1 ml/kg] with the indicated amount of SLx-4090. Animals were acclimated to the vivarium for at least 5 days before use. Food and water was available ad libitum except for a 8- to 10-h fast before dosing. Food was returned to the animals ~1 h postdose. Blood samples were collected (K₃EDTA) via implanted venous cannula at the indicated times postdose. The samples were reduced to plasma via centrifugation and stored frozen at -20°C until analyzed for SLx-4090 and SLx-4099 as described under *LC-MS/MS Analysis*.

Pharmacokinetics (Portal Vein). Adult male Sprague-Dawley rats previously implanted with portal vein cannula were obtained from Hilltop Laboratory Animals, Inc. and acclimated to vivarium conditions for 3 days before use. Food and water was available ad libitum except for a 8- to 10-h fast before dosing. Food was returned to the animals ~1 h postdose. SLx-4090 (25 or 100 mg/kg) was administered via oral gavage (0.4% MC; 10 ml/kg), and blood samples (~0.5 ml) were collected into K₃EDTA at predose and 0.5, 1, 2, 4, 8, 12, and 24 h postdose. The samples were reduced to plasma via centrifugation and stored frozen at -20°C until analyzed for SLx-4090 and SLx-4099 as described below.

MTP inhibitors were extracted from rat plasma by precipitation with acetonitrile in a 96-well Captiva filtration plate (Agilent Technologies, Santa Clara, CA) and quantitated using liquid chromatography in conjunction with mass spectrometry. Plasma (50 μl) was added to each well of the Captiva filtration plate followed by the acetonitrile (150 μl) containing 1.6 μM haloperidol (internal standard) and water (50 μl). The wells were sealed and the samples were mixed by inverting the plate several times manually. The samples were then slowly filtered under vacuum and subsequently analyzed by LC-MS/MS.

LC-MS/MS Analysis. The LC-MS/MS system consisted of a binary pump, a plate autosampler with temperature control unit degasser, and a 2000QTrap LC-MS/MS system (Applied Biosystems, Foster City, CA) fitted with TurboIonspray interface. Samples were chromatographed on an Xterra MS C₁₈ 2.1 × 20 mm, 3.5-μm column (Waters, Milford, MA), using the fast mobile phase gradient [mobile phase A, 0.1% formic acid in MilliQ water (v/v); mobile phase B,

0.085% formic acid in acetonitrile (v/v)] with 1-min equilibration to aqueous phase. The flow rate was 1 ml/min, with 1:10 split before the ion source and the injection volume was 5 μl. The mass spectrometer was operated in the positive ion mode with a source temperature of 350°C, using nitrogen as nebulizing and heating gas. MS-MS analysis was carried out in the Selected Reaction Monitoring mode, and the analytes were monitored using an appropriate the precursor-product ion pair. The LC-MS/MS data were calculated using the Analyst 1.4 quantitation software supplied by Applied Biosystems. Statistical analysis was used to generate the means, standard deviations, coefficient of variation, and regression parameters. The peak area ratio of the test item/internal standard was calculated for each sample. A calibration line was generated using the test item/internal standard area ratio plotted against the actual test item concentration to generate a standard calibration line. The typical assay range was from 5 nM to 10 μM, and the LOQ for the assay was found to be 5 ng/ml (equivalent to ~10 nM).

Data Analysis. Statistical analyses were performed using Prism V4 (GraphPad Software Inc., San Diego, CA). Inhibition constants or ED₅₀ values were calculated using the variable slope sigmoidal model. Group mean analyses were performed using a one-way analysis of variance with Tukey's post hoc test to determine the significance of differences among groups. Comparisons between two groups were made using Student's two-tailed *t* test with or without Welch's correction for uneven variance.

Results

Inhibition of MTP-Mediated Lipid Transfer. Incubation of MTP isolated from human liver microsomes and donor vesicles containing quenched fluorescent lipids with varying concentrations of MTP inhibitors resulted in a concentration-dependent inhibition of MTP-mediated TG transfer between vesicles (Table 1). The IC₅₀ value for the selective inhibitor of MTP, CP-346086 (2.0 nM), was consistent with previously published findings (Chandler et al., 2003; Li et al., 2007). The IC₅₀ values obtained for the tetrahydroisoquinoline derivatives SLx-4090 and SLx-4099 (M1 metabolite of SLx-4090) (Fig. 1A) were determined to be 8.0 ± 0.8 and 262 ± 30 nM, respectively. Data for SLx-4090 and SLx-4099 inhibition of MTP-mediated lipid transfer are shown in Fig. 1B.

TABLE 1

Biochemical characterization of inhibition of SLx-4090 and its M1 metabolite SLx-4099 and CP-346086, a MTP inhibitor reference compound

Compound	Assay Type	Value
MTP-mediated lipid transfer ^a	SLx-4090	8.0 ± 0.8 (3)
	SLx-4099	262 ± 30 (3)
	CP-346086	2.0
ApoB secretion (primary hepatocytes) ^a	SLx-4090	81 ± 15 (5)
	SLx-4099	2720 ± 490 (5)
	CP-346086	N.D.
ApoB secretion (Caco-2 cells) ^a	SLx-4090	9.6 ± 1.6 (5)
	SLx-4099	530 ± 50 (5)
	CP-346086	10
ApoA1 secretion (primary hepatocytes or Caco-2) ^b	SLx-4090	>30,000
	SLx-4099	>30,000
	CP-346086	>30,000
Caco-2 drug permeability (apical to basal) ^c	SLx-4090	0
	SLx-4099	0
	CP-346086	14
Caco-2 compound permeability (basal to apical) ^c	SLx-4090	0
	SLx-4099	1.01
	CP-346086	12

N.D., not determined.

^a IC₅₀ nM ± S.E.M. (*n*).

^b Highest concentration tested.

^c *P*_{app} × 10⁻⁶ cm/s.

