Tissue-Specific Induction of Intestinal ABCA1 Expression With a Liver X Receptor Agonist Raises Plasma HDL Cholesterol Levels

Liam R. Brunham, Janine K. Kr uit, Terry D. Pape, John S. Parks, Folkert Kuipers, Michael R. Hayden

ABCA1 controls the rate-limiting step in HDL particle formation and is therefore an attractive molecular target for raising HDL levels and protecting against atherosclerosis. Intestinal ABCA1 significantly and independently contributes to plasma HDL cholesterol levels in mice, suggesting that induction of intestinal ABCA1 expression may raise plasma HDL cholesterol levels. We evaluated the ability of a synthetic Liver X Receptor (LXR) agonist, GW3965, to raise plasma HDL cholesterol levels in control mice and mice with liver- or intestinal-specific deletion of the Abca1 gene. Oral treatment with GW3965 increased the expression of ABCA1 by ~6-fold (P=0.004) as well as other LXR target genes in the intestines of mice, with no change in the hepatic expression of these genes. This resulted in a significant ~48% elevation of plasma HDL cholesterol levels in wild-type mice (P<0.01) with no change in plasma triglycerides. A similar increase in HDL cholesterol was observed in mice lacking hepatic ABCA1, indicating that the increase in plasma HDL cholesterol was independent of hepatic ABCA1. This effect was completely abrogated in mice lacking intestinal ABCA1. These data indicate that intestinal ABCA1 may be an attractive therapeutic target for raising HDL levels while avoiding the hepatic lipogenesis and hypertriglyceridemia typical of systemic LXR activation.

The ATP-binding cassette transporter, subfamily A, member 1 (ABCA1) mediates the rate-limiting step in the biogenesis of high density lipoprotein (HDL), the assembly of free cholesterol with apolipoprotein A-I. Absence of ABCA1, as occurs in Tangier disease and familial HDL deficiency, results in reduced HDL cholesterol levels and increased risk for coronary artery disease.1 ABCA1 is expressed in many tissues,2 but it has recently been shown that hepatic3 and intestinal4 ABCA1 are the major sites of HDL biogenesis in vivo, establishing these two tissues as the major targets for therapeutic activation of ABCA1 to raise HDL cholesterol levels.

Natural and synthetic agonists of the LXR are potent inducers of ABCA1 expression, and significantly raise HDL cholesterol levels5 and prevent atherosclerosis in murine models.6 However, systemic LXR activation is associated with excessive triglyceride synthesis, hypertriglyceridemia, and hepatic steatosis.5,7 The availability of synthetic LXR agonists with relative specificity for the intestine,8 together with the identification of intestinal ABCA1 as an important and independent contributor to plasma HDL cholesterol levels,4 raised the possibility that activation of intestinal ABCA1 may increase plasma HDL levels without inducing hepatic steatosis and hypertriglyceridemia. Here we evaluated the ability of a synthetic LXR agonist, GW3965, to raise HDL levels in mouse models of tissue-specific ABCA1 deficiency.

Materials and Methods

Abca1 intestinal and liver-specific knock-out mice3,4 were fed 0.7 mg of GW3965 or vehicle control by intragastric gavage and blood was drawn from 4 hour–fasted mice at baseline and after 9 days of feeding. All experiments were performed under the approval of the University of British Columbia Animal Care Committee. Western blotting,2 real-time PCR,9 and plasma lipid analysis1 were performed as previously described. Additional experimental details are provided in the online data supplement available at http://circres.ahajournals.org.

