Full Paper

A Novel Mouse Model of Combined Hyperlipidemia Associated With Steatosis and Liver Injury by a Single-Dose Intragastric Administration of Schisandrin B/Cholesterol/Bile Salts Mixture

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Abstract. Hyperlipidemia is referred to as hypercholesterolemia, hypertriglyceridemia, or both in combined hyperlipidemia. Here, a novel mouse model of combined hyperlipidemia is described. Mice were orally given a single dose of a modeling agent (MA) made of a mixture of schisandrin B/cholesterol/bile salts (1/2/0.5 g/kg) suspended in olive oil. MA treatment increased serum triglycerides (TG) and total cholesterol (TC) (up to 422% and 100% at 12 – 96 h post-treatment, respectively) and hepatic TG and TC (up to 220% and 26%, respectively) in a time- and dose-dependent manner, associated with elevation of high-density lipoprotein and low-density lipoprotein levels. Serum alanine/aspartate aminotransferase activities, indicators of liver cell damage, were also elevated (up to 198%) at 48 and 72 h post-MA treatment. Fenofibrate blocks MA-induced hyperlipidemia, lipid accumulation in the liver, as well as liver injury. Oral administration of a mixture of schisandrin B, cholesterol, and bile salt could generate an interesting mouse model of combined hyperlipidemia associated with hepatic steatosis and steatohepatitis.

Keywords: combined hyperlipidemia, hepatic steatosis, hepatomegaly, schisandrin B, fenofibrate

Introduction

It is well known that hyperlipidemia (including hypercholesterolemia and hypertriglyceridemia, or their combination) is a prevalent health problem in modern societies. Due to their association with atherosclerosis, hyperlipidemia constitutes one of the major risk factors for vascular diseases such as coronary artery disease, a leading cause of death in developed countries, as well as hepatic steatosis (1, 2). Fatty liver-induced steatohepatitis, a specific pattern of injury within the spectrum of non-alcoholic fatty liver disease, can gradually lead to the development of fibrosis, cirrhosis, liver failure, and even hepatocellular carcinoma (3). In view of the adverse health consequences caused by hyperlipidemia, the

search for lipid-lowering drugs has been an area of intensive research. As such, the development of animal models of hyperlipidemia is instrumental to expedite the discovery of drugs to treat this problem. Experimental models of hyperlipidemia to date include high-fat diet- or fructose-enriched diet-fed mice or rats (4, 5), apolipoprotein E-deficient (ApoE^{−/−}) mice (6), LDL receptor-deficient (*Ldlr*^{−/−}) hypercholesterolaemic mice (7), glutamate-cysteine ligase modifier subunit gene-deficient [*Gclm*(^{−/−}) mice (8), *APOE*3Leiden.cholesteryl ester transfer protein* (*E3L.CETP*)] (9), or ApoCIII transgenic mice or pigs (10) as well as combination of *Ldlr*^{−/−} and high-fat diet-fed mice (7). Interestingly, ApoCIII transgenic animals showed significantly increased plasma triglyceride (TG) levels, but with minimal changes in plasma cholesterol (TC) concentrations (11).

Previous studies in our laboratory have shown that Schisandrin B (Sch B) treatment significantly increased serum and hepatic TG, but not TC, levels in mice, which

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led to the setting up of a mouse model of hypertriglyceridemia (12, 13). In the present study, a mouse model of combined hyperlipidemia (i.e., hypertriglyceridemia/hypercholesterolemia) was created by a single intragastric administration of a mixture of Sch B, cholesterol (Ch), and a bile salt (Bs) suspended in olive oil. As hepatosteatosis is often associated with liver damage (14), we also examined the effects of the mouse model of dyslipidemia on liver damage.

Materials and Methods

Chemicals and reagents

Sch B was purified from the petroleum ether extract of dried fruits of *Schisandra chinensis*, as previously described (15). The purity of Sch B, as determined by high performance liquid chromatography analysis, was higher than 95%. Fenofibrate (certificate No. 0405030) was bought from Beijing Yongkang Medical, Ltd. (Beijing, China). Ch (certificate No. 20110324) and Bs (certificate No. F20101214) were purchased from Beijing Chemical Reagent Co. (Beijing, China). Sodium carboxymethylcellulose (CMC, certificate No. 971230) was obtained from Beijing Xudong Chemical Plant (Beijing, China). Olivioila extra virgin olive oil was purchased from a local market. Fenofibrate was dissolved with 0.5% CMC solution. Assay kits for measuring TC, TG, high density lipoprotein (HDL), and low density lipoprotein (LDL) levels, as well as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were bought from Zhongsheng Beikong Biotechnology and Science, Inc. (Beijing, China) or Beijing Leadman Biochemistry Co., Ltd. (Beijing, China).

Animal care

All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Beijing University of Chinese Medicine. Male ICR mice (Grade II, certificate No. SCXK(jing)2006-0009), weighing 18–20 g, were purchased from Vital River Lab Animal Co., Ltd. (Beijing, China). They were maintained at 20°C–21°C, with a relative humidity of 50%–55% and allowed free access to water and food. Experiments were performed when the animals had attained a body weight of 24–26 g.

Preparation of the modeling agent

The modeling agent (MA) tested in the present study was a mixture of Sch B, Ch, and Bs suspended in olive oil. A full dose of MA was made up of Sch B (1 g/kg), Ch (2 g/kg), and Bs (0.5 g/kg), with olive oil being administered at 20 mL/kg. A half dose of MA was exactly one half of a full dose of MA: Sch B, 0.5 g/kg;

Ch, 1 g/kg; and Bs, 0.25 g/kg, suspended in olive oil at 10 mL/kg. Both MAs were administered orally once for generating a mouse model of combined hypertriglyceridemia and hypercholesterolemia.

Experimental design

Design 1: In this study, mice received either Sch B (1 g/kg) or Ch/Bs (2/0.5 g/kg), with both being suspended in olive oil (10 mL/kg). Control animals were orally administered vehicle (10 mL/kg) only. Twenty-four hours following the treatment, animals were sacrificed. Blood, collected from the orbital vein under light ether anesthesia, and liver tissue samples were obtained and subjected to biochemical analysis.

Design 2: Mice were divided into two groups; 1) for the half-dose of MA group, animals were intragastrically treated with Sch B/Ch/Bs (0.5/1/0.25 g/kg, suspended in olive oil, given at the dose of 10 mL/kg); 2) in the full-dose of MA group, animals received Sch B/Ch/Bs (1/2/0.5 g/kg, suspended in olive oil, given at the dose of 20 mL/kg). Control (i.e., non-Sch B/Ch/Bs-treated) animals were given vehicle only. Mice were sacrificed at 12, 24, 48, 72, 96, and 120 h post-treatment.

Design 3: The aim of this experimental design was to explore the effect of hypolipidemic drug intervention in this model. Fenofibrate is a drug used to decrease blood TC and TG levels in the clinic. In this study, mice were intragastrically treated with fenofibrate [0.1 g/kg, suspended in 0.5% (w/v, in H₂O) CMC] once daily for 3 days. The fenofibrate-untreated group received the vehicle (10 mL/kg) orally for 3 days. On the fourth day of the experiment, mice were treated with fenofibrate dissolved in olive oil (20 mL/kg) alone or a combination of fenofibrate and full-dose of MA. Fenofibrate-untreated animals were orally administered MA only. Control animals received vehicle only (olive oil, 20 mL/kg). Blood and liver samples were obtained at 24, 48, and 72 h after the last treatment.

Measurement of lipid contents

Serum samples, which were prepared by centrifuging the whole blood for 8 min at 2000 × g, were analyzed for lipid profiles using a Biocode Hyclis Lisa 300 Hematology analyzer. Liver tissue samples were homogenized in 9 volumes of saline using two 10-s bursts of a tissue disintegrator at 13,500 rpm, and the homogenate was then centrifuged at 2000 × g for 15 min to obtain the supernatants. The hepatic supernatants (10 or 40 μL) were used to determine the TG and TC levels, respectively, using assay kits according to the manufacturer's instructions. In essence, the enzyme-coupled reactions resulted in the formation of quinone imide, which can be measured spectrophotometrically (Spectrophotometer

Unic UV-2000; Unico Instruments Co., Ltd., Shanghai, China) at 500 nm. The concentration of TG or TC in the assay sample was estimated from the equation: (sample OD / standard OD) $\times$ standard concentration. Under the standard assay conditions described in the manufacturer's manual, the analytical range of the assay kit is 0–11.29 mmol/L for TG and 0–12.93 mmol/L for TC. Serum HDL and LDL levels, as well as ALT and AST activities were detected by an automatic Biochemistry Analyzer (Beckman Coulter Synchron CX4 PRO; Beckman, Brea, CA, USA).

Measurement of hepatic index

Liver weight was measured and hepatic index was estimated from the ratio of liver weight to body weight (liver weight / body weight $\times$ 100).

Statistical analyses

All group data are shown as the mean $\pm$ S.E.M. Statistical comparisons were determined using either one-way ANOVA followed by Dunnett's multiple comparison test, Student's *t*-test, or post-hoc analysis using SPSS 16.0 for Windows. Differences were considered significant at $P < 0.05$.

Results

Effects of Sch B, Ch/Bs, or their combination treatment on serum TG and TC levels

Figure 1A shows that Sch B/olive oil (1 g/10 mL / kg) had no significant effect on TC, but serum TG levels were markedly increased (by 399%) at 24 h post-dosing, when compared with the control; in contrast, Ch/Bs/olive oil (2 g/0.5 g/10 mL / kg) treatment increased TC (by 54%) but decreased TG (by 41%) under the same conditions. Changes in serum TG and TC levels produced by Sch B, Ch, and Bs combination treatment were time-dependent. Sch B/Ch/Bs at the dose of 0.5/1/0.25 g/kg (half-dose MA) increased the serum TG levels by 210%, 157%, 36%, 19%, and 13% at 12, 24, 48, 72, and 96 h post-treatment, respectively. After Sch B (1 g/kg), Ch (2 g/kg), and Bs (0.5 g/kg) (full-dose MA) combination treatment, serum TG levels were increased by 84%, 422%, 202%, 120%, and 56% at 12, 24, 48, 72, and 96 h, respectively (Fig. 1B). Serum TC concentrations in mice receiving half-/full-dose of MA were increased by 57/86%, 64/85%, 36/101%, 33/81%, and 25/60% at 12, 24, 28, 72, and 96 h, respectively, post-treatment (Fig. 1C).

Effects of Sch B, Ch/Bs, or their combination treatment on hepatic TG and TC contents

Treatment of mice with Ch/Bs (2/0.5 g/kg) resulted

Fig. 1. Schisandrin B/cholesterol/bile salt-induced changes in serum triglyceride (TG) and total cholesterol (TC) levels in mice. Mice were orally administered Schisandrin B (Sch B) at 1 g/kg, cholesterol/bile salts (Ch/Bs) at 2/0.5 g/kg (A), half-dose of MA (left panel in B and C), or full-dose of MA (right panel in B and C). Control mice received vehicle (olive oil). Serum TG and TC levels were measured at 12, 24, 48, 72, 96, and 120 h post-MA treatment or at 24 h post-vehicle treatment (control mice). However, all serum samples including control mice were collected on the same day. Our preliminary test showed that vehicle did not impact on the serum biomarkers determined in the present study (data not shown) after 12–96-h treatment, compared with the water treatment. Therefore the control data at 12, 48, 72, 96, and 120 h after vehicle alone treatment was not presented. Values given are the mean $\pm$ S.E.M., with $n = 10$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control (Ctrl), using a one-way ANOVA followed by Dunnett's multiple comparisons test or Student's *t*-test. MA is the abbreviation for the modeling agent. Half-dose of MA treatment implies that Sch B/Ch/Bs (0.5/1/0.25 g/kg, suspended in olive oil 10 mL/kg) is given to mice. Full-dose of MA treatment implies that Sch B/Ch/Bs (1/2/0.5 g/kg, suspended in olive oil 20 mL/kg) is given to mice.

Fig. 2. Schisandrin B/cholesterol/bile salt-induced changes in hepatic triglyceride (TG) and total cholesterol (TC) levels in mice. Experimental details are as described in Fig. 1. Hepatic TG and TC levels were measured at 12, 24, 48, 72, 96, and 120 h post-treatment. Values given are the mean $\pm$ S.E.M., with $n = 10$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control (Ctrl), using a one-way ANOVA followed by Dunnett's multiple comparisons test or Student's t -test.

in an increase (131%) in hepatic TC but a reduction (37%) in hepatic TG levels after 24 h. In contrast, administration of Sch B (1 g/kg) increased hepatic TG by 209%, whereas hepatic TC levels decreased by 43% at the same time point (Fig. 2A). The effects of half- or full-dose MA treatment on hepatic TG and TC levels were investigated. The half dose of MA decreased hepatic TG by 25% and 20% at 12 and 24 h post-

treatment, respectively, but increased hepatic TG by 24% at 96 h. However, hepatic TG content was increased by 127%, 220%, 126%, 103%, 34%, and 22% at 12, 24, 48, 72, 96, and 120 h, respectively, after a full dose of MA treatment (Fig. 2B). The half-dose MA treatment lowered hepatic TC (by 15%–20%) at 12, 24, and 96 h post-treatment. Hepatic TC content was elevated (by 20%) at 72 and 96 h post-MA treatment at full-dose (Fig. 2C).

Effects of Sch B, Ch/Bs, or their combination treatment on hepatic function

Treatment with Ch/Bs, but not Sch B, caused increases in activities of serum ALT (by 100%) and AST (by 39%), indicative of hepatic injury at 24 h post-treatment (Fig. 3A). However, half-dose of MA treatment produced no detectable effect on serum ALT and AST activities up to 72 h post-treatment. MA-treated mice at full-dose showed elevated activities of serum ALT (by 198%) and AST (by 64%) at 72 h post-treatment (Fig. 3: B and C). Serum transaminase activities were slightly suppressed at 96 and 120 h after MA treatment at both doses.

Effects of Sch B, Ch/Bs, or their combination treatment on hepatic mass

Sch B, but not Ch/Bs, treatment increased hepatic index (the ratio of liver weight to body weight) by 41% at 24 h post-treatment (Fig. 4A). MA markedly increased the hepatic index by 23%, 25%, 32%, 17%, and 12% at 12, 24, 48, 72, and 96 h, respectively, after half-dose of MA treatment. MA at full dose increased the hepatic index by 10%, 27%, 45%, 66%, 42%, and 15% at 12, 24, 48, 72, 96, and 120 h post-treatment, respectively (Fig. 4B).

Effect of fenofibrate pretreatment on serum lipid profiles in combined hyperlipidemic mice

In addition to elevating serum TG and TC levels, Table 1 showed that MA treatment at full dose also increased serum HDL level (by 36% and 44%, at 48 and 72 h post-treatment, respectively). LDL levels were also elevated by 58 and 97% at 24 and 48 h, respectively, following the full-dose MA treatment. Fenofibrate pretreatment alone caused decreases in serum TG (by 54% and 45% at 24 and 48 h post-treatment, respectively) and TC (by 13% at 24 h, respectively) levels. However, it increased serum TC by 35% at 48 h post-treatment. Combined treatment with fenofibrate and MA decreased serum TC, TG, and HDL levels by 27%, 52%, and 22%, respectively, at 72 h post-treatment, when compared with those of fenofibrate-untreated animals with MA. However, serum HDL levels in animals treated with both

Fig. 3. Schisandrin B/cholesterol/bile salt-induced changes in hepatic function in mice. Experimental details are as described in Fig. 1. The serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, biochemical indices of liver injury, were measured at 12, 24, 48, 72, 96, and 120 h post-treatment. Values given are the mean $\pm$ S.E.M., with $n = 10$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control (Ctrl), using a one-way ANOVA followed by Dunnett's multiple comparisons test or Student's t -test.

MA and fenofibrate showed a further increase at 48 h after administration by 15% and 19% compared with MA and fenofibrate treatment alone, respectively. No detectable changes in these serum lipid parameters were apparent at 24 post-treatment (Table 1).

Effect of fenofibrate pretreatment on hepatic lipids contents in combined hyperlipidemic mice

MA induced the elevation of hepatic TC and TG levels. Hepatic TG levels were lowered by 46%, 37%, and 25% at 24, 48, and 72 h after treatment with fenofibrate alone for 4 days, when compared with the corresponding control group, respectively. Combination of fenofibrate and full-dose MA treatment reduced TG levels by 32%, 56%, and 46% at 24, 48, and 72 h after the last dosing, compared with the corresponding model group untreated with fenofibrate (Fig. 5A). Fenofibrate pretreatment effectively inhibited the MA-induced elevation of TC by 63% and 31% at 24 and 72 h after treatment, respectively (Fig. 5B). However, fenofibrate did not affect the hepatic TC content in mice receiving MA at 48 h post-treatment.

Effects of fenofibrate pretreatment on hepatic index and function in combined hyperlipidemic mice

Figure 6 shows that both fenofibrate and MA treatment at full dose individually markedly increased the hepatic index (by 17% – 67%), when compared with the control group. Combined treatment with fenofibrate and MA treatment further increased the hepatic index (by 76% – 139%) (Fig. 6A). Serum ALT activity was elevated (by 50% and 31% at 24 and 48 h post-dosing) in mice treated with fenofibrate. MA increased ALT activity by 34% and 103% at 48 and 72 h post-treatment, respectively. Combined treatment with fenofibrate and MA further increased ALT activity at 48 h post-treatment. However, fenofibrate pretreatment completely suppressed the MA-induced elevation in serum ALT activity at 72 h post-dosing (Fig. 6B).

Discussion

Over the last twenty years, several strains of genetically modified mice have been established to serve as animal models of lipid metabolism disorders. However, fat and/or cholesterol-enriched diets with variations in the lipid and carbohydrate components are still commonly used to induce hyperlipidemia in a variety of experimental animals including mouse, rat, golden hamster, guinea pig, rabbit, pigeon, and quail (16 – 19). While an elevation of blood cholesterol level is always found in animal models of diet-induced hyperlipidemia, blood TG levels show inconsistent changes. Thus, elevations (20, 21), decreases (17), or no changes (22, 23) of TG levels in the blood have been observed. Recently, Gómez-Pérez et al. reported that blood TG levels are increased in female mice and unchanged in male mice fed with the same high-fat diet (13% protein, 33% carbohydrate, and 54% lipid) (24).

Fig. 4. Schisandrin B/cholesterol/bile salt-induced changes in hepatic index in mice. Experimental details are as described in Fig. 1. Hepatic index was measured at 12, 24, 48, 72, 96, and 120 h post-treatment. Values given are the mean $\pm$ S.E.M., with $n = 10$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control (Ctrl), using a one-way ANOVA followed by Dunnett's multiple comparisons test or Student's t -test.

Table 1. Effects of fenofibrate pretreatment on serum lipid profiles in combined hyperlipidemic mice

Groups	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
24 h post treatment				
Control	3.52 $\pm$ 0.13	1.49 $\pm$ 0.07	3.64 $\pm$ 0.12	0.31 $\pm$ 0.02
Model	4.49 $\pm$ 0.28**	5.57 $\pm$ 0.44***	3.81 $\pm$ 0.21	0.49 $\pm$ 0.07*
Fenofibrate	3.05 $\pm$ 0.15*	0.68 $\pm$ 0.05***	3.29 $\pm$ 0.15	0.31 $\pm$ 0.03
Model/fenofibrate	4.31 $\pm$ 0.19**##	5.19 $\pm$ 0.27***##	3.69 $\pm$ 0.12	0.46 $\pm$ 0.06*#
48 h post treatment				
Control	3.11 $\pm$ 0.15	1.35 $\pm$ 0.12	3.30 $\pm$ 0.16	0.35 $\pm$ 0.02
Model	5.41 $\pm$ 0.28***	3.86 $\pm$ 0.82***	4.49 $\pm$ 0.25***	0.69 $\pm$ 0.04***
Fenofibrate	4.19 $\pm$ 0.16***	0.74 $\pm$ 0.11***	4.36 $\pm$ 0.11***	0.53 $\pm$ 0.03***
Model/fenofibrate	5.88 $\pm$ 0.23***##	3.93 $\pm$ 0.57***##	5.17 $\pm$ 0.13***##	0.64 $\pm$ 0.03***#
72 h post treatment				
Control	3.42 $\pm$ 0.13	1.39 $\pm$ 0.07	3.54 $\pm$ 0.12	0.30 $\pm$ 0.02
Model	5.31 $\pm$ 0.33***	2.83 $\pm$ 0.14***	5.10 $\pm$ 0.31	0.41 $\pm$ 0.10*
Fenofibrate	3.54 $\pm$ 0.10	1.44 $\pm$ 0.17	3.65 $\pm$ 0.10	0.30 $\pm$ 0.04
Model/fenofibrate	3.87 $\pm$ 0.12††	1.26 $\pm$ 0.14††	3.99 $\pm$ 0.12††	0.32 $\pm$ 0.05†

Mice were pretreated with fenofibrate (0.1 g/kg per day for 3 day, p.o.). On day 4 of the experiment, combined hyperlipidemic mice were intragastrically administered with a full dose of modeling agent, a mixture of schisandrin B/cholesterol/bile salt (1/2/0.5 g/kg), with and without fenofibrate co-treatment. Control mice received vehicle. Serum samples were obtained at 24, 48, and 72 h after the last treatment and were analyzed for total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), and low density lipoprotein (LDL) levels. Values given are the mean $\pm$ S.E.M., with $n = 10$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control group; † $P < 0.05$, †† $P < 0.01$ vs. model group; # $P < 0.05$, ## $P < 0.001$ vs. fenofibrate group. Differences were determined using a one-way ANOVA followed by Dunnett's post-hoc analysis.

Despite the fact that contemporary genetic models of hyperlipidemia are very sophisticated, they only focus on some genes that influence lipid metabolism on a specific target(s). For example, *Ldlr*^{-/-} and *ApoE*^{-/-} mice only emerge as a model of hypercholesterolaemia (6, 7); hypertriglyceridemia is observed in hypertriglyceridemic *ApoCIII* transgenic mice (10). However the pathogenesis of human hyperlipidemia is much more complex than

that of genetic models except for familial hyperlipidemia. When compared with the existing genetic and diet-based models, our model has the advantages of simplicity and rapidity of development. The model reproduces manifestations of clinical hyperlipidemia such as hypertriglyceridemia, hypercholesterolemia, hyperlipoproteinemia, and fatty liver, as well as steatohepatitis.

Sch B is an active dibenzocyclooctadiene derivative

Fig. 5. Effects of fenofibrate pretreatment on hepatic triglyceride (TG) and total cholesterol (TC) contents in combined hyperlipidemic mice. Experimental details are as described in Table 1. Hepatic TG (A) and TC (B) contents were measured at 24, 48, and 72 h after the last treatment. Values given are the mean $\pm$ S.E.M., with $n = 10$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control (Ctrl); † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$ vs. model; # $P < 0.05$, ### $P < 0.001$ vs. fenofibrate. Differences were determined using a one-way ANOVA followed by Dunnett's post-hoc analysis.

Fig. 6. Effects of fenofibrate pretreatment on hepatic index and function in combined hyperlipidemic mice. Experimental details are as described in Table 1. Hepatic index and serum alanine aminotransferases (ALT) were measured at 24, 48, and 72 h after the last treatment. Values given are the mean $\pm$ S.E.M., with $n = 10$. ** $P < 0.01$, *** $P < 0.001$ vs. control (Ctrl); † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$ vs. model; # $P < 0.01$, ### $P < 0.001$ vs. fenofibrate. Differences were determined using a one-way ANOVA followed by Dunnett's post-hoc analysis.

isolated from the fruit of *Schisandra chinensis*, a deciduous woody vine native to forests of Northern China and the Russian Far East, which has been used as tonic and restorative adaptogen with notable liver protecting effects (25, 26). In the present study, Sch B increased TG levels in serum and liver tissue, resulting in hypertriglyceridemia and fatty liver. While high intake of cholesterol results in hypercholesterolemia, Bs (sodium salts of bile acids) can facilitate the intestinal absorption of lipids (27, 28), as well as induce liver injury by virtue of their pro-oxidant activity (29). Current investigations have shown that bile acids stimulate the regulation of early growth response factor-1 (Egr-1) via mitogen-activated protein kinase (MAPK) signaling in the liver during cholestasis, which causes liver injury (30). Although the exact mechanism of Sch B-induced hypertriglyceridemia is still unclear, the formulation of the current MA is very practical in that a mouse model of combined hyperlipidemia is able to serve the needs of research and development of lipid-lowering drugs.

MA used in the current study was found to markedly increase serum TG and TC levels in mice from 12 to 96 h post-treatment. The extent of MA-induced elevation of TC was not as large as that of TG in the liver. A representative microscopic picture of a hematoxylin and eosin-stained liver tissue section indicated that hepatic lipid deposits, which appeared as small vacuoles within the cytoplasm of liver cells, were found in model mice (data not shown). Hepatic TC and TG levels were reduced in the mice treated with half-dose MA. This may be attributed to the fact that Ch/Bs and Sch B *per se* can produce a triglyceride- and cholesterol-lowering effect on the liver, respectively (13). However, the hepatic TC

and TG were increased in the mice administered with high-doses Sch B, Ch, and Bs (full-dose MA). The observation that Ch/Bs treatment increased hepatic TC, but decreased the TG level, and that Sch B treatment increased hepatic TG, but decreased TC content, may be related to the possible reciprocal regulation of hepatic TC and TG levels in mice. The elevation of serum HDL level may be an event secondary to MA-induced hyperlipidemia and/or the increased serum LDL level. HDL particles remove TC from within artery atheroma and transport cholesterol back to the liver for excretion and/or re-utilization (31). In contrast, LDL particles deposit cholesterol onto the vessel wall and lead to an increased risk of CAD (32).

Serum ALT and AST activities, which are biochemical parameters of liver cell injury, were increased at 24 h after the combined treatment with Ch and Bs. The Ch/Bs-induced liver injury is likely caused by the Bs component (30, 33). However, the combined treatment with a mixture of Ch/Bs and Sch B only slightly increased serum ALT and AST activities at 24 h post-treatment. This apparent hepatoprotective effect is likely related to the antioxidant activity and modulation of the NF- κ B pathway of Sch B (34–36). Recently, it was found that gomisins N, the active component of Sch B, can inhibit the DNA damage checkpoint signaling by stereospecifically interacting with ataxia telangiectasia and Rad-3-related protein kinase (37). However, progressive increases in serum ALT and AST activities were observed in MA-treated mice from 48 to 72 h post-treatment. The result suggested that liver injury associated with steatosis/fatty liver (likely due to steatohepatitis) developed in a time-dependent manner in this mouse model of combined hyperlipidemia induced by MA.

To investigate potential applications of the mouse model, the effects of fenofibrate pretreatment on MA-induced changes were examined. Fenofibrate (a fibric acid agent) used alone or in combination with other agents has long been shown to have a beneficial effect in patients with hyperlipidemia. In essence, fenofibrate was found to reduce serum concentrations of LDL and TG, with an associated increase in serum HDL. In the present study, fenofibrate treatment for 4 days was shown to reduce serum TG levels at 24 and 48 h after the last dosing. However, serum TC, HDL, and LDL levels were increased at 48 h following the drug withdrawal in non-hyperlipidemic mice. Fenofibrate treatment lowered hepatic TG contents (at 24, 48, and 72 h after the last dosing) and TC contents (at 24 and 72 h, but not 48 h, after the last dosing) in MA-induced hyperlipidemic mice. Fenofibrate-induced decreases in serum levels of TC and TG were found only at 72 h

after the last dosing in MA-induced hyperlipidemic mice. The results indicated that fenofibrate is more effective in reducing hepatic levels than serum TC and TG levels in both normal and hyperlipidemic mice. The inability of fenofibrate to decrease hepatic TC content in hyperlipidemic mice at 48 h post-dosing may be related to liver damage caused by the combined treatment with fenofibrate and MA treatment.

Although current clinical data showed that fenofibrate reduced lymphocyte release of interleukin-2, interferon- γ , and tumor necrosis factor- α (38), it can cause acute cholestatic hepatitis in patients given this drug at the dose of 300 mg daily for 10–14 days (39–41). In the present study, fenofibrate treatment (100 mg/kg per day for 4 day) was found to cause acute liver injury, which seemed to be reversed at 72 h after the final dosing. While the combined treatment with fenofibrate with MA further increased the extent of liver damage at 48 h post-treatment, fenofibrate pretreatment suppressed the MA-induced increase in serum ALT activity at 72 h after the last dosing. This hepatoprotective action of fenofibrate may, at least in part, be attributed to the fenofibrate-induced decline in hepatic lipids.

In conclusion, in the present study, a MA consisting of a mixture of Sch B, cholesterol, and Bs suspended in olive oil was investigated by examining its effect on plasma and hepatic lipid profiles as well as hepatic function. The results showed that this mouse model of combined hyperlipidemia, which simulates almost all aspects of hyperlipidemia in humans in a very short period of time (within 12–72 h), including hypertriglyceridemia and hypercholesterolemia as well as

Fig. 7. A summary of the current research.

significant elevations of the serum LDL and HDL level and ALT activity, was established by this mixture. The greatest extents of hyperlipidemia and hepatomegaly/liver injury were observed at 24 and 72 h, respectively, after MA administration. While hypercholesterolemia was maintained from 12 to 120 h post-treatment, fenofibrate pretreatment effectively suppressed the extents of hypertriglyceridemia, hypercholesterolemia, hepatic steatosis, and hepatic injury in MA-induced hyperlipidemic mice. In contrast, the combined treatment with fenofibrate and MA caused hepatomegaly in an apparently additive manner (summarized in Fig. 7). The mouse model of combined hyperlipidemia induced by Sch B/Ch/Bs may prove useful for effectively assessing the effects of potential lipid-lowering drugs and related mechanisms underlying the regulation of lipid metabolism.

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