Inhibition of ApoB and ApoA1 Secretion from Human Primary Hepatocytes and Caco-2 Cells. SLx-4090 was a potent inhibitor of ApoB secretion in primary human hepatocytes and Caco-2 cells (Table 1). In contrast, SLx-4099, the primary metabolite of SLx-4090, was 33- and 55-fold less active than the parent molecule in primary human hepatocytes and Caco-2 cells, respectively. Neither SLx-4090 nor SLx-4099 inhibited the secretion of ApoA1 at the highest concentration tested (30 μ M), demonstrating the specificity of SLx-4090 for MTP-mediated events.

Permeability of Caco-2 Cells to MTP Inhibitors. SLx-4090 and the reference inhibitor CP-346086 were evaluated for both apical-to-basal transport and basal-to-apical transport. As expected, CP-346086 was permeable in this model as determined by its high apparent drug permeability coefficient ($P_{app} \sim 14 \times 10^{-6}$ cm/s) (Table 1) consistent with the oral bioavailability of this drug. SLx-4090 was designed to selectively accumulate in the apical plasma membrane of the enterocyte, and, consistent with this strategy, it was not permeable in the human Caco-2 cell assay. The M1 metabolite of SLx-4090, SLx-4099, displayed no apical-to-basal permeability, but a small basal-to-apical permeability was detected.

Effect of SLx-4090 on Postprandial Triglycerides and Free Fatty Acids. Oral gavage of SLx-4090 to male Sprague-Dawley rats resulted in a dose-dependent reduction of PP serum TG increases (Fig. 2A). The estimated ED₅₀ values for SLx-4090 (the dose reducing postprandial TG increases by 50%) was approximately 7 mg/kg (Fig. 2B). The action was rapid in onset, being apparent when animals were challenged with a corn oil/sugar gavage within 1 h of compound administration, and was dose-dependent (Fig. 2C). In contrast to SLx-4090, CP-346086 showed a trend in lowering TG levels below predose baseline.

The serum TG-lowering effects of SLx-4090 were long-lasting. After administration of 30 mg/kg SLx-4090 significantly inhibited the PP spike in serum TG for up to 6 h. A nonsignificant trend toward lowering serum TG levels relative to the vehicle control was observed for up to 20 h (Fig. 2D).

In contrast to the effects of CP-346086, which inhibits both enterocytic and hepatic MTP, SLx-4090 did not result in a decrease in serum TG levels below baseline levels (Fig. 2C). Total serum TG pools are a combined response to both enterocyte TG absorption and hepatic TG release. Compounds that inhibit only one of these pools would not be expected to completely block the PP TG rise, which is mediated by both sources of lipid. The finding is consistent with the suggestion that SLx-4090 acts at the level of the enterocyte. Both SLx-4090 and CP-346086 inhibited the PP increase in serum free fatty acids (Fig. 2E).

Enterocyte-Specific Action of SLx-4090. In fasted animals serum TG levels are predominantly of hepatic origin with little or no TG being secreted by the intestine. Tyloxapol-treated groups showed a linear increase in serum TG over several hours, reflecting a baseline rate of hepatic TG secretion (Fig. 3). SLx-4090 (30 mg/kg) did not affect TG levels in tyloxapol-treated animals, suggesting that it does not inhibit hepatic MTP. CP-346086, a systemically available MTP inhibitor, completely blocked the secretion of serum TG from the liver (Fig. 3). These results indicate that SLx-4090 was not active in the liver and are consistent with the acute PP TG data, indicating that SLx-4090 is a nonsystemically available compound that retains enterocytic MTP inhibitory activity.

Effects of SLx-4090 on Body Weight, Food Consumption, and Serum Lipid Measures in ApoE(-/-) Mice. To further evaluate the effects of SLx-4090 on metabolic status SLx-4090 was administered as an admix in a HFD or LFD for 10 weeks to ApoE(-/-) mice (Table 2). In the ApoE(-/-) mouse, a well known dyslipidemic animal model, the severity of the hyperlipidemia was exacerbated by high dietary fat.

Body weights were similar among all groups at the start of the treatment period. Based on food consumption measures and body weight records the average daily dose of SLx-4090 was 94 mg/kg per day for animals maintained on a LFD. In the three dose groups maintained on a HFD, the average daily dose of SLx-4090 was 5.8 (HFD-LD), 18.9 (HFD-MD), and 66.8 (HFD-HD) mg/kg per day during the 10-week treatment period.

At the end of treatment, LFD-4090 mice had similar weight gain and food consumption as control LFD mice (Table 2). Both the high- and mid-dose HFD-SLx-4090 treatment groups had significantly reduced weight gain compared with control animals fed the HFD alone or the HFD and the lowest dose of SLx-4090 (Table 2). The reduced weight gain during SLx-4090 treatment occurred despite similar per-day food consumption among all treatment groups.

Visceral fat weight, normalized to body weight, was significantly reduced by the middle (38.9 ± 7.2 mg of fat/g body weight; $p < 0.05$) and high dose (27.2 ± 4.4 mg/g; $p < 0.01$) SLx-4090 treatment compared with control HFD diet and low-dose SLx-4090 animals (58.4 ± 2.6 mg/g) (Fig. 4). HFD mice administered the lowest dose of SLx-4090 had similar visceral fat-body weight ratio as control HFD animals.

Serum TG levels were similar between LFD and LFD-SLx-4090 mice. In contrast, both mid- and high-dose SLx-4090 treatment in HFD-fed mice significantly reduced serum TG in mice compared with animals receiving a HFD and vehicle (Fig. 5).

SLx-4090 treatment altered cholesterol homeostasis in both LFD- and HFD-fed mice (Fig. 6). LFD-4090 mice had significant reduction of 22 and 26% (both $p < 0.01$) in total and LDL-C levels and a significant increase of 14% ($p < 0.05$) in HDL-C levels compared with control LFD mice. In HFD-fed mice, the mid- and high-dose mice had significantly decreased total cholesterol (-41%, $p < 0.01$ and -68%, $p < 0.001$, respectively) and LDL-C (-42%, $p < 0.01$ and -70%, $p < 0.001$, respectively) and significantly increased HDL-C (+67%, $p < 0.01$ and +123%, $p < 0.001$, respectively) compared with control HFD mice. Low-dose HFD mice had similar total and LDL-C as control HFD, but had a significant increase in HDL-C (+37%, $p < 0.05$).

Toxicology. SLx-4090, when administered via oral gavage once daily for 90 consecutive days to male and female Sprague-Dawley rats at doses of 40, 200, and 1000 mg/kg per day, resulted in no mortality or adverse findings for clinical or cage-side observations, body weights, body weight changes, food consumption, clinical pathology, gross pathology, or histopathology. The no-observed-adverse-effect level was at least 1000 mg/kg in male and female rats (P. Sweetnam, unpublished data).

Effects on Serum Vitamin Levels. In animals fed a standard diet with no additional vitamin supplementation SLx-4090 or CP-346086 (30 mg/kg) administered for 4 or 14 days had no effect on endogenous levels of vitamin A (Table 3). There was no effect of 4 days of drug administration on vitamin E and a marginal, but nonsignificant, 24% decrease in mean circulat-

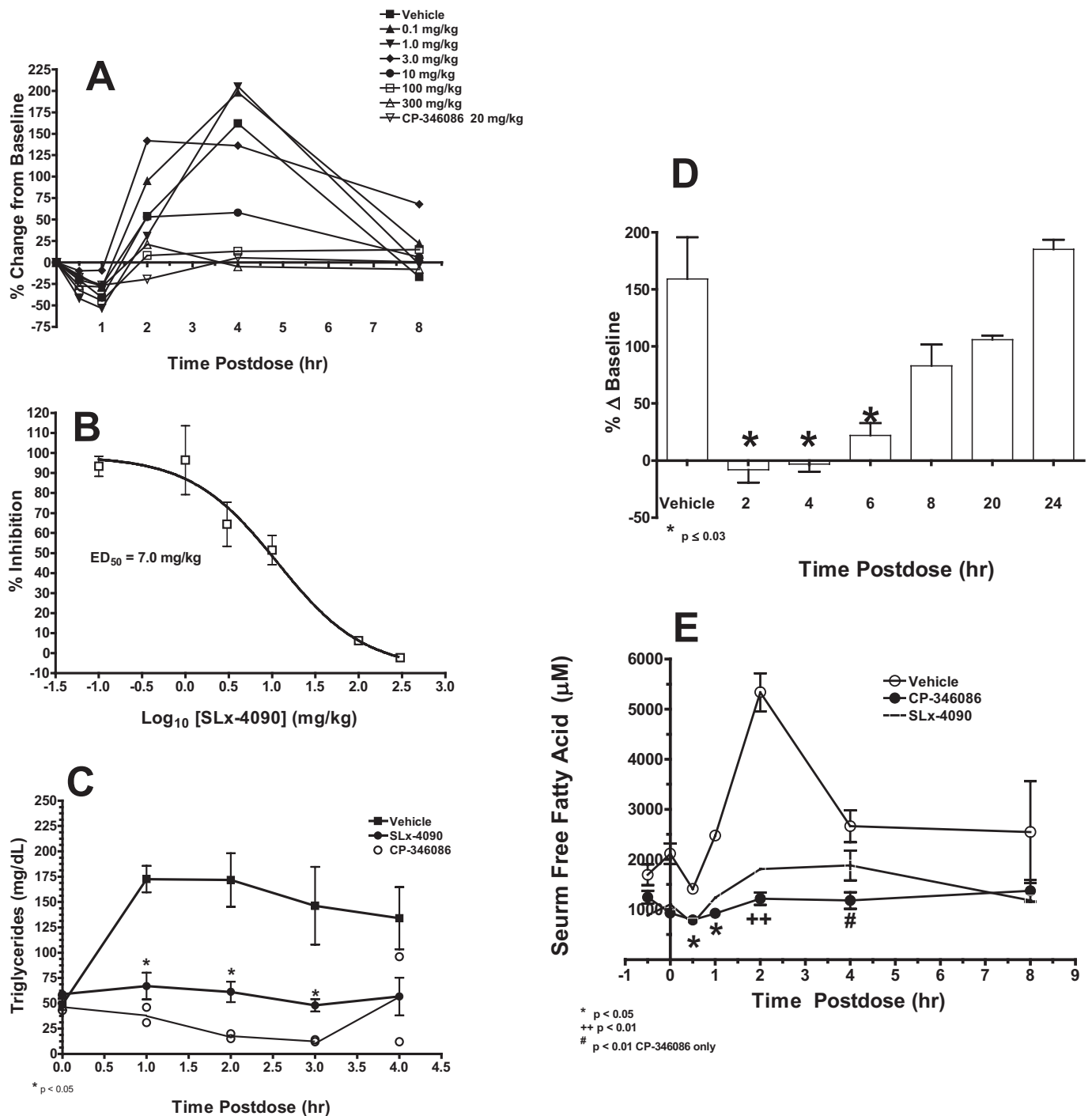


Fig. 2. Inhibition of PP serum lipid increases by SLx-4090, dose response, and duration of action. A, SLx-4090 was administered 20 min before the oral gavage of a corn oil/sucrose challenge. Blood samples were drawn at the indicated times for determination of serum TG levels. B, the ED₅₀ value for SLx-4090 to inhibit PP serum TG increases was calculated using the *t* = 4-h data from A and was estimated to be 7 mg/kg. C, animals were treated with SLx-4090 (30 mg/kg), the systemically active MTP inhibitor CP-346086 (30 mg/kg), or vehicle and challenged 20 min later with a corn oil/sucrose meal (*, *p* < 0.05 to vehicle, with the exception of CP-346086 where both replicates for *n* = 2 have been plotted). D, SLx-4090 (30 mg/kg) was administered via oral gavage and followed by a corn oil/sucrose challenge at 0, 2, 4, 6, 8, or 20 h after drug administration (*, *p* ≤ 0.03). E, using a dosing regimen identical to that in D, serum free fatty acids were determined at the indicated times postdose. Values are the mean ± S.E.M. of three to five separate determinations at each time point. *, *p* < 0.05; ++, *p* < 0.01; #, *p* < 0.01 for CP-346086 only.

ing levels of this vitamin after 14 days of SLx-4090 administration. Both 4- and 14-day administration of CP-346086 resulted in a greater decrease (−26 and −46%, respectively) in vitamin E. However, this effect was not statistically significant. Short-term administration of either compound had no effect on the water-soluble vitamin C.