Results

Abca1 floxed mice (Abca1i/i) or mice with intestinal (Abca1i/i–/i) or hepatic (Abca1L/L–/L) inactivation of ABCA1 were treated with 0.7 mg of GW3965 by intra-gastric gavage daily for 9 days. Plasma HDL cholesterol at baseline was ~80% lower in Abca1L/L–/L mice and ~30% lower in Abca1i/i–/i mice compared with Abca1o/o mice (supplemental Figure 1), consistent with previous reports.3,4 After 9 days of treatment with GW3965 total plasma cholesterol levels were significantly increased in Abca1o/o and Abca1i/i–/i mice, but not in Abca1i/i–/i mice or Abca1o/o mice treated with a vehicle control (Figure 1A). These changes in total plasma cholesterol were accounted for by a 48% increase in HDL cholesterol in Abca1o/o mice (P<0.01) (Figure 1B). Abca1i/i–/i mice, with very low baseline levels of plasma HDL cholesterol, also displayed a significant 42% increase in plasma HDL cholesterol after GW3965 treatment (P<0.05) (Figure 1B), indicating that the effect of GW3965 on HDL levels is independent of hepatic ABCA1. Notably, this effect was completely abrogated in Abca1i/i–/i mice, which displayed no significant difference in HDL levels at baseline compared with after treatment. This finding indicates that the increase in HDL observed in Abca1o/o mice is dependent on the activity of intestinal ABCA1. Plasma triglycerides were not altered by
GW3965 in any group of mice (Figure 1C). Separation of plasma lipoprotein classes by FPLC further indicated that the increase in plasma cholesterol in Abca1fl/fl mice treated with GW3965 was attributable to a large increase in the HDL fraction (Figure 1D). A slight shift toward smaller HDL particles could be observed from the FPLC profile. The amount of cholesterol in the VLDL and LDL fractions was not appreciably altered.

Treatment with GW3965 significantly increased the expression of intestinal Abca1 by ~6-fold ($P < 0.004$) (Figure 2A) in Abca1fl/fl mice compared with Abca1fl/fl mice treated with a vehicle control, but had no effect on the expression of hepatic Abca1 (Figure 2B), confirming the selectively of this compound for intestinal rather than hepatic LXR targets in mice. Other LXR target genes, such as Abcg8, Abcg1, and Srebp1c were also significantly upregulated in the intestines of GW3965-treated mice, whereas none of these genes were significantly upregulated in the livers of GW3965-treated mice. The simultaneous rise in intestinal Abca1 mRNA levels and HDL levels in Abca1fl/fl mice, together with the lack of this effect in Abca1−/− mice, further indicate that the effect of GW3965 on plasma HDL levels is predominantly attributable to its effect on intestinal ABCA1. Western blotting revealed that ABCA1 protein levels were highly induced in the intestines but not livers of mice treated with GW3965, in accordance with the Abca1 mRNA levels in these mice (Figure 2C).

Because ABCG1, which mediates the efflux of cholesterol to HDL, was also significantly upregulated in the intestine on GW3965 treatment, we measured intestinal Abcg1 mRNA levels in Abca1fl/fl and Abca1−/− mice to determine whether the induction of ABCG1 could contribute to the increase in HDL cholesterol levels observed in wild-type mice. Abcg1 expression was increased in both Abca1fl/fl and Abca1−/− mice (Figure 2D). Indeed, Abcg1 expression was induced to an...
even greater extent in \textit{Abca1}\textsuperscript{–/–} compared with \textit{Abca1}\textsuperscript{+/+} mice, yet resulted in no change in plasma HDL cholesterol levels, suggesting that the induction of this gene was unlikely to have contributed to the rise in HDL cholesterol observed in wild-type mice. The higher expression of \textit{Abcg1} in the intestines of \textit{Abca1}\textsuperscript{–/–} compared with \textit{Abca1}\textsuperscript{+/+} mice suggests that ABCG1 expression may be increased in the absence of ABCA1, as has been previously reported.\textsuperscript{10}

**Discussion**

LXRs are nuclear hormone receptors that act as oxysterol sensors and play a central role in lipid metabolism in multiple tissues by controlling the expression of a variety of genes involved in cholesterol homeostasis. Synthetic agonists of LXR result in a variety of beneficial metabolic and inflammatory changes\textsuperscript{11} and reduce atherosclerosis,\textsuperscript{6} establishing LXRs as important pharmacological targets in the treatment of cardiovascular and metabolic disease. However, the usefulness of LXRs as pharmacological targets has largely been compromised by the effect of systemic LXR activation on the expression of hepatic lipogenic genes directly and via activation of hepatic sterol regulatory element binding protein 1c (SREBP1C) leading to hypertriglyceridemia and hepatic steatosis.\textsuperscript{5,7} Successful development of LXR-based therapeutics will therefore require methods to exploit the beneficial aspects of LXR activation whereas avoiding these unwanted side effects.

