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Title

JTP-109192, a novel G protein-coupled receptor 119 agonist, prevents atherosclerosis by improving hypercholesterolemia in congenic spontaneously hyperlipidaemic mice

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Short title

JTP-109192 prevents atherosclerosis by improving hypercholesterolemia

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Abstract

G protein-coupled receptor 119 (GPR119) expression in pancreatic β-cells and intestinal L-cells is a potential therapeutic target for the treatment of type 2 diabetes. Previously, we have reported that the GPR119 agonist JTP-109192 improves glucose metabolism with single and repeated administration. Conversely, overexpression of the *Gpr119* gene reportedly regulates cholesterol transporter expression in animal models, and a natural GPR119 agonist, oleoylethanolamide (OEA), improves atherosclerosis. Therefore, improving dyslipidaemia is considered a possible feature of GPR119 agonists. In the present study, the lipid-lowering effect of JTP-109192 was examined in BALB/c background spontaneously hyperlipidaemic (SHL) mice with repeated administration, once daily for 12 weeks. On repeated administration, JTP-109192 revealed a cholesterol-lowering effect and improved atherosclerosis following histopathological examination. With further investigation, the cholesterol-lowering effect and subsequent antiatherosclerotic effect of JTP-109192 was attributed to changes in intestinal cholesterol metabolism gene expression. Based on these results, JTP-109192 represents a new potential antihypercholesterolemic agent for the treatment of dyslipidaemia.

Keywords: G protein-coupled receptor 119, atherosclerosis, dyslipidaemia, spontaneously hyperlipidaemic mouse.

1. Introduction

The global prevalence of hypercholesterolemia among adults is 39%, and elevated total cholesterol remains a major cause of disease burden worldwide, presenting a risk factor for ischemic heart disease and stroke according to the World Health Organization[1]. For treating hyperlipidaemia, hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors (statins) are listed as first-line therapeutic agents. Large-scale clinical trials have shown that lowering low-density lipoprotein cholesterol (LDL-C) prevents coronary artery disease[2]. According to the guidelines for the prevention of atherosclerotic disease published in 2017 in Japan, the primary prevention target value for patients has been established based on assessment of the risk considering age, sex, smoking, blood pressure, high-density lipoprotein cholesterol (HDL-C), LDL-C, glucose intolerance, and family history[3]. Although the United States guidelines, as revised in 2013, failed to specify a control target value for LDL-C, the importance of lowering LDL-C remains unchanged. Recently, proprotein convertase subtilisin/kexin type 9 (PCSK9) antibodies (Evolocumab and Alirocumab) have been launched; however, the annual cost remains high (US$7000/year) as it is an antibody therapy[4].

G protein-coupled receptor 119 (GPR119) expression in the pancreas and intestine is involved in insulin- and glucagon-like peptide-1 (GLP-1) secretion[5]. As previously reported, JTP-109192, a novel GPR119 agonist, enhances glucose-stimulated insulin secretion and GLP-1 secretion *in vitro* and *in vivo,* improving insulin sensitivity with repeated administration in Zucker fatty (ZF) rats[6]. In apolipoprotein E (*Apoe*)-deficient mice, overexpression of the *Gpr119* gene regulates cholesterol transporter expression in the intestinal lumen and liver to reduce lipid and inflammatory cytokine levels, improving atherosclerotic lesions[7]. Therefore, GPR119 agonists are considered not only antidiabetic but also lipid-lowering drugs. Oleoylethanolamide (OEA), a natural ligand of GPR119, shows antiatherosclerotic effects in *Apoe*-deficient mice[8], while OEA itself is reported to be a ligand of peroxisome proliferator-

activated receptor α (PPARα) and transient receptor potential vanilloid 1 (TRPV1) in addition to GPR119[9]. In contrast, GSK1292263 is a GPR119 agonist synthesized by Glaxo SmithKline plc. as a drug for treating dyslipidaemia [NCT01218204], and reportedly increased HDL and decreased LDL-C following a 2-week administration during a clinical study[10]. For preclinical investigations of atherosclerosis, normal mice are generally resistant to developing atherosclerosis even under a high-cholesterol diet owing to high HDL-C levels in the plasma[11]. Conversely, congenic spontaneously hyperlipidaemic (SHL) mice exhibit hyperlipidaemia owing to a spontaneous *Apoe* gene deficiency, and thus SHL mice are easier to handle than genetically modified mice such as *Apoe*-deficient mice or other larger experimental animals such as rabbits.

In the present study, we investigated the *in vivo* effects of JTP-109192 on lipid metabolism in SHL mice with repeated administration, once daily for 12 weeks. Then, we performed a histopathological examination following repeated JTP-109192 administration to SHL mice. Additionally, lipid metabolism gene expression was evaluated following a single administration of the JTP-109192 derivative to clarify the mode of action.

2. Results

2.1 Relationship between plasma total cholesterol and atherosclerosis formation in C57BL/6 and BALB/c background SHL mice

Before repeated JTP-109192 administration, we characterized the relationship between plasma total cholesterol and atherosclerotic lesions for 4 months in C57BL/6 and BALB/c background SHL mice, fed a western or normal diet. Following 4 months of feeding, the area of atherosclerotic lesions expanded in a time-dependent manner in both strains. Scatter plots of the data are shown in Fig. 1A–D. C57BL/6 background SHL mice exhibited an exponential increase in the area of atherosclerotic lesions, while those in BALB/c background SHL mice

increased linearly over the 4 months. Thus, the antiatherosclerotic effect of JTP-109192 was investigated in BALB/c background SHL mice owing to the linearity of atherosclerosis formation in these mice.

2.2 Effects of JTP-109192 on food intake, body weight, and plasma lipid parameters in SHL mice.

During 12 weeks of repeated JTP-109192 (100 mg/kg) or vehicle administration, food intake and body weight increased, with no differences observed between the two groups (Fig. A, 2B). For every sampling period, the plasma total cholesterol levels were lower in the JTP-109192 100 mg/kg group ($P < 0.01$). Based on the plasma cholesterol composition analysis, the non-HDL-C levels decreased significantly, with HDL-C levels significantly increased in the JTP-109192 100 mg/kg group ($P < 0.01$) (Fig. 2C, 2D).

2.3 Effect of JTP-109192 on cholesterol and bile acid in faeces

We measured faecal cholesterol and bile acid contents to clarify whether the decrease in plasma cholesterol levels can be attributed to suppressed cholesterol absorption, as well as the promotion of cholesterol excretion. On days 0–7, 21–28, and 77–84, faecal cholesterol was increased in the JTP-109192 100 mg/kg group ($P < 0.01$) (Fig. 3A). On days 21–28 and 77–84, bile acid excretion was decreased in the JTP-109192 100 mg/kg group ($P < 0.01$) (Fig. 3B).

2.4 Effects of JTP-109192 on atherosclerosis

To investigate the effect of JTP-109192 on atherosclerotic lesions in mice, histopathological specimens of thoracic and abdominal aortas were prepared, and the areas of atherosclerotic lesions were measured by staining fat deposits. On consuming a western diet, SHL mice in the vehicle group developed atherosclerosis over 9% of the whole aortic area, approximately

consistent with our preliminary study; at a dose of 100 mg/kg, JTP-109192 significantly decreased the area of atherosclerotic lesions (Fig. 4B). Representative examples are shown in Fig. 4A.

2.5 Effect of JTP-109192 derivative on intestinal gene expression involved in cholesterol metabolism

To elucidate the mechanism through which GPR119 agonists lower plasma cholesterol and improve atherosclerosis, the expression of lipid metabolism-related genes was evaluated. The JTP-109192 derivative increased mRNA levels of *Abca1, Abcg5,* and *Abcg8* in the upper small intestine and colon ($P < 0.01$) (Fig. 5A–D). *Npc1l1* mRNA levels were lower in the colon of the JTP-109192-treated group.

3. Discussion

Atherosclerosis is a major risk factor for cardiovascular disease, and hypercholesterolemia is a well-established key factor for developing atherosclerosis. Furthermore, lowering plasma cholesterol levels has been extensively investigated, and the principle of “the lower, the better” has been well established[12]. HMG-CoA inhibitors, termed as statins, are used as the first-line of treatment for hypercholesterolemia. However, the cardiovascular residual risk remains high despite intensive statin therapy[13]. Although good adherence to medication is necessary for the continuation of statin treatment in clinical practice, patients may fail to consistently comply with prescriptions. Appropriate assessment of side effects, such as myopathy, is important to improve adherence. However, some patients with mild myopathies have been considered to be intolerant to statins owing to the concern of statin-induced rhabdomyolysis, with insufficient cholesterol control reported in some patients[14]. Furthermore, such myopathies, including mild myopathies and severe rhabdomyolysis, can lead to poor adherence, dose decrease, and

discontinuation of treatment resulting in sub-optimal cholesterol control[15]. Therefore, the need for an effective cholesterol-lowering drug remains. In 2015, the blockbuster PCSK9 antibody was launched, and since then, treatment costs have been relatively realistic when compared with those at the time of launch; however, therapy remains expensive as it is a biological therapeutic agent. Thus, new potent small molecule drugs are necessary for the treatment of hypercholesterolemia.

In the present study, prior to repeated treatment with JTP-109192, two SHL mouse strains (derived from C57BL/6 and BALB/c) that spontaneously develop atherosclerosis were obtained, and the relationship between changes in plasma total cholesterol levels and atherosclerosis progression were compared to investigate which strain would be more suitable for experimentation. In these strains, we monitored the relationship between plasma cholesterol levels and atherosclerosis for 4 months using both western and normal diets. C57BL/6 background SHL mice exhibited an exponential increase in the area of atherosclerotic lesions, whereas those in BALB/c background SHL mice increased linearly over the 4 months. In BALB/c background SHL mice, the progression of atherosclerosis is considered to be closely related to an increase in plasma cholesterol levels. A comparison of these two strains was reported by Matsushima *et al.*[16]. In brief, the progression of aortic atherosclerotic lesions is relatively moderate in BALB/c SHL mice when compared with C57BL/6 SHL mice. Shirai *et al*. have reported that C57BL/6 and BALB/c SHL mice are prototypical Th1- and Th2-type mouse strains[17]. Therefore, in our experiment, the exponential increase in the area of atherosclerotic lesions in C57BL/6 SHL mice may be attributed to factors other than plasma total cholesterol (such as inflammatory cytokines). In BALB/c background SHL mice, the area of atherosclerotic lesion was increased with vehicle administration, while significantly decreasing after 12 weeks of JTP-109192 treatment following a decrease in plasma total cholesterol level. The increase in faecal cholesterol excretion was thought to occur owing to

suppressed absorption or enhanced excretion. Diarrhoea, which may flush gastrointestinal contents such as cholesterol, was not observed during repeated administration in both groups. Faecal bile acid excretion increased during the middle and end of the administration period, while no change was observed when compared with vehicle administration during the first 7 days of administration. These results suggest that the decrease in faecal bile acid excretion was secondary to a decrease in the body cholesterol pool. Moreover, liver cholesterol content was found to be reduced (data not shown). A single treatment with the JTP-109192 derivative partially disclosed a mode of action for the observed cholesterol-lowering effect, as well as the subsequent antiatherosclerotic effect. Following treatment with the JTP-109192 derivative, *Abca1* and *Abcg5* mRNA expressions were increased in the upper intestine and colon of SHL mice. The former probably contributed to increased HDL in the blood, while the latter may promote the faecal excretion of dietary cholesterol. Our results are partly concordant with those reported by Hu *et al*. who used *Apoe*-deficient mice with a *Gpr119*-coded lentivirus and demonstrated an increase in intestinal ABCA1 protein expression, protection against atherosclerosis, and decrease in ABCG5 protein level[7]. However, contrary to their results, our findings revealed increased *Abcg5* mRNA level. This difference is attributable to the off-target effect of JTP-109192 on a receptor other than GPR119 or on other molecular targets. In our experiment, *Gpr119* expression did not increase after a single treatment with the JTP-109192 derivative in SHL mice (data not shown). Additionally, a similar divergence was observed in *Npc1l1* mRNA and NPC1L1 protein expression. Our results demonstrated that a decrease in colonic *Npc1l1* mRNA expression may contribute to the cholesterol-lowering effect. Considering that JTP-109192 may not only possess antidiabetic but also antiatherosclerotic effects, we believe JTP-109192 is an attractive candidate owing to its unique profile. However, there is a ~10-fold discrepancy regarding the dose that exhibits antiatherosclerotic and antidiabetic effects. One rationale for the divergence may be explained by the differing

pharmacokinetic or metabolic profiles of JTP-109192 between rats and mice. In liver microsomes, the stability of JTP-109192 was lower in mice than in rats (data not shown), although the agonistic potency of JTP-109192 toward GPR119 is similar in both species[6]. Prior to 3 months of repeated administration, we performed a preliminary experiment with 2 weeks of repeated JTP-109192 administration, at 10, 30, and 100 mg/kg, in SHL mice for dose determination. The results showed that plasma cholesterol levels were decreased in a dose-dependent manner, and the maximum cholesterol-lowering effect was observed at 100 mg/kg. It would be worthwhile to investigate the efficacy of a combination of either a statin or ezetimibe with JTP-109192, considering the differences in the mechanism of action. Thus, we are considering such an investigation as the subject of future research. A scatter plot between the area under cholesterol-time curve (TC-AUC) and atherosclerotic area ratio for three animal groups (BALB/c SHL mice receiving a western diet, BALB/c SHL mice on a normal diet, and JTP-109192 treated BALB/c SHL mice receiving a western diet) suggests that the TC-AUC values in all groups up to day 84 positively correlated with atherosclerotic area ratio (Fig. 4C). Therefore, we consider that the cholesterol-lowering effect of JTP-109192 may contribute to its atherosclerosis-improving effect. In contrast to our expectations, the plasma triglyceride (TG) and glucose levels were not affected by JTP-109192 in SHL mice in this study. We did not observe any correlation between plasma TG-AUC and atherosclerotic area ratio (data not shown). Based on these results, we postulate that the atherosclerotic area in this animal model is largely dependent on plasma total cholesterol levels. In terms of the plasma glucose levels, we speculate that the enhancement of glucose-dependent insulin secretion by JTP-109192 is unlikely to occur in this strain. Here, one possible explanation is that the plasma glucose levels in HFD-fed SHL mice were within the normal range. Our previous report has shown the glucose-lowering effect of JTP-109192 in insulin-resistant ZF rats, which exhibit postprandial hyperglycaemia[6]. Further investigations are necessary to verify whether JTP-109192 exhibits

these antiatherosclerotic effects in clinical settings. One expectation is based on the increased *Abca1* mRNA expression observed in the human colon carcinoma cell line, Caco-2, treated with JTP-109192 for 24 h during a preliminary experiment. Thus, we believe that JTP-109192 may be a candidate drug for dyslipidaemia. To the best of our knowledge, this is the first report presenting antiatherosclerotic effects with small molecule GPR119 agonists.

However, the following limitations of this study need to be addressed. We speculate that the mode of action of JTP-109192 involves altering mRNA expression levels in the gastrointestinal tract following a single administration of the JTP-109192 derivative as this is partially consistent with a previous report[7]. However, a comprehensive understanding of the antiatherosclerotic mechanism of GPR119 antagonists, clarifying the expression of proteins related to lipid metabolism, as well as transcription factors (such as liver X receptor) that regulate *Abca1*, *Abcg5*, and *Abcg8* gene expression in the gastrointestinal tract, remains important. Moreover, analysing cellular components in aortic sinus sections may provide further evidence to examine the atherosclerotic plaque content, as well as atherosclerotic area.

In conclusion, the cholesterol-lowering effect of JTP-109192 leads to an improvement in lipid abnormalities and atherosclerotic lesions in SHL mice, along with increased faecal cholesterol excretion. The cholesterol-lowering effect of JTP-109192 may be attributed to changes in intestinal cholesterol metabolism-related gene expression. Therefore, JTP-109192 represents a new potential antihypercholesterolemic agent for the treatment of dyslipidaemia, in addition to diabetes.

4. Materials and Methods

4.1 Animals

C57BL/6 and BALB/c background C.KOR/StmSlc-*Apoe*^{shl} (SHL) mice were purchased from Japan SLC, Inc. (Shizuoka, Japan). Animals were treated in accordance with the National

Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978). Animal study protocols were approved by the Institutional Animal Care and Use Committee of the Central Pharmaceutical Research Institute, Japan Tobacco Inc. The animal housing conditions were maintained as follows: room temperature 23.0 ± 3.0°C, humidity 55 ± 15%, 12 h:12 h light:dark cycle (lights on at 8 a.m., lights off at 8 p.m.), F2WTD (sucrose 34%, salt- free butter 20%, 417 kcal; Oriental Yeast Co., Ltd., Tokyo, Japan) as western diet or CRF-1 (Oriental Yeast Co., Ltd., Tokyo, Japan) as normal diet supplied *ad libitum*, UV-irradiated tap water supplied *ad libitum*, and individual housing. JTP-109192 and its derivative were administered as a food admixture, a less invasive procedure. The concentrations of JTP-109192 were calculated from food intake and body weight every week, and the food admixture was renewed once weekly.

4.2 Chemicals

The structure-activity relationship of GPR119 agonist development has been previously reported by Harada *et al.* [18]. The test agent, JTP-109192, 4-[5-(3,3-dimethyl-2-oxa-spiro[4.5] dec-8-yl)-pentyloxy]-N,N-dimethyl-benzamide, and its derivatives were synthesized at the Central Pharmaceutical Research Institute of Japan Tobacco Inc. (Osaka, Japan).

4.3 Characterization of SHL mouse strain

C57BL/6 and BALB/c background SHL mice were fed a western or normal diet to compare the relationship between atherosclerotic lesions and plasma total cholesterol levels. Male mice (7 weeks old) were fed the respective diets for 2, 3, and 4 months. Two-dimensional scatter plots of TC-AUC values and atherosclerotic lesions were assessed in these two strains receiving two diets. Linear and non-linear regression fitting was performed using GraphPad Prism version 7.0 for Windows (GraphPad Software, San Diego, CA).

4.4 JTP-109192 repeated treatment in SHL mice

Sixteen male BALB/c background SHL mice (7 weeks old) were selected from 20 mice as follows. Four days before the start of treatment administration, blood samples (100 µL) were collected from the tail vein under isoflurane anaesthesia into heparinized tubes and centrifuged at 10000 × *g* at 4°C for 5 min to obtain plasma samples. Total cholesterol concentrations were measured using a commercial kit (Roche Diagnostics, Switzerland) and an automatic analyser (Hitachi, Japan). HDL and non-HDL levels were measured using a high-speed automatic electrophoresis system (Helena, Japan). On the day of administration (defined as day 0), the body weight was measured. After excluding 4 mice presenting outlier values for these parameters from the group allocation, 16 mice were assigned to two groups with 8 mice in each group (JTP-109192 100 mg/kg or vehicle group), to avoid any bias concerning these parameters between the two groups. During the 84 days of treatment, food intake and body weight were measured weekly. The blood biochemical parameters (total cholesterol levels) were evaluated every one or two weeks. HDL and non-HDL levels were measured on days 0, 47, and 84.

4.5 Measurement of cholesterol and bile acids in faeces

On days 0–7, 21–28, and 77–84, for 7 consecutive days during each period, faecal cholesterol and bile acid were measured. During each sampling period, faeces were collected and dried for 16 h at 90°C. After measuring the dried weight, the faeces were ground using a mixer mill. Extraction was performed with the addition of 100% ethanol. The extract was dried and resolved in 2-propanol for measurement using a commercial kit (bile acid: FUJIFILM Wako Pure Chemical Corporation, Japan).

4.6 Analysis of atherosclerotic lesions

To determine the atherosclerotic plaque area, thoracic and abdominal aortas were dissected on day 84 and stained with Oil red O. The aortic specimens were randomized for evaluation in a single-blinded manner. A digital image of the intravascular lumen of each aortic specimen was obtained. The atherosclerotic and non-atherosclerotic areas were measured using image processing software (Adobe Photoshop CS, Version 8.0.1; Adobe Systems Incorporated) and analysis software (WinROOF Version 5.01; Mitani Corporation). The ratio of the atherosclerotic area relative to the total area of the evaluated region (percent area of atherosclerotic region) was calculated for the thoracic and abdominal aortas.

4.7 Single administration of the JTP-109192 derivative and mRNA quantification with real-time quantitative PCR

Fourteen male C57BL/6 background SHL mice were assigned to two groups, with 7 mice in each group (JTP-109192 derivative 100 mg/kg or vehicle group). The JTP-109192 derivative was administered as a food admixture in the western diet, which mice accessed *ad libitum* for 16 h. The mice were sacrificed under isoflurane anaesthesia and the upper intestine (duodenum and jejunum), lower intestine (jejunum and ileum), and colon were collected and frozen at –80°C until total RNA extraction. Total RNA was extracted from tissues using the miRNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. RNA was transcribed into complementary DNA using M-MLV reverse transcriptase and random primers (Invitrogen, Carlsbad, CA). The reaction mixture was incubated for 10 min at 25°C, 2 h at 37°C, and 5 min at 85°C. Real-time PCR quantification was performed in a 20 μL reaction mixture, with an automated sequence detector combined with ABI Prism7700 Sequence Detection System software (Applied Biosystems, Foster City, CA). The reaction mixture contained 50 ng of synthesized cDNA, TaqMan Universal PCR Master Mix, and 0.9 μM primers/0.25 μM probes or TaqMan primer/probe mix (Applied Biosystems). The cycle parameters were 10 min

at 95°C, followed by 40 cycles of 15 s at 95°C, and 1 min at 60°C. mRNA expression levels were normalized to 18s rRNA levels. The following primers and FAM-conjugated probes were designed using Primer Express software (Applied Biosystems): *Abca1* (probe, Mm00442646_m1), *Abcg5* (probe, Mm00446241_m1), *Abcg8* (probe, Mm00445970_m1), *Npc1l1* (probe, Mm01191972_m1), and 18s rRNA (purchased from Applied Biosystems).

4.8 Statistical analyses

Significance tests were performed using the following procedures: for comparisons between two groups, homogeneity of variance was tested using the F-test; subsequently, the Student's *t*-test or Aspin-Welch's *t*-test was performed for homoscedastic data or heteroscedastic data, respectively. A 2-tailed significance level of 5% was applied. SAS System version 8.2 and SAS Preclinical Package version 5.0 (SAS Institute Japan Ltd., Tokyo, Japan) were used for all statistical analyses. The R^2 value was calculated from a linear regression line using GraphPad Prism version 7.0 for Windows (GraphPad Software, San Diego, CA).

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Disclosure

The authors declare that there are no conflicts of interest regarding this article.

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Figure 1

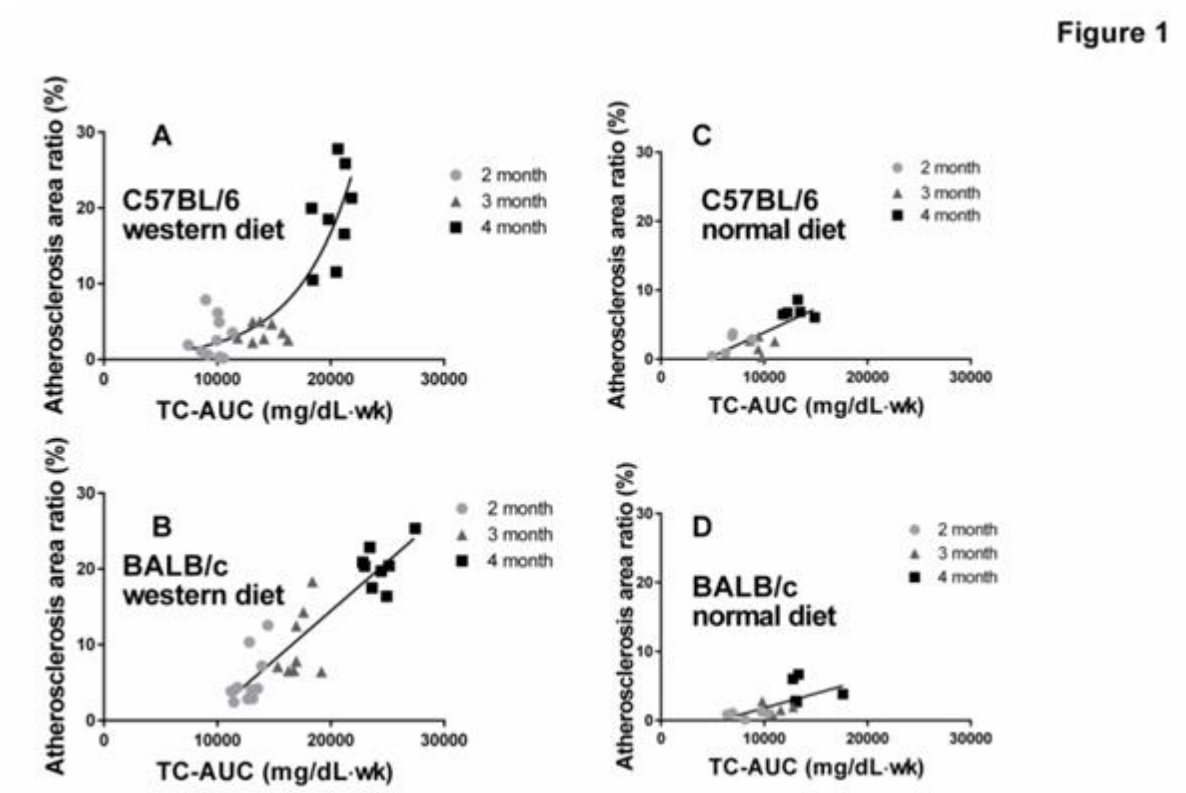

Figure 1. Plasma total cholesterol and atherosclerosis formation in C57BL/6 and BALB/c background SHL mice.

The relationship between the area under the curve (AUC) values of plasma total cholesterol and atherosclerotic lesions in (A) C57BL/6 SHL mice receiving a western diet, (B) BALB/c SHL mice receiving a western diet, (C) C57BL/6 SHL mice on a normal diet, and (D) BALB/c SHL mice on a normal diet. Eight to ten male mice (7 weeks old) received a western diet for 2, 3, and 4 months. Five male mice (7 weeks old) were used for each rearing period with a normal diet.

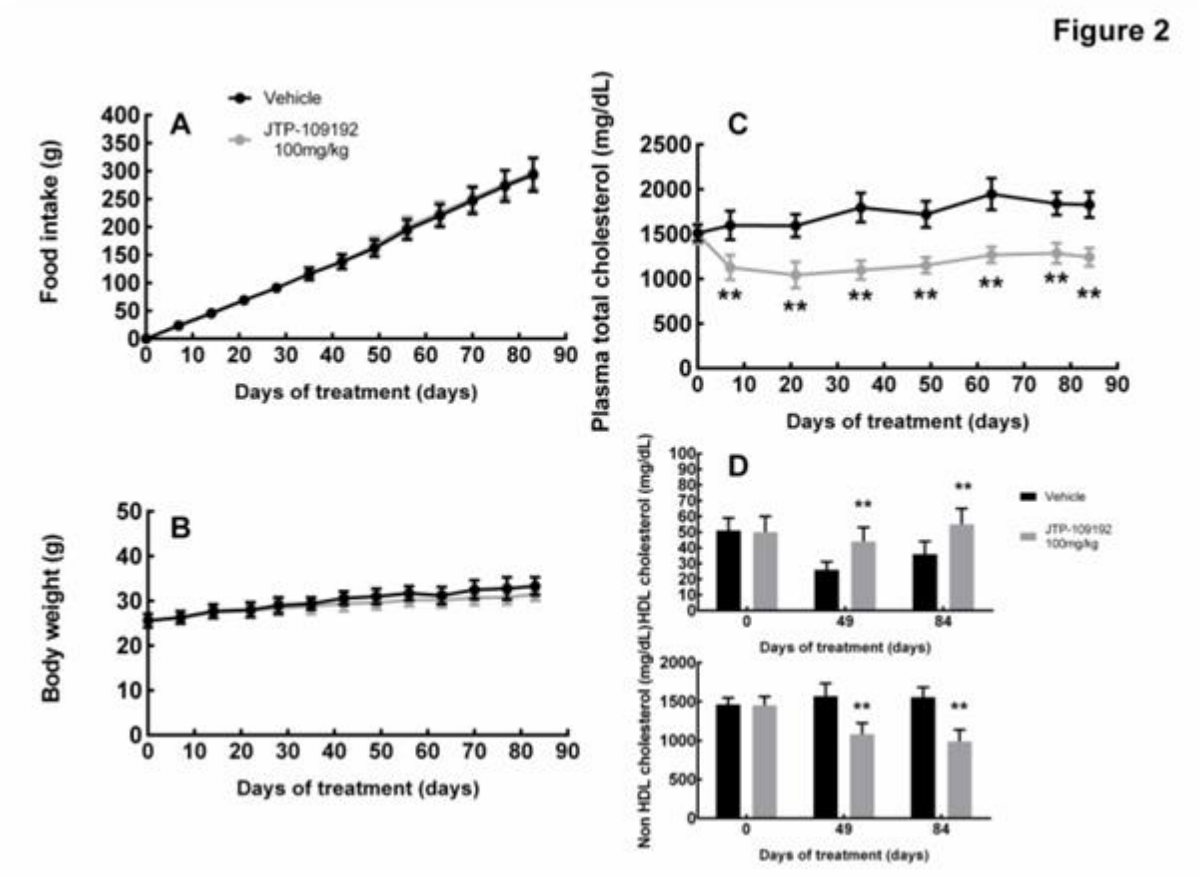

Figure 2. Effects of JTP-109192 on food intake, body weight, and plasma cholesterol in SHL mice.

(A) Food intake and (B) body weight during repeated administration. No significant changes are observed between JTP-109192 100 mg/kg and vehicle groups. (C) shows the plasma total cholesterol levels during repeated administration. (D) presents HDL and non-HDL-C levels on day 0, 49, and 84. Data are expressed as the mean ± standard deviation; n = 8. $^{**}P < 0.01$ vs. vehicle group (Student's *t*-test). HDL-C; high-density lipoprotein cholesterol

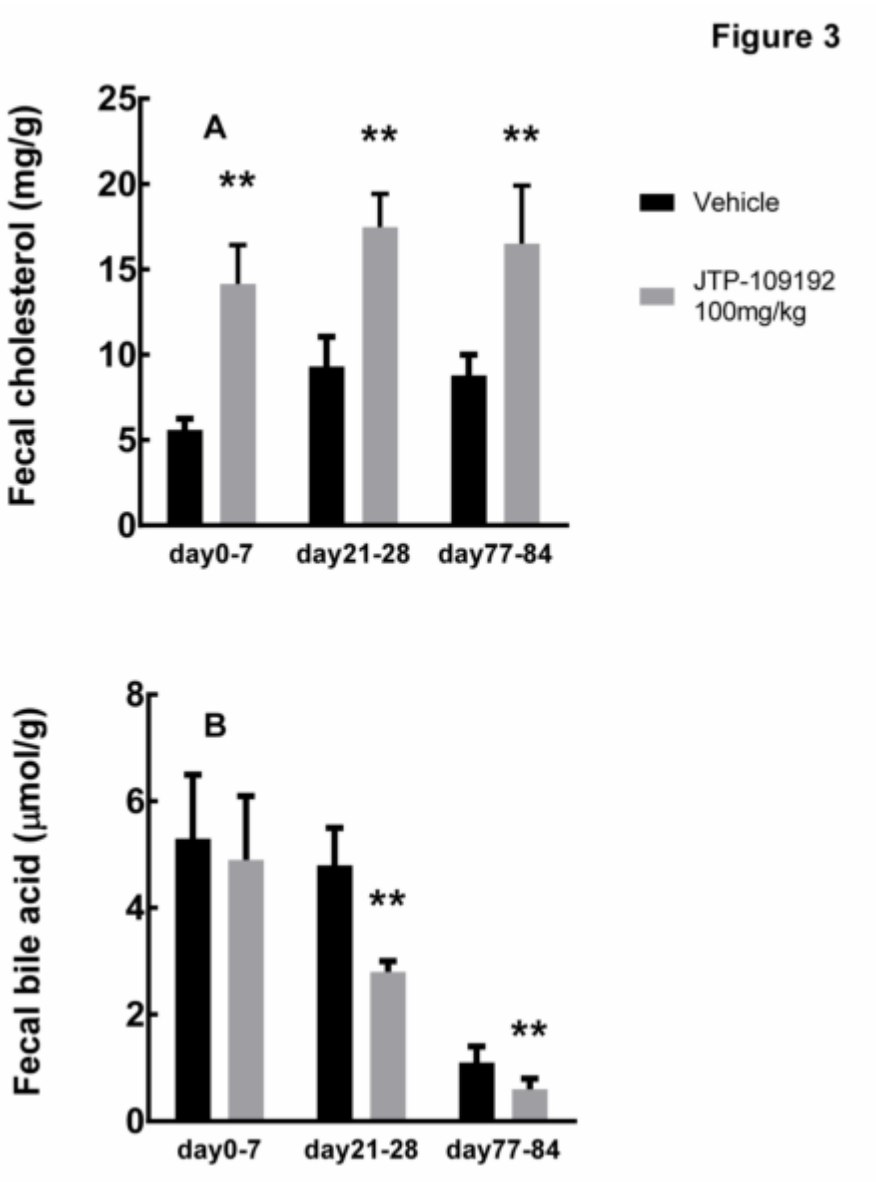

Figure 3. Effects of JTP-109192 on faecal cholesterol and bile acid excretion.

(A) Cholesterol and (B) bile acid in faeces on days 0–7, 21–28, and 77–84. Data are expressed as the mean ± standard deviation; n = 8. $^{**}P < 0.01$ vs. vehicle group (Student's *t*-test).

Figure 4

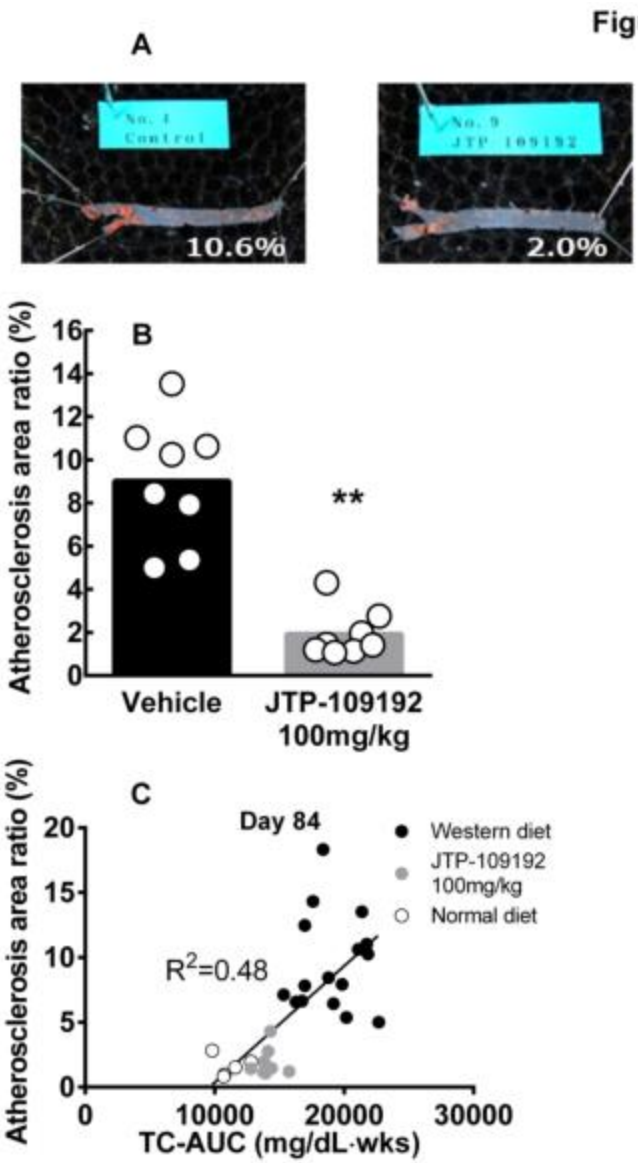

Figure 4. Effect of JTP-109192 on atherosclerotic lesions.

(A) shows representative examples of the aortic specimens in the vehicle and JTP-109192 100 mg/kg groups. (B) shows the atherosclerotic area ratio. Data are expressed as the mean and scatter plots; n = 8. $^{**}P < 0.01$ vs. vehicle group (Student's *t*-test). (C) Scatter plot between TC-AUC and atherosclerotic area ratio of three animal groups up to day 84 (closed circle: BALB/c SHL mice receiving a western diet, open circle: BALB/c SHL mice on a normal diet, grey circle: JTP-109192 treated BALB/c SHL mice receiving a western diet). The R^2 value is 0.48. TC-AUC; area under the cholesterol-time curve.

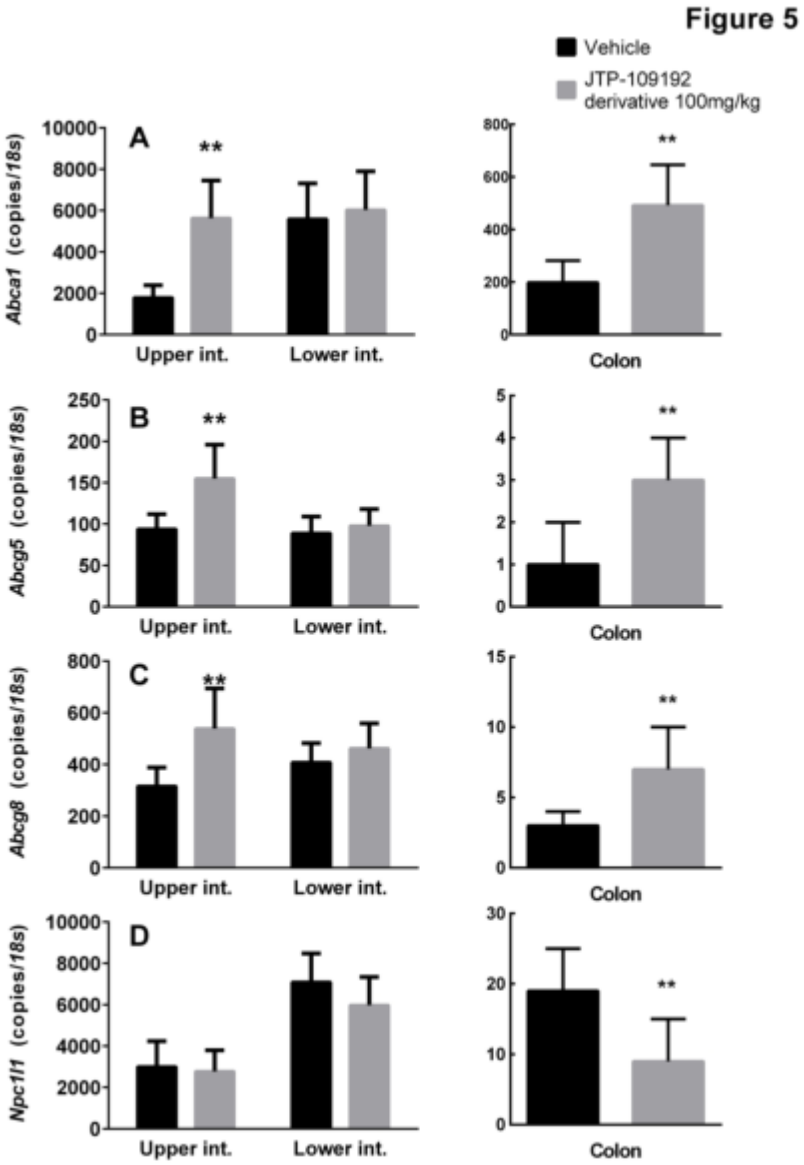

Figure 5. Effects of JTP-109192 derivative on gastrointestinal tract mRNA expression involved in cholesterol metabolism.

(A) *Abca1*, (B) *Abcg5*, (C) *Abcg8*, and (D) *Npc1l1* mRNA expression in the JTP-109192 derivative 100 mg/kg and vehicle groups in the upper intestine, lower intestine, and colon. Data are expressed as the mean ± standard deviation; n = 7. $^{**}P < 0.01$ vs. vehicle group (Student's *t*-test).