In Vitro Metabolism in Human Liver Microsomes. The in vitro half-life of SLx-4090 was determined using a commercial preparation of human liver microsomes. Reactions were carried out for 60 min in the presence and absence of reductase NADPH. Samples were assayed for parent compound and metabolites using LC-MS/MS methods. The re-

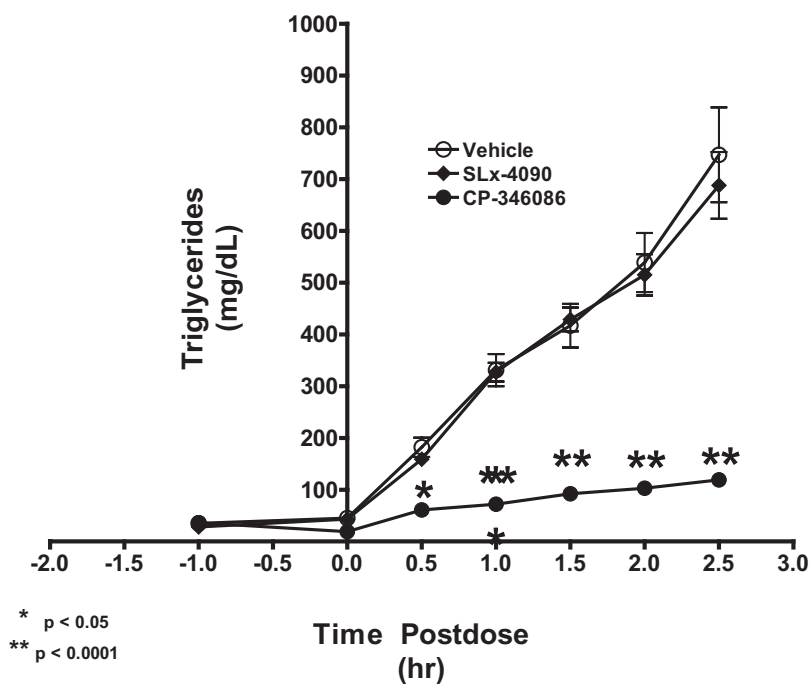


Fig. 3. Effect of tyloxapol on serum TG levels after SLx-4090 or CP-346086 administration to rats. Rats were fasted overnight and administered tyloxapol (1.0 ml; 125 mg/ml i.v.) and vehicle, SLx-4090 (30 mg/kg), or CP-346086 (30 mg/kg). Serum TG (without any meal challenge) were determined at the indicated time after drug administration. Tyloxapol inhibits serum lipases. In the absence of a dietary source of TG, the serum increase in TG reflects the release of TG from hepatic stores. Values are the mean \pm S.E.M. of three to five separate determinations at each time point. *, $p < 0.05$; **, $p < 0.0001$.

TABLE 2

Body weight and food intake in control and SLx-4090-treated during low- or high-fat diet

Food consumption represents the mean food consumption for all animals in a single cage. Individual animal food consumption was not determined because the animals were group-housed.

Treatment (n)	Body Weight (Mean \pm S.E.M.)			Food Consumption
	Initial	Final	Gain	
			<i>g</i>	
LFD (8)	21.1 \pm 0.6	30.3 \pm 0.9	9.2 \pm 0.8	4.0
LFD-4090 (8)	22.0 \pm 0.4	30.0 \pm 0.7	7.9 \pm 0.5	4.2
HFD (7)	22.0 \pm 0.7	39.1 \pm 1.7	17.1 \pm 1.2	3.1
HFD-LD (7)	22.1 \pm 0.5	38.2 \pm 1.8	16.1 \pm 1.5	2.8
HFD-MD (8)	21.5 \pm 0.4	33.4 \pm 1.0*	11.9 \pm 0.9**	2.9
HFD-HD (8)	21.4 \pm 0.4	31.3 \pm 1.3**	10.0 \pm 1.0***	3.0

HFD-LD = SLx-4090 5.8 mg/kg per day; HFD-MD = SLx-4090 18.9 mg/kg per day; HFD-HD = SLx-4090 66.8 mg/kg per day.

* $P < 0.05$ vs. HFD; ** $P < 0.01$ vs. HFD; *** $P < 0.001$ vs. HFD.

sults demonstrated a rapid metabolism of SLx-4090 to SLx-4099 (see Fig. 1) in the presence of NADPH with a SLx-4090 half-life of approximately 8 min. Microsomal reaction of SLx-4090 in the absence of reductase NADPH failed to produce metabolite SLx-4099, indicating that it is exclusively formed via cytochrome P450 oxidative metabolism. In similar studies using rat and dog hepatic or intestinal microsomes, only SLx-4099 has been identified as a metabolic product, suggesting the metabolic fate of SLx-4090 is similar in all species (data not shown).

Pharmacokinetics (Systemic). The intrinsic PK of SLx-4090 in rats determined via intravenous drug administration is shown in Fig. 7. After intravenous administration of SLx-4090 (1 mg/kg) significant levels of SLx-4090 were evident at the first time point (5 min). Levels of SLx-4090 rapidly declined such that they were less than 50 ng/ml by the 2-h time point. The $t_{1/2}$ value for SLx-4090 after intravenous dosing was 1.60 h. Substantially lesser amounts of SLx-4099 were detected in these animals.