We have recently shown that intestinal-specific deficiency of \textit{Abca1} in mice results in a 30% reduction in plasma HDL cholesterol,\textsuperscript{4} indicating that intestinal ABCA1, in addition to hepatic ABCA1, is crucial for the maintenance of plasma HDL cholesterol levels. These data suggested that selectively increasing intestinal ABCA1 may lead to elevated plasma HDL cholesterol levels.

To test this hypothesis, we treated mice with the synthetic LXR agonist GW3965. GW3965 significantly raised plasma HDL cholesterol levels in wild-type mice and mice lacking hepatic ABCA1, and this effect was completely abrogated in mice lacking intestinal ABCA1, thereby providing proof-of-principle that activation of intestinal ABCA1 can lead to an increase in HDL levels.

GW3965 is a nonsteroidal carboxylic acid derivative of a tertiary amine identified to possess LXR activity and has previously been shown to effectively raise HDL cholesterol levels\textsuperscript{12} and inhibit the development of atherosclerosis\textsuperscript{8} without causing significant induction of lipogenic genes in the liver.\textsuperscript{5} Under our experimental conditions, this compound was highly selective for intestinal rather than hepatic LXR targets. GW3965 has previously been demonstrated to exhibit different patterns of coactivator recruitment compared with nonselective LXR agonists\textsuperscript{8} which may account for its tissue-selectivity.

Our data provide the first direct evidence that plasma HDL levels can be raised by raising intestinal ABCA1. The recent identification of partial agonists of LXRs with greater tissue-specificity\textsuperscript{13} and LXR ligands with target specificity\textsuperscript{14} indicates that it may be possible to design LXR agonists that activate specific genes in a tissue-specific manner. Our data suggest that intestinal ABCA1 may represent a promising therapeutic target for raising HDL levels by transcriptional mechanisms while avoiding the hepatic steatosis and hypertriglyceridemia normally associated with systemic LXR activation.

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**Disclosures**

None.

**References**


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Supplemental Methods

Animals

Abca1 intestinal and liver-specific knock-out mice have been previously described\textsuperscript{1,2}. GW3965 was kindly provided by GlaxoSmithKline, Stevenage, UK. Fourteen week-old female mice were fed 0.7 mg of GW3965 (corresponding to ~34 mg/kg/day) or vehicle control by intragastric gavage. On each day of the study, GW3965 was dissolved in DMSO under sterile conditions to a concentration of to 70 mg/L, then dissolved in one volume of Cremaphor followed by 9 volumes of 5% mannitol. Two-hundred microliters of this solution, corresponding to 0.7 mg of GW3965, were delivered to each mouse by intragastric gavage using feeding needles (Popper and Sons).

Blood was drawn from 4 hour-fasted mice at baseline and after 9 days of feeding. Liver and small intestine (proximal jejunum to distal ileum) were harvested, snap-frozen in liquid nitrogen and stored at -80°C. All experiments were performed under the approval by the University of British Columbia Animal Care Committee.

Real-time PCR and Western Blotting

Total RNA was extracted from livers and intestines using the RNeasy kit (Life Technologies). Two µg of DNase treated RNA were reverse transcribed using Superscript II (Life Technologies). Rnase treated cDNA was used for real time PCR using SYBR® Green PCR Master Mix (Applied Biosystems) in an ABI
Prism 7700 Sequence Detection. Gapdh was used as the invariant control.
mRNA levels in control mice were arbitrarily set as 1.

Western blotting was performed as previously described \(^3\). Briefly, liver or intestine tissues from Abca1\(^{fl/fl}\) mice treated with vehicle or GW3965 were homogenized in 20 mM Hepes, 5 mM KCl, 5 mM MgCl\(_2\), 0.5% (v/v) Triton X-100, and complete protease inhibitor (Roche), and protein concentration was determined by the Lowry assay. Eighty milligrams of total protein were separated by SDS-PAGE, transferred to PVDF membranes and probed with anti-ABