Effects of K-877, a novel selective PPAR modulator, on small intestine contribute to the amelioration of hyperlipidemia in low-density lipoprotein receptor knockout mice

Full paper

Effects of K-877, a novel selective PPARα modulator, on small intestine contribute to the amelioration of hyperlipidemia in low-density lipoprotein receptor knockout mice

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ABSTRACT

Peroxisome proliferator-activated receptor α (PPARα) is a well-known therapeutic target for treating hyperlipidemia. K-877 is a novel selective PPARα modulator (SPPARMα) that enhances PPARα transcriptional activity with high selectivity and potency, resulting in reduced plasma lipid levels. This study aimed to evaluate the effects of K-877 on hyperlipidemia in low-density lipoprotein receptor knockout ($Ldlr^{-/-}$) mice, a mouse model of atherosclerosis. We revealed that K-877 administration significantly decreased plasma triglyceride (TG) and total cholesterol (TC) levels and increased plasma high-density lipoprotein cholesterol (HDL-C) levels in $Ldlr^{-/-}$ mice. K-877 administration to $Ldlr^{-/-}$ mice efficiently increased the gene expression of PPARα and its target genes related to fatty acid oxidation in the liver and small intestine. The same treatment significantly increased ATP-binding cassette a1 gene expression in the liver and small intestine and reduced Niemann Pick C1-like 1 gene expression in the small intestine, suggesting that K-877 administration induced HDL-C production in the liver and small intestine and reduced cholesterol absorption in the small intestine. In conclusion, K-877 administration had pronounced effects on the liver and small intestine in $Ldlr^{-/-}$ mice. K-877 is an attractive PPARα-modulating drug for treating hyperlipidemia that works equally well in both the liver and small intestine.

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1. Introduction

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily comprising three subtypes: PPARα, PPARβ/δ, and PPARγ. Upon ligand binding, PPARs form heterodimers with the retinoid X receptor and interact with PPAR response elements to regulate target gene expression. PPARα is ubiquitously expressed and is activated by hypolipidemic fibrate-class drugs (fibrates). It controls lipid flux in the liver by modulating fatty acid transport and β-oxidation and improves plasma lipid profiles by decreasing TG and increasing HDL-C levels. Fibrates such as gemfibrozil, bezafibrate, and fenofibrate (Feno) decrease plasma TG levels and increase HDL-C levels in patients with hyperlipidemia and type 2 diabetes and can prevent coronary heart disease and stroke.[1–5]

Pharmaceutical approaches that aimed to affect cholesterol metabolism target PPARα to treat or prevent the development of cardiovascular disease, particularly atherosclerosis. The activation of liver X receptor (LXR) by synthetic ligands increases HDL-C levels and reverses cholesterol transport, resulting in reduced atherosclerotic plaque formation in mice. LXR activation upregulates the

transcription of several genes including ATP-binding cassette a (*Abca*)1, *Abcg1*, and *Abcg5/g8*. ABCA1 mediates the rate-controlling step in HDL particle formation by promoting the efflux of cholesterol and phospholipids to apolipoprotein a1 (ApoA-I).[6,7] ABCA1 deficiency in Tangier disease is characterized by HDL deficiency, high plasma TG levels, elevated tissue sterol levels, and premature coronary atherosclerosis in some kindreds.[8] Studies on tissue-specific ablation have shown that hepatic and intestinal ABCA1 are responsible for approximately 70 and 30% of plasma HDL, respectively.[9,10] Cholesterol absorption has been extensively studied because of its significant positive correlation with plasma cholesterol levels and atherosclerosis.[11–13] Cholesterol absorption is defined as the transport of dietary lipids from the intestinal lumen across enterocytes into the plasma.[12] The uptake of dietary and biliary cholesterol occurs in the proximal small intestine. Cholesterol uptake from the intestinal lumen by enterocytes is the rate-limiting step in cholesterol absorption[14], and studies have demonstrated that NPC1L1, which is efficiently inhibited by ezetimibe (a drug that specifically binds to the NPC1L1 protein), is involved in this process.[15–17]

PPARα agonists reduce plasma LDL cholesterol levels in part by decreasing cholesterol absorption.[18–21] Feno suppresses *Npc1l1* expression in the small intestine, thereby limiting cholesterol absorption at the level of cholesterol transport across the apical membrane of enterocytes.[22] However, fibrates are weak agonists of PPARα and have poor PPAR subtype selectivity and their efficient application requires high clinical doses. Therefore, a potent and selective PPARα agonist is needed for treating patients with metabolic syndrome. K-877 (pemafibrate) is a novel selective PPARα modulator (SPPARMα) that has been developed to maximize receptor-mediated effects and diminish side effects and elicits.[23,24]

Here the effects of K-877 on lipid metabolism in LDL receptor knockout (*Ldlr*$^{-/-}$) mice, a mouse model of atherosclerosis, were studied.

2. Materials and methods

2.1. Reagents

K-877, which is shown in Fig. 1, was kindly provided by Kowa Co. Ltd, Tokyo Japan. Feno was purchased from Sigma–Aldrich.