When dosed orally at 10 mg/kg, no detectable amounts of SLx-4090 or its metabolite, SLx-4099, were found at any time

point up to 24 h postdose (lower LOQ 5 ng/ml). The oral bioavailability after a single oral dose of 10 mg/kg was 0%. When dosed orally at 30 mg/kg, plasma samples from five of the six rats showed no quantifiable amounts of SLx-4090 or its metabolite, SLx-4099. One animal in this group showed an amount of SLx-4090 slightly above the LOQ at one time point although no SLx-4099 was detected. The oral bioavailability after a single oral dose of 30 mg/kg was calculated to be 0.0011%. These data indicate that the absolute oral bioavailability of SLx-4090 after single pharmacologically relevant doses of 10 and 30 mg/kg is effectively 0%.

Pharmacokinetics (Portal Vein). When blood was collected from the portal vein of animals dosed orally with 25 mg/kg SLx-4090 no SLx-4090 was detected in portal vein plasma up to 24 h postdose. In three of four animals evaluated no levels of the metabolite SLx-4099 could be quantified up to 24 h postdose. In the fourth animal a small amount of SLx-4099 (12.6 ng/ml) was seen at a single time point (12 h). After oral gavage of 100 mg/kg SLx-4090, no levels of SLx-4090 or SLx-4099 could be quantified in rat plasma from blood drawn from the portal vein up to 24 h postdose in any of the four animals studied. These data indicate that the absorption of SLx-4090 after doses of 25 and 100 mg/kg is extremely low with no SLx-4090 detected in the portal circulation.

Discussion

The enterocyte, which lines the luminal wall of the intestine, has an expansive apical surface area and the intracellular machinery needed to produce lipoprotein vesicles that pass through the basal surface and into systemic circulation. This unique design optimizes the transfer of dietary nutrients into circulation (Mansbach and Siddiqi, 2010). One of the key players in the formation of lipoprotein vesicles or "chylomicrons" is MTP, making it a seemingly ideal target to disrupt dietary contributions to systemic lipid and cholesterol loads (Wierzbicki et al., 2009). Unfortunately, MTP is also found in a number of additional organs with notably

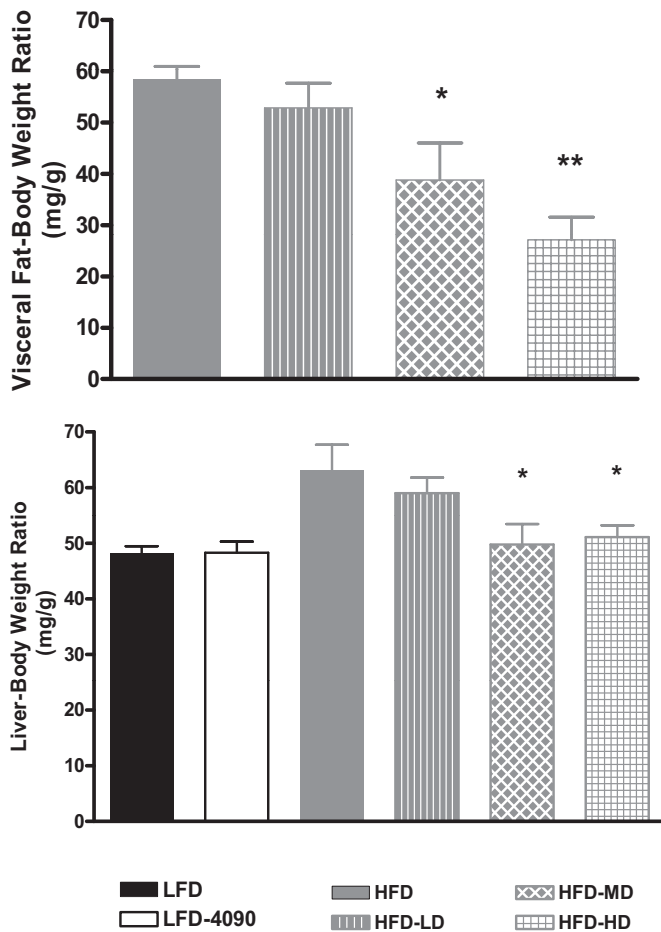


Fig. 4. Visceral fat and liver weights adjusted for body weight in control and SLx-4090-treated ApoE(-/-) mice during low- or high-fat diet. Visceral fat pads were obtained from control HFD-fed (HFD, $n = 7$) ApoE(-/-) mice and mice ingesting HFD containing LD ($n = 7$), MD ($n = 8$ fat, $n = 7$ liver), or HD ($n = 8$) SLx-4090 at 10 weeks and normalized to terminal body weight. Livers were obtained from control LFD-fed mice ($n = 8$), SLx-4090-treated LFD-fed ApoE(-/-) mice (LF-4090, $n = 8$), and HFD mice ingesting the control HFD ($n = 7$) or LD ($n = 7$), MD ($n = 8$ fat, $n = 7$ liver), or HD ($n = 8$) SLx-4090 at 10 weeks and normalized to terminal body weight. *, $p < 0.05$ versus HFD; **, $p < 0.01$ versus HFD.

high levels in the liver, retina, and heart (Borén et al., 1998; Li et al., 2005). Inhibition of hepatic MTP decreases the ability of the liver to rid itself of fat, and chronic treatment with first-generation MTP inhibitors resulted in unacceptable rises in liver transaminases and hepatic steatosis in rodent models and humans (Lammens et al., 1999; Lilly and

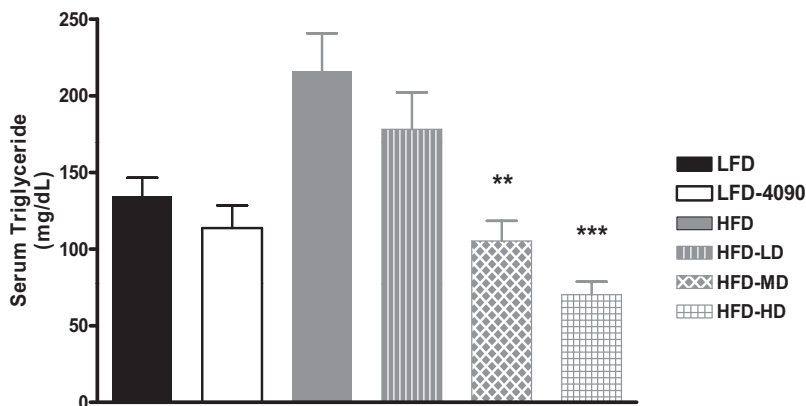


Fig. 5. Triglyceride levels in control and SLx-4090-treated ApoE(-/-) mice during low- or high-fat diet. Serum TG levels were obtained from control LFD-fed ($n = 8$), SLx-4090-treated LFD-fed ApoE(-/-) mice (LF-4090, $n = 8$), and HFD mice ingesting the control HFD ($n = 7$) or LD ($n = 7$), MD ($n = 8$ fat, $n = 7$ liver), or HD ($n = 8$) SLx-4090 at 10 weeks. **, $p < 0.01$ versus HFD; ***, $p < 0.01$ versus HFD.

Rader, 2007; Miyazaki et al., 2007; Wren et al., 2007). Inhibition of MTP in the heart and the retina may also elicit adverse effects (Borén et al., 1998; Li et al., 2005). The development of small-molecule therapeutics that selectively target the enterocyte but do not affect the lipid metabolism in organs such as the liver could provide a novel strategy to develop new therapies for the treatment of metabolic diseases. If these agents were efficacious they could provide a margin of safety superior to currently available drugs used in the treatment of metabolic disease. Based on this hypothesis, a strategy was implemented to develop an MTP inhibitor that was available to the intracellular space of the enterocyte (enterocytic availability) but did not enter the systemic circulation (nonbioavailability). Intestinal and hepatic MTP are highly conserved, making the development of an enterocytic selective compound a pharmacokinetic rather than a pharmacodynamic strategy.

SLx-4090 is an inhibitor of MTP that preferentially accumulates within the enterocyte and effectively blocks the formation of chylomicrons in vitro and in vivo. For example, in mass balance/tissue distribution studies with [14 C]SLx-4090 approximately 10% of the administered dose is recovered within the small intestine, whereas less than 1% is recovered from all other tissues (data not shown). Our studies establish that SLx-4090 preferentially inhibits enterocytic MTP, resulting in the improvement of metabolic endpoints and lacks the hepatic toxicities associated with systemically available MTP inhibitors.

SLx-4090 is a potent and selective inhibitor of MTP activity as demonstrated in human hepatic microsomal preparations and was comparable in potency to the clinical reference agent CP-346086 (Chandler et al., 2003). At the cellular level SLx-4090 was equipotent to CP-346086 in the inhibition of ApoB secretion from primary human hepatocytes and, like CP-346086, had no effect on the non-MTP-mediated secretion of ApoA1. The latter is significant because ApoB, but not ApoA1, requires the activity of MTP for lipoprotein vesicle formation. SLx-4090, the principal metabolite of SLx-4090, was significantly less potent than SLx-4090 at inhibiting MTP at the molecular and cellular level.

To confirm the enterocytic selectivity of SLx-4090, a series of in vitro and in vivo studies were performed. Data obtained using the differentiated Caco-2 cell permeability model were consistent with the idea that SLx-4090 would have limited systemic availability when dosed orally. SLx-4090 did not transit through the Caco-2 cell barrier when applied to either

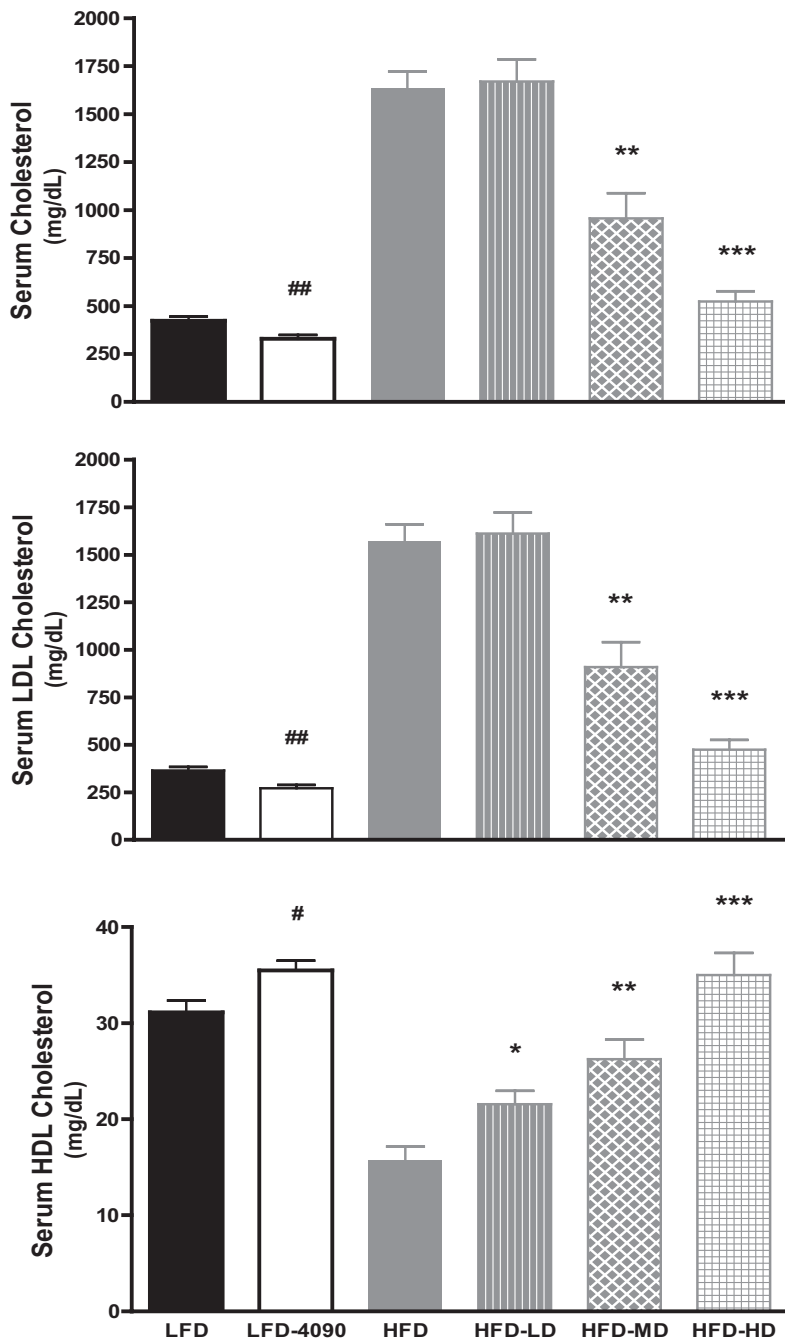


Fig. 6. Total, LDL-C, and HDL-C levels in control and SLx-4090-treated ApoE(-/-) mice during low- or high-fat diet. Total, LDL-C, and HDL-C levels were obtained from control LFD-fed ($n = 8$), SLx-4090-treated LFD-fed ApoE(-/-) mice (LF-4090, $n = 8$), and HFD mice ingesting the control HFD ($n = 7$) or LD ($n = 7$), MD ($n = 8$ fat, $n = 7$ liver), or HD ($n = 8$) SLx-4090 at 10 weeks. #, $p < 0.05$ versus LFD; ##, $p < 0.01$ versus LFD; *, $p < 0.05$ versus HFD; **, $p < 0.01$ versus HFD; ***, $p < 0.01$ versus HFD.

TABLE 3

Effect of SLx-4090 on serum levels of vitamins E, A, and C in Male Sprague-Dawley rats

Values are the mean \pm S.E.M. of the number of animals shown in parentheses and are reported as $\mu\text{g/ml}$. There were no significant differences among groups or treatments on any measure.

Dose Regimen	Four Daily Doses			14 Daily Doses		
	Vehicle	SLx-4090 (30 mg/kg)	CP-346086 (30 mg/kg)	Vehicle	SLx-4090 (30 mg/kg)	CP-346086 (30 mg/kg)
			$\mu\text{g/ml}$			
Vitamin E	1.27 \pm 0.17 (4)	1.49 \pm 0.16 (5)	0.94 \pm 0.13 (4)	2.11 \pm 0.91 (2)	1.61 \pm 0.23 (4)	1.14 \pm 0.09 (4)
Vitamin A	0.76 \pm 0.09 (4)	0.76 \pm 0.05 (5)	0.68 \pm 0.08 (4)	0.68 \pm 0.00 (2)	0.75 \pm 0.08 (4)	0.92 \pm 0.13 (4)
Vitamin C	2.73 \pm 0.34 (5)	2.78 \pm 0.25 (5)	2.60 \pm 0.33 (5)	N.D.	N.D.	N.D.

N.D., not determined.

the apical or basal cell surface. Although these data suggested that SLx-4090 would have limited systemic availability they did not demonstrate the enterocytic availability of

the SLx-4090. The ability of SLx-4090 to inhibit the secretion of ApoB from the Caco-2 cells (Table 1) is consistent with the notion that SLx-4090 is capable of entering the enterocyte

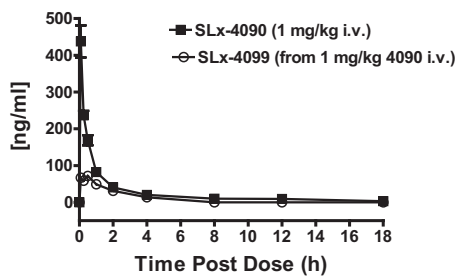


Fig. 7. Intrinsic pharmacokinetics of SLx-4090 and SLx-4099 after intravenous administration to rats. Shown is the pharmacokinetic profile of SLx-4090 and its principal metabolite, SLx-4099, after intravenous administration of 1 mg/kg to male Sprague-Dawley rats. Values are the mean \pm S.E.M. of at least three separate determinations at each time point.

and inhibiting MTP found within. It should be noted that other drugs (e.g., ezetimide) that inhibit cholesterol absorption (Van Heek et al., 1997) do not alter Apo-B secretion kinetics (Tremblay et al., 2006), suggesting that this site of action is not responsible for the actions noted for SLx-4090.

The systemic absorption of SLx-4090 is limited by its conversion to SLx-4099 by cytochrome P450 enzymes located in the enterocytes. This metabolism, as well as the natural turnover of the enterocytes from the intestinal lining (Thomson et al., 1994), limits the systemic absorption of SLx-4090.

Initial PK (LOQ 5 ng/ml) revealed that single doses (≤ 30 mg/kg) of SLx-4090 had an absolute bioavailability of 0% and the major metabolite SLx-4099 could not be detected in serum. It remained possible, however, that SLx-4090 was absorbed and transported via the portal vein to the liver where it might undergo first-pass metabolism. When SLx-4090 was dosed orally up to 100 mg/kg, neither SLx-4090 nor SLx-4099 were detected in plasma obtained from portal vein cannulated animals. These data are consistent with the lack of absorption of SLx-4090 from the gut and absence of SLx-4090 exposure to the liver.