2.2. Animals

C57BL/6J (wild-type, WT) mice were obtained from CLEA Japan. B6; 129S4-*Ppara*^{tm1Gonz}/J (*Ppara*$^{-/-}$) and *Ldlr*$^{-/-}$ mice were purchased from Jackson Laboratory. Eight-week-old male WT and

Ppara$^{-/-}$ mice were fed a modest fat (MF) diet supplemented with Feno or K-877 for 1 week. Eight-week-old male *Ldlr*$^{-/-}$ mice were fed an MF diet supplemented with K-877 for 1 week. All animal husbandry procedures and experiments were compliant with the University of Tsukuba's Regulations for Animal Experiments and were approved by the Animal Experiment Committee at the University of Tsukuba.

2.3. Metabolic measurements

Plasma levels of TG, non-esterified fatty acid (NEFA), TC, HDL-C, aspartate aminotransferase, alanine aminotransferase, and intestinal TC were measured as previously described.[25]

2.4. Fecal cholesterol output

Fecal cholesterol outputs were measured as previously described.[26,27] Briefly, mice were individually housed for fecal collection. The feces were dried, weighed, and crushed into powder. Fecal cholesterol was extracted from powdered feces with a chloroform/methanol mixture (2:1 vol/vol).[27]

2.5. Postprandial TG response

Mice were starved for 16 h; this was followed by the oral administration of 200 μl of olive oil.[28] Blood samples were collected at 0, 3, 6, and 9 h post administration.

2.6. Determination of plasma LPL activity

Mice were injected with 100 U/kg body weight of heparin (Novo Heparin, Mochida Pharmaceutical Co., Ltd) via the tail veins. Blood samples were collected at 20 min post administration. Plasma LPL activity was determined using an LPL activity assay kit (Roar Biochemical, Inc) according to the manufacturer's instructions.

2.7. Gene expression analysis

Total RNA from tissues was extracted using TRIzol reagent (Invitrogen). Prior to real-time PCR analysis, total RNA was reversed transcribed into cDNA using a reverse transcriptase, according to the manufacturer's instructions (Invitrogen). Real-time PCR was performed using an ABI Prism 7300 system (ABI) with SYBR Green Master Mix (Roche).[29] Primer sequences are listed in Table 1. The expression levels of mRNA were normalized to those of Cyclophilin.

2.8. Statistical analyses

Comparisons of treatment groups were made using unpaired Student's *t*-tests or Tukey–Kramer post hoc tests, and differences were considered significant when p < 0.05. All data are expressed as mean ± standard error of the mean.

3. Results

3.1. K-877 reduced plasma TG and TC levels and increased plasma HDL-C levels in Ldlr$^{-/-}$ mice

To determine the effects of K-877 on lipid metabolism, *Ldlr*$^{-/-}$ mice were fed with an MF diet supplemented with 0.001% K-877 for 1 week. The dose of K-877 was decided according to a previous report.[30] K-877 administration significantly reduced plasma TG levels but did not change plasma NEFA levels (Fig. 2A). K-877 administration significantly reduced plasma TC levels and increased HDL-C levels in *Ldlr*$^{-/-}$ mice (Fig. 2A). Thus, these
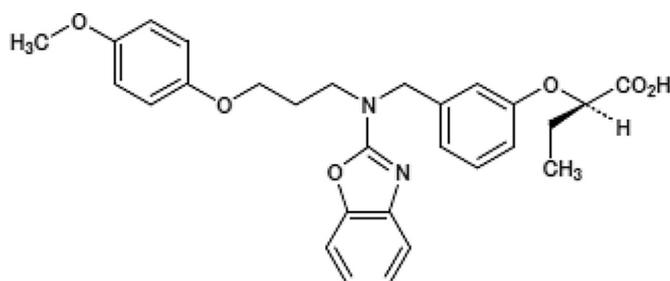
$C_{28}H_{30}N_2O_6$

MW: 490.55

Fig. 1. The structure of K-877.

findings indicate that the ratio of plasma HDL-C/TC levels was higher with K-877 administration than that with no administration, suggesting that K-877 has anti-atherogenic effects. To check plasma TG metabolism by K-877, we studied the postprandial TG response in these mice. K-877-treated $Ldlr^{-/-}$ mice were significantly lower than non-treated- $Ldlr^{-/-}$ mice during this test (Fig. 2B), suggesting an increase in TG clearance in K-877-treated $Ldlr^{-/-}$ mice.

3.2. K-877 regulates gene expression related to TG and HDL-C metabolism in the liver of Ldlr⁻/⁻ mice

Hepatic gene expression analysis revealed that K-877 administration significantly increased the expression of *Ppara* and its target genes such as *Acox1*, *Cpt1a*, *Creb3l3*, and *Fgf21*, which activate fatty acid oxidation, in the liver of $Ldlr^{-/-}$ mice (Fig. 3A). There were no differences in the hepatic gene expression of the lipoprotein lipase (LPL) activators *Apoa5* and *Apoc2* between K-877-treated and untreated mice. However, the expression of the LPL inhibitor *Apoc3* significantly decreased in the liver of K-877-treated $Ldlr^{-/-}$ mice compared with that in the liver of untreated mice (Fig. 3A). These results suggest that K-877-treated $Ldlr^{-/-}$ mice have higher plasma LPL activity than non-treated $Ldlr^{-/-}$ mice. To confirm this, we measured the plasma LPL activity in these mice. K-877 administration significantly increased the plasma LPL activity compared with non-treated $Ldlr^{-/-}$ mice (Fig. 3B). These findings suggest that K-877 activates fatty acid oxidation in the liver and plasma LPL activity, contributing to the decrease in plasma TG levels. The expression of *Abca1* also significantly increased in the liver of K-877-treated $Ldlr^{-/-}$ mice (Fig. 3A), contributing to the increase in plasma HDL-C levels. However, the expression of *Apoa1* was unchanged (Fig. 3A).

3.3. K-877 regulates cholesterol and TG metabolic gene expression in the small intestine of Ldlr⁻/⁻ mice

K-877 administration to $Ldlr^{-/-}$ mice remarkably increased the expression of *Ppara* and its target genes such as *Creb3l3*, *Acox1*, and *Cpt1a* in the small intestine (Fig. 4A). Moreover, K-877 administration significantly increased the expression of *Lxra* and *Abca1*, but not that of *Lxrb*, *Abcg5*, and *Apoa1* (Fig. 4A). Conversely, K-877 administration significantly reduced *Npc1l1* expression in the small intestine of $Ldlr^{-/-}$ mice (Fig. 4A). K-877 administration tended to reduce the expression of *Apob* (Fig. 4A). These results suggested that K-877 induced intestinal fatty acid oxidation and intestinal HDL-C secretion and reduced cholesterol absorption in the

intestine and chylomicron secretion from the intestine. Taken together, the effect of K-877 on the liver and small intestine improved lipid metabolism in a mouse model of hyperlipidemia.

To confirm the effects of K-877 on the small intestine of $Ldlr^{-/-}$ mice, we determined intestinal and fecal TC levels. Fecal cholesterol levels significantly increased in K-877-treated $Ldlr^{-/-}$ mice (Fig. 4B). There were no differences in intestinal TC levels between K-877-treated and untreated mice (Fig. 4B). These findings indicated that K-877 reduces cholesterol absorption in the small intestine of $Ldlr^{-/-}$ mice.

3.4. The effects of K-877 and Feno on intestinal gene expression is mediated via PPARα

To confirm that the effect of K-877 was mediated by PPARα, WT and $Ppara^{-/-}$ mice were treated with Feno (0.2%) or K-877 (0.001%) for 1 week. There were no apparent differences in the food intake among all groups (data not shown). The dose of the agonists Feno (0.2%) and K-877 (0.001%) significantly decreased the plasma TG and TC levels in the treated mice compared with those in the untreated WT mice, and there were no differences in plasma TG and TC levels between K-877 and Feno (Takei, submitted data). K-877 and Feno significantly increased the expression of *Ppara* and its target genes such as *Cpt1a*, and *Creb3l3* in the small intestine of WT mice compared with that in the small intestine of untreated WT mice, and these changes induced by PPARα agonists were blunted in $Ppara^{-/-}$ mice (Fig. 5). K-877 significantly increased the expression of *Acox1*, but Feno did not (Fig. 5). Moreover, K-877 administration had a stronger effect on the expression of these genes than Feno in spite of the dose differences (Fig. 5). Our findings indicated that K-877 more efficiently increased the PPARα transcriptional activity than Feno in the small intestine. K-877 and Feno significantly decreased the expression of *Npc1l1* in treated mice compared with the untreated WT mice; there were no differences between K-877 and Feno (Fig. 5). K-877 increased the expression of *Lxra* and *Abca1*, but not that of *Lxrb*, *Abcg5*, and *Apoa1*. K-877 and Feno administration to $Ppara^{-/-}$ mice had no detectable effects on the expression of the genes mentioned above (Fig. 5). These results indicate that both PPAR agonists have an effective ability in the small intestine of WT mice and that the functions of both PPAR agonists are mediated via PPARα.

4. Discussion

In this study, we showed that K-877 administration efficiently induced PPARα activity not only in the liver but also in the small intestine of $Ldlr^{-/-}$ mice. K-877-treated mice had the increased expression of PPARα and its target genes, i.e., fatty acid oxidation genes in the liver as well as small intestine. Moreover, K-877 administration significantly decreased the expression of *Npc1l1*, encoding the rate-limiting transporter for cholesterol absorption, and with an increase expression of *Abca1*, which was related to HDL-C secretion, in the liver and small intestine. These changes resulted in the reduction in TG and TC levels in plasma and the increase in plasma HDL-C levels in $Ldlr^{-/-}$ mice. Therefore, K-877 administration improved the dysregulation of lipid metabolism in $Ldlr^{-/-}$ mice through the increased activity of PPARα in the liver and small intestine.

Whole body cholesterol homeostasis is maintained by a complex network of biosynthetic, trafficking, secretory, and regulatory mechanisms.[1] Among these mechanisms, the enterohepatic circulation of cholesterol and bile salts plays a critical role.[2] For the maintenance of cholesterol homeostasis in adult organisms, intestinal cholesterol absorption and endogenous cholesterol synthesis must be matched by biliary cholesterol and bile acid

Table 1
Primer sets used for real-time PCR.

| Gene name	Primer (forward)	Primer (reverse)
Abca1	AAAACCGCAGACATCCTTCAG	CATACCGAAACTCGTTCACCC
Abcg5	AGGGCCTCACATCAACAGAG	GCTGACGCTGTAGGACACAT
Acox1	CGATCCAGACTTCCAACATGAG	CCATGGTGGCACTCTTCTTAACA
Apoa1	TCACCCACACCCTTCAGGAT	CTGGCTCCCTGTCAGGAAGA
Apoa5	GCGAGTTCTGCCGTAGGAC	CCCAACCCCATCAAATGTGA
Apob	TTGGCAAACTGCATAGCATCC	TCAAATTGGGACTCTCCTTTAGC
Apoc2	CCAAGGAGGTTGCCAAAGAC	TGCCTGCGTAAGTGCTCATG
Apoc3	TACAGGGCTACATGGAACAAGC	CAGGGATCTGAAGTGATTGTCC
Cpt1a	CCTGGAACCTGGCAACTTCAT	GGACGCCACTCACGATGTT
Creb3l3	CCTGTTTGATCGGCAGGAC	CGGGGGACGATAATGGAGA
Cyclophilin	TGGCTCACAGTTCTTCATAACCA	ATGACATCCTTCAGTGGCTTGTC
Fgf21	AGATCAGGGAGGATGGAACA	TCAAAGTGAGGCGATCCATA
Lxrα	AGCAACAGTGTAACAGGCGCT	ACGATGGCCAGCTCAGTAAAGT
Lxrβ	ATGTCTTCCCCCACAAGTTCT	GACCACGATGTAGGCAGAGC
Npc1l1	ATCCTCATCCTGGGCTTTGC	GCAAGGTGATCAGGAGGTTGA
Pparα	ACGCGAGTTCCTTAAGGAACCTG	GTGTCATCTGGATGGTTGCTCT
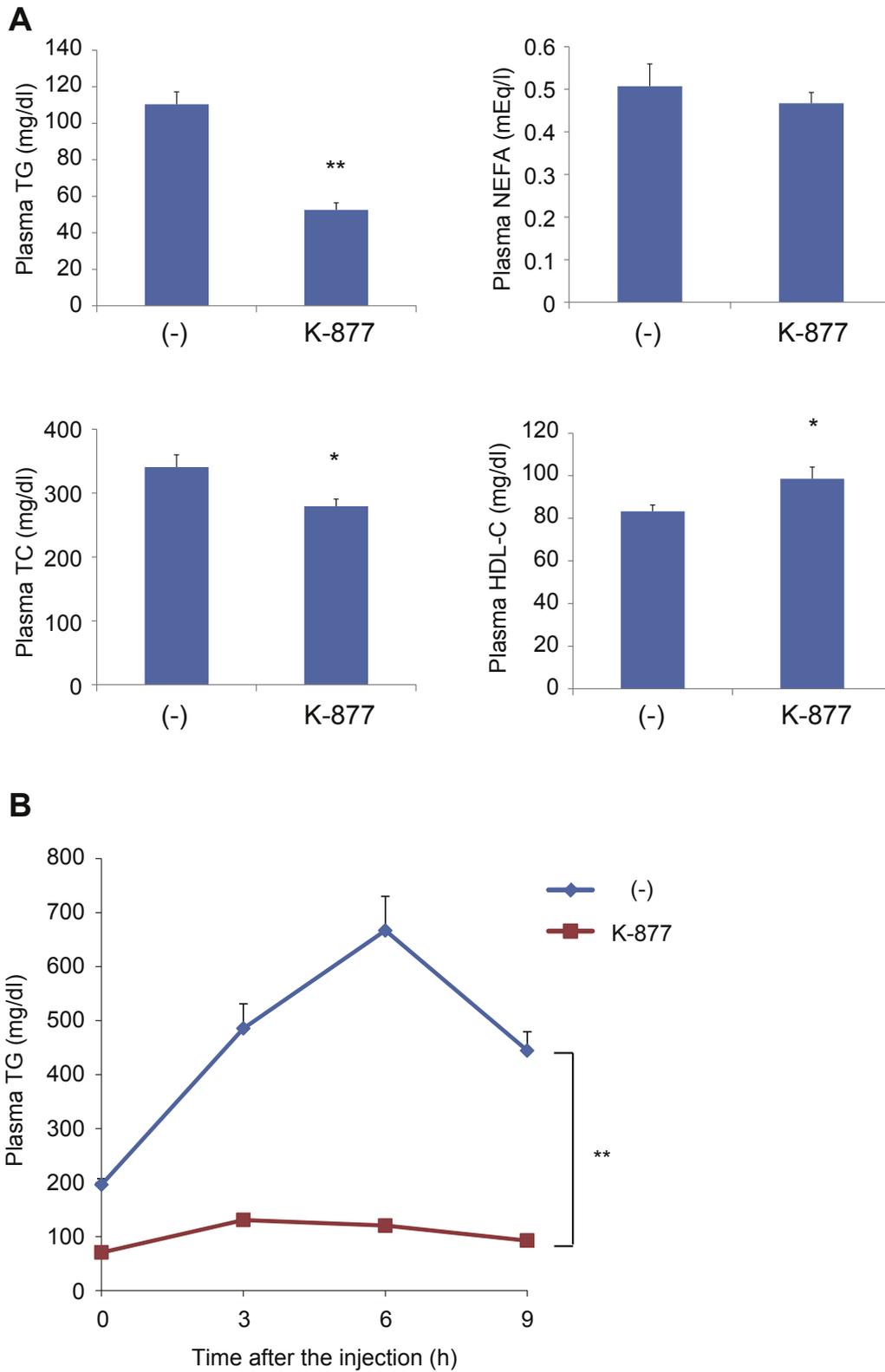
Fig. 2. K-877 reduced plasma triglyceride and total cholesterol levels and increased the plasma HDL cholesterol levels in *Ldlr*−/− mice. (A) Eight-week-old male *Ldlr*−/− mice were fed MF diets containing K-877 (0.001%) for 1 week. Plasma TG, TC, HDL-cholesterol, and non-esterified fatty acid (NEFA) levels were measured; n = 8−9 per group; *p < 0.05 and **p < 0.01. (B) Eight-week-old male *Ldlr*−/− mice were fed MF diets containing K-877 (0.001%) for 1 week. Mice were fasted for 16 h; olive oil was then orally administered. Plasma TG levels were measured. Data are represented as mean ± SEM. Significant differences were determined by repeated-measures two-way ANOVA with the Bonferroni post hoc t-test and were denoted as **p < 0.01. *n* = 5−6 per group.
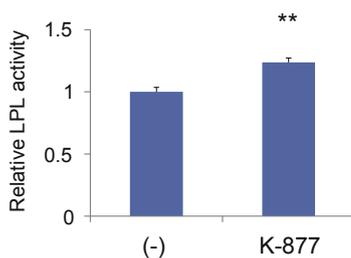
Liver

A
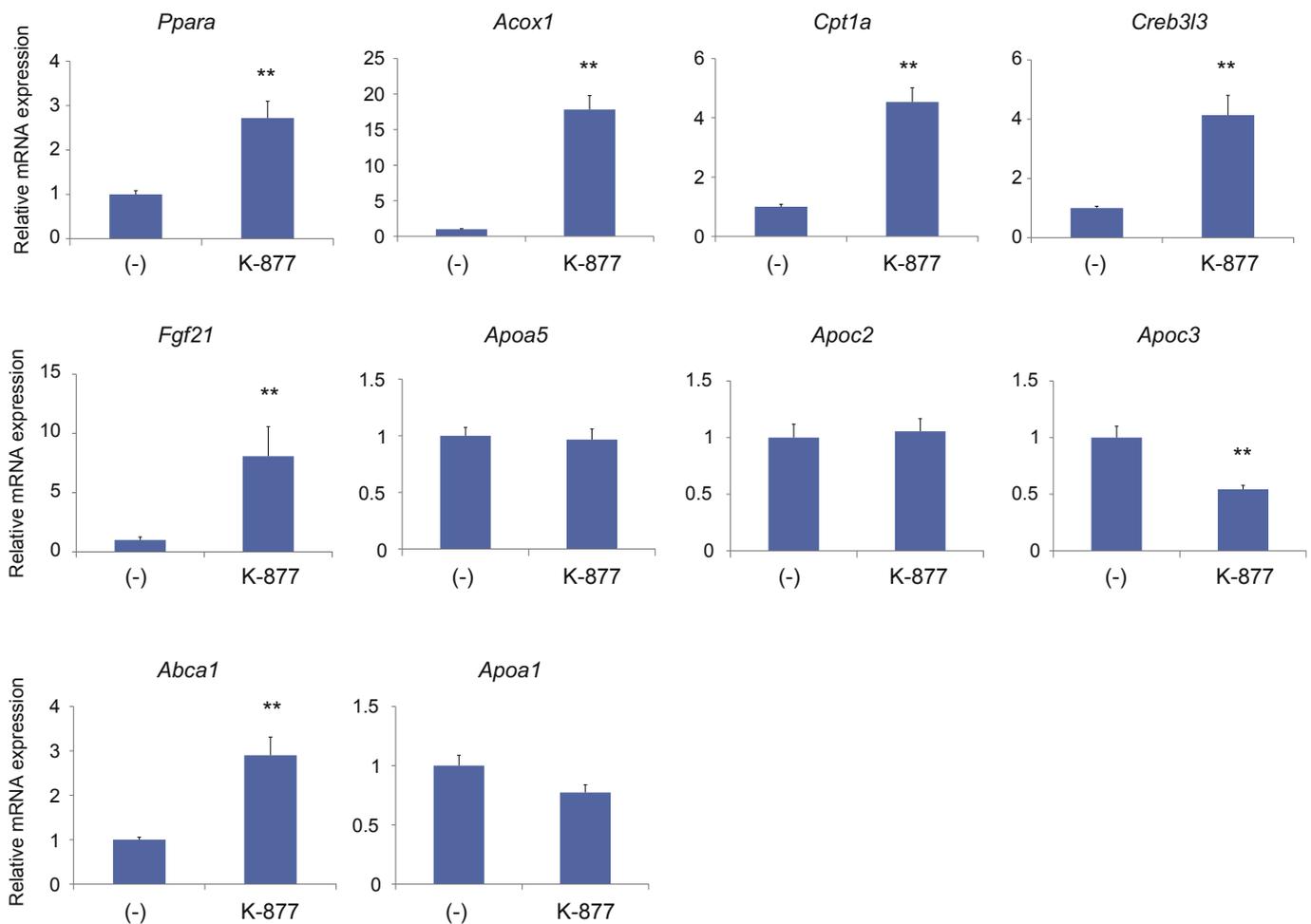
B

Fig. 3. Hepatic gene expression related to fatty acid oxidation was significantly increased in *Ldlr*$^{-/-}$ mice treated with K-877. (A) Eight-week-old male *Ldlr*$^{-/-}$ mice were fed MF diets containing K-877 (0.001%) for 1 week. Hepatic gene expression profiles of *Ldlr*$^{-/-}$ mice were analyzed; n = 8–9 per group; *p < 0.05 and **p < 0.01. (B) Plasma LPL activity in 8-week-old male *Ldlr*$^{-/-}$ mice fed with MF diets containing K-877 (0.001%) for 1 week and injected with 100 U/kg body weight of heparin via the tail veins. Blood samples were collected at 20 min post administration. Plasma LPL activity was determined; n = 5–6 per group; **p < 0.01.

secretion. The hypotriglyceridemic effect of fenofibrate in the postprandial state may be not only due to the well-established rapid clearance of TG from circulation due, in part, to an increased LPL activity[6] but also due to the decreased secretion of TG into circulation from the small intestine.

K-877, a highly specific PPARα-agonist with SPPARMα properties, has a superior lipid-modifying efficacy at considerably lower doses in humans with dyslipidemia.[31] PPARα is expressed in many tissues including skeletal muscle, adipose intestine, kidney, and heart tissues. Recently, liver-specific PPARα knockout mice were
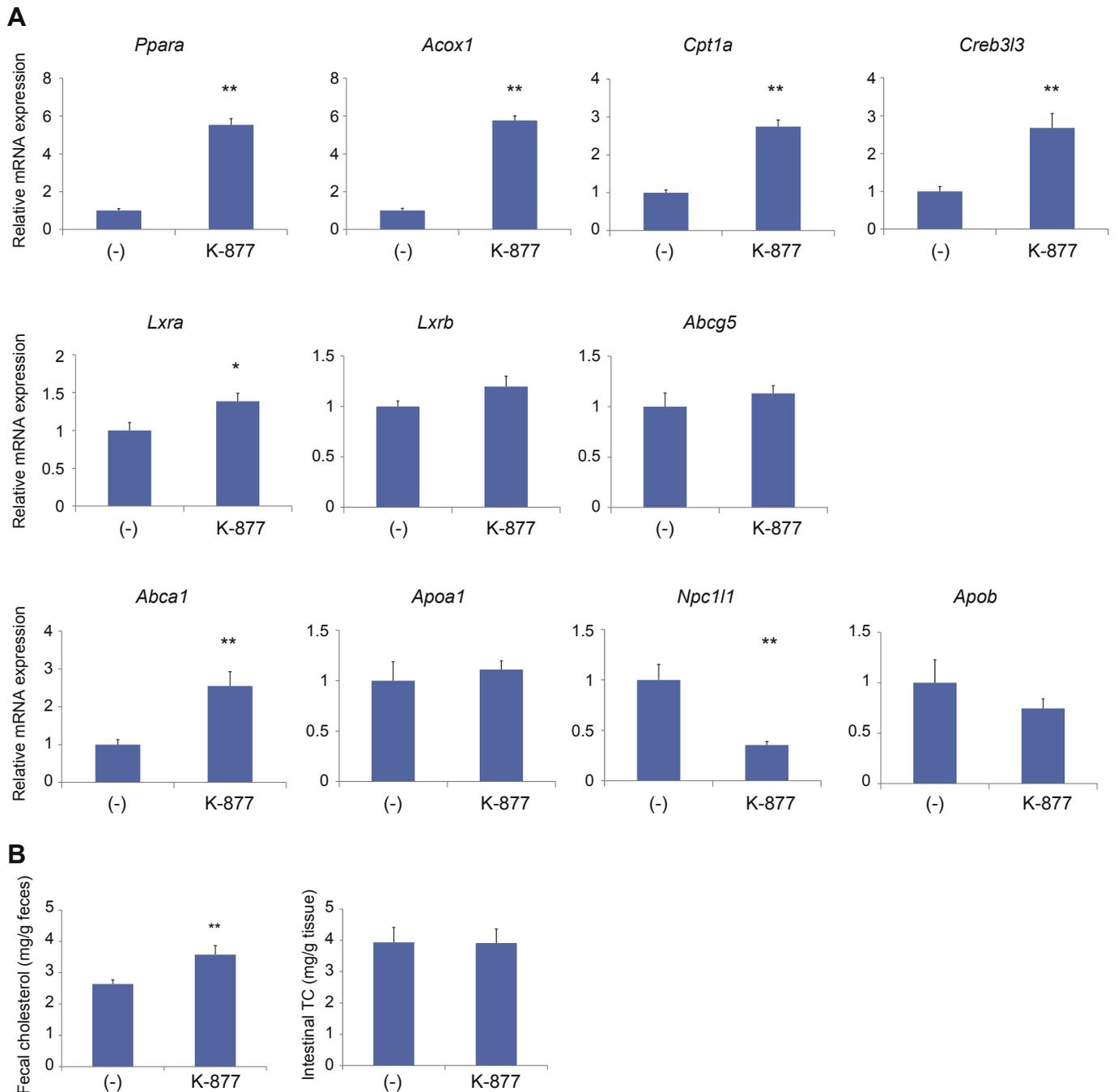
Fig. 4. Intestinal gene expression in *Ldlr*^{−/−} mice treated with K-877. Eight-week-old male *Ldlr*^{−/−} mice were fed MF diets containing K-877 (0.001%) for 1 week. (A) Profiles of intestinal gene expression. (B) Fecal and intestinal TC levels; n = 8–9 per group; *p < 0.05 and **p < 0.01.

generated, which indicated that the liver-restricted deletion of PPARα is sufficient to promote hyperlipidemia.[32]

The lowering effects of plasma TG and TC levels by PPARα agonists mainly depend on the liver and small intestine. K-877 significantly decreased the postprandial TG response, suggesting that K-877 increases plasma LPL activity and TG absorption from the small intestine. K-877 efficiently increased the expression of PPARα target genes such as *Acox1*, *Cpt1a*, and *Creb3l3* in the liver and small intestine of *Ldlr*^{−/−} mice, thereby activating fatty acid oxidation in both tissues. Moreover, the activation of intestinal fatty acid oxidation by the PPARα agonist bezafibrate suppresses postprandial lipidemia in WT mice.[33] Thus, this change in the liver

and small intestine could decrease the TG supply for its secretion into the blood circulation. PPARα activation leads to the stimulation of the catabolism of TG-rich lipoproteins that is caused not only by the upregulation of plasma LPL activity but also by the decreased mRNA expression of *Apoc3*, which has inhibitory effects on LPL activity.[34,35] K-877 administration to *Ldlr*^{−/−} mice decreased *Apoc3* expression. We confirmed that K-877 significantly activated plasma LPL activity, which contributes to a decrease in plasma TG levels. In agreement with previous reports[36,37], we confirmed that K-877 increased hepatic *Fgf21* expression that results in the amelioration of hyperlipidemia by inducing lipid catabolism.
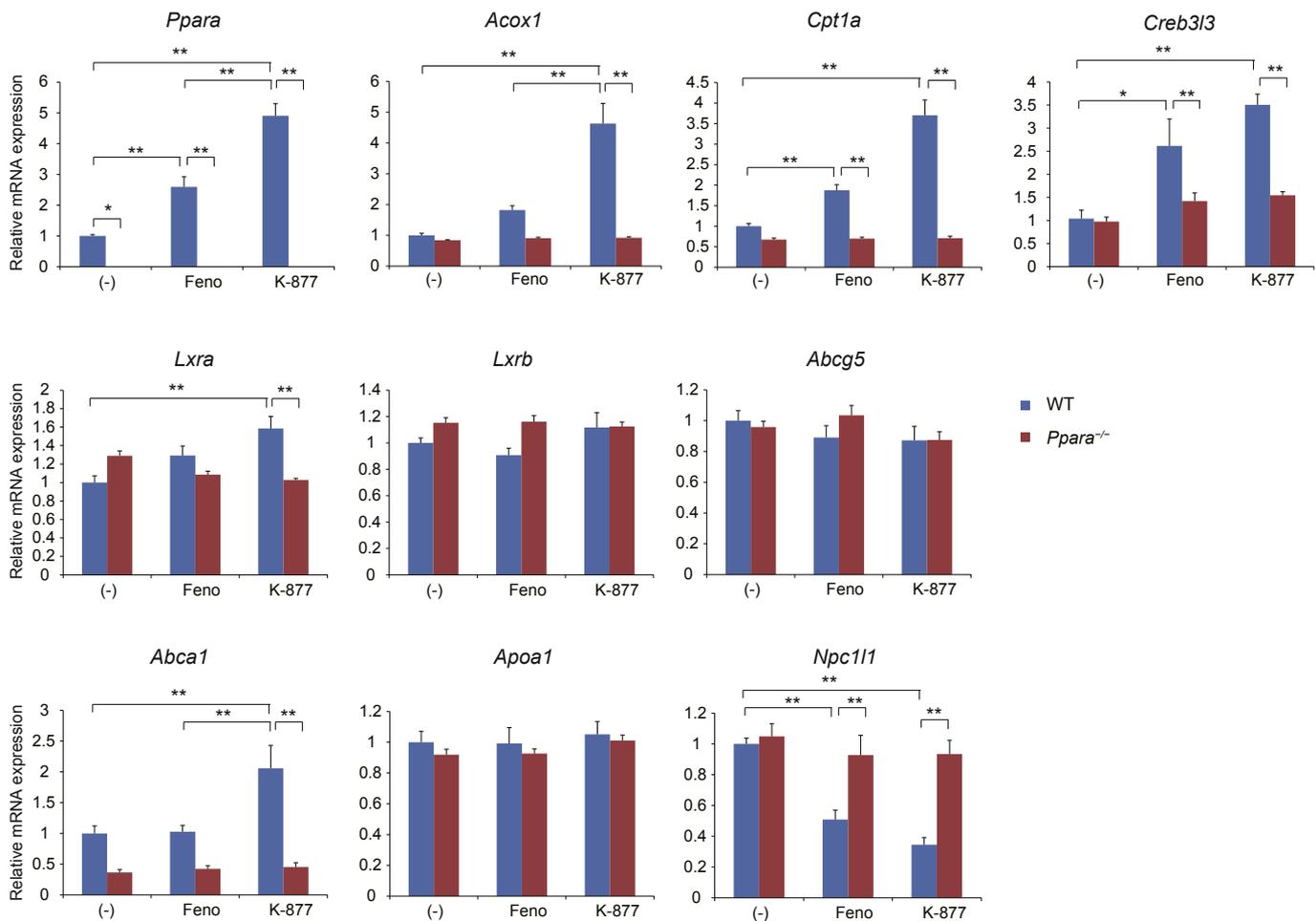
Small intestine

Figure 5. K-877 reduced the expression of *Npc1l1* and increased the expression of *Abca1* in the small intestine of WT, but not in *Ppara*$^{-/-}$, mice. Eight-week old male WT and *Ppara*$^{-/-}$ mice were fed MF diets containing Feno (0.2%) or K-877 (0.001%) for 1 week. Intestinal gene expression in WT and *Ppara*$^{-/-}$ mice was analyzed; n = 10–11 per group; *p < 0.05 and **p < 0.01.

As cholesterol metabolism is maintained by enterohepatic circulation, the function of the small intestine is important, particularly for cholesterol absorption from food. NPC1L1 is a rate-limiting transporter for cholesterol absorption in the small intestine of mice. A high dose of Feno reduces intestinal cholesterol absorption via the PPARα-dependent modulation of *Npc1l1* expression in mice.[22] We showed that a low dose of administrated K-877 correlated with more efficient reduction of *Npc1l1* expression in the small intestine than a high dose of Feno. However, it remains unknown if the mouse *Npc1l1* promoter has a PPRE sequence, the PPAR-response element, while it was reported that human *NPC1L1* promoter has a PPRE sequence.[38] Conversely, human *NPC1L1* promoter activity is upregulated by PPARα.[38] Thus, there is species difference between mice and humans in the regulation of the *NPC1L1* expression by PPARα. In addition, there is a difference in tissue distribution; human *NPC1L1* is expressed in the liver and small intestine, and mouse *Npc1l1* is expressed only in the small intestine. In both species, a PPARα agonist could decrease plasma cholesterol levels via different mechanisms. In humans, a PPARα agonist increases *NPC1L1* expression in the liver and small intestine. A PPARα agonist efficiently transfers the absorbed cholesterol from the small intestine to the liver, thereby decreasing plasma cholesterol levels. In contrast, in mice, cholesterol absorption in the small intestine decreases in response to a PPARα agonist, thereby decreasing plasma cholesterol levels. Therefore, although there is species difference, a PPARα agonist would be a drug for hypercholesterolemia. There is a candidate molecule to regulate mouse *Npc1l1* promoter activity by K-877. Recently, we have reported that intestinal CREB3L3 overexpression decreases cholesterol absorption in the small intestine by directly downregulating the expression of *Npc1l1*.[39] Thus, there is a possibility that K-877-induced CREB3L3 expression contributes to the regulation of *Npc1l1* expression. Ezetimibe, an inhibitor for NPC1L1, decreased the absorption of intestinal cholesterol and *Abca1* and *Abcg5* expression but had no influence on the levels of serum HDL-C or ApoA-I.[40] Thus, K-877 could be the most efficient drug to improve lipid metabolism. Feno increases plasma HDL-C levels in *Ldlr*$^{-/-}$ mice only when human ApoA-I is overexpressed in these animals.[41] GW7647, a PPARα agonist, has no effect on the lipoprotein profile in *Ldlr*$^{-/-}$ mice fed an atherogenic diet.[42] Thus, K-877 is a promising drug for improving lipid profile in *Ldlr*$^{-/-}$ mice. ABCA1 is an important molecule to produce HDL-C by transporting intracellular cholesterol from the liver and small intestine. Intestinal ABCA1 deficiency leads to deficient HDL biogenesis, thereby reducing cholesterol influx into the circulation.[10,43] Feno and Wy14643 increase *Abca1* expression in the small intestine.[18] In this study, K-877

apparently increased *Abca1* expression in WT mice, but Feno did not. Our data indicate that K-877 more efficiently works in the small intestine in comparison with Feno. Similar to the *Npc1l1* promoter, it remains unknown if the mouse *Abca1* promoter has a PPRE sequence. A previous report showed that PPARα agonist-induced *Abca1* expression is mediated by LXRs.[18] The increase in *Abca1* expression by K-877 was blunted in *Ppara*$^{-/-}$ mice. K-877 slightly increased the expression of LXRα expression but not that of LXRβ. The mouse LXRα promoter has a PPRE consensus sequence.[44] Therefore, K-877 partially increases *Abca1* expression via PPARα-induced LXR activation.

To conclude, we reported that K-877 administration reduced plasma TG and TC levels and increased plasma HDL-C levels in *Ldlr*$^{-/-}$ mice. K-877 administration regulated hepatic and intestinal fatty acid oxidation and apolipoprotein mRNA expression, thereby reducing plasma TG levels. K-877 administration remarkably reduced *Npc1l1* expression and increased *Abca1* expression in the small intestine. These data clearly showed that K-877, acting via PPARα, reduced cholesterol absorption in part by altering the intestinal expression of *Npc1l1*. K-877 administration increased hepatic and intestinal *Abca1* expression, contributing to an increase in plasma HDL-C levels. Selective activation of PPARα by K-877 in the small intestine was associated with beneficial changes in cholesterol metabolism genes, suggesting the potential of this novel agent in treating cardiovascular diseases.

Funding information

Conflict of interest

The authors declare that they have no conflict of interest.

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