To determine whether SLx-4090 was enterocytically available after oral administration, rats were challenged with a bolus of corn oil/sucrose, and levels of PP TG and free fatty acids were determined. SLx-4090 dose-dependently inhibited the rise in PP lipids, but did not result in a lowering of serum lipids below baseline levels. The effect was long-lasting with a maximally efficacious dose (30 mg/kg) resulting in a decrease in meal-driven serum lipid levels for 6 h. In contrast to SLx-4090, the systemically available MTP inhibitor CP-346086 seemed to reduce lipids to below baseline levels. Serum lipid levels represent the sum of lipids contributed by multiple sources, including those derived from dietary intake and those released from tissue sources, principally the liver. The response to MTP inhibitors acting at both the enterocyte and the liver are typically characterized by a decrease in serum lipids to levels below the predose fasting baseline. SLx-4090, acting only at the level of the enterocyte, affects only that portion of the serum lipid burden arising from dietary sources and does not lower baseline TG after a single dose. Finally, SLx-4090 administered at 30 mg/kg significantly inhibited the raise of PP free fatty acids, similar to CP-346086. This suggests that dietary lipids broken down in the intestinal lumen by lipases are not transported into the systemic circulation by a non-MTP-mediated mechanism.

To further demonstrate the enterocytic specificity of SLx-

4090, animals were treated with the serum lipase inhibitor tyloxapol. After an overnight fast the contribution of dietary lipids to the total serum lipid load is negligible. Under these conditions and in the presence of tyloxapol, the release of lipids from hepatic stores results in an increase in serum lipids. In the presence of tyloxapol, and the absence of dietary lipids, the rate of serum lipid increase is a direct reflection of the rate of hepatic lipid release. Consistent with an enterocytic site of action, SLx-4090 did not change the rate of serum lipid accumulation in these experiments. The systemically active MTP inhibitor CP-346086 blunted the increase in serum lipids as predicted by the inhibition of hepatic MTP.

Experimental studies have demonstrated that MTP inhibition leads to reductions in circulating lipids. Systemic MTP inhibitors have been shown to reduce TG and cholesterol in normal rodents and humans (Chandler et al., 2003; Aggarwal et al., 2005; Lilly and Rader, 2007; Miyazaki et al., 2007), HFD ApoE(-/-) mice (Ueshima et al., 2005), and mice with intestinal-specific MTP deletion (Xie et al., 2006).

ApoE(-/-) mice maintained on a HFD and SLx-4090 for 10 weeks had improved lipid profiles (lower baseline TG and total cholesterol and LDL-C and higher HDL-C levels) compared with vehicle-treated animals. In addition, SLx-4090 decreased the body weight gain in ApoE(-/-) mice over the course of treatment and had a positive impact on liver and fat stores as evidenced by reductions in fat and liver weight to body weight ratios. The overall reduction in body weight compared with vehicle animals was not the result of a decrease in food intake that has been reported for the MTP inhibitor dirlotapide (Wren et al., 2007). The enterocytic selectivity of SLx-4090 was further supported by the lack of elevation of the liver transaminases even at the highest dose tested. In contrast, the systemic MTP inhibitor implitapide significantly elevated liver transaminases in these mice (P. Sweetnam, unpublished data). Unlike other MTP inhibitors (Chandler et al., 2003; Ueshima et al., 2005; Miyazaki et al., 2007), SLx-4090 also significantly increased HDL-C.

The major impediment to the development of clinically useful MTP inhibitors has been the rapid development of liver toxicities (Lammens et al., 1999; Lilly and Rader, 2007; Miyazaki et al., 2007). In contrast, SLx-4090 demonstrated no indication of hepatic damage from either a histological or a clinical chemistry perspective in toxicological studies as long as 90 days with doses as high as 1000 mg/kg (rat) or 100 mg/kg (dog) (P. Sweetnam, unpublished data). These findings extended to all organ systems. The resistance of animals to hepatic damage is attributed to two primary characteristics of SLx-4090, its low absorption and rapid metabolism to SLx-4099, a compound with low potency to inhibit MTP.

SLx-4090 had only a minor, if any, impact on the levels of endogenous fat-soluble vitamins, in contrast to other MPT inhibitors (Anwar et al., 2007). Because blockade of essential nutrient absorption could be of concern in a chronic dosing scenario these data suggest that impact of SLx-4090 will be negligible in this regard.

Current dyslipidemic agents work predominantly by targeting the liver. The statins inhibit HMG-CoA reductase, whereas the fibrates target peroxisome proliferator-activated receptor α . Ezetimibe, a cholesterol absorption inhibitor thought to demonstrate the utility of targeting the absorption of cholesterol by the enterocyte, has been shown to in part work through blocking the same uptake site in the liver. The

combination of SLx-4090 with any of these agents presents a cotherapy strategy that would target two organs important in fat metabolism, the intestine and the liver. In addition, the reduction of circulating chylomicrons should ultimately reduce the number of circulating chylomicron remnants, reducing the risk of atherosclerotic plaque formation (Botham, 2008). The enterocytic lining of the intestinal lumen sits at the vertex of a host of metabolic diseases that are caused or exacerbated by improper dietary behaviors common in today's society. For anatomical reasons, this tissue is an easily accessible target for therapies that reduce the passage of dietary lipid and cholesterol. This approach provides a direct method to induce caloric restriction and improve metabolic endpoints. It is noteworthy that it is a strategy that does not involve the direct interaction with the liver and the complications that are often associated with doing so. This approach opens the way to look at other mechanisms to treat not only metabolic disorders but inflammation and potential cancers of the gastrointestinal tract.

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Authorship Contributions

Participated in research design: Kim, Wong, Cole, Kuo, Ellis, Ferkany, and Sweetnam.

Conducted experiments: Kim, Wong, and Kuo.

Contributed new reagents or analytic tools: Kim, Campbell, and Schueller.

Performed data analysis: Kim, Wong, Kuo, Ellis, Ferkany, and Sweetnam.

Wrote or contributed to the writing of the manuscript: Schueller, Cole, Kuo, Ferkany, and Sweetnam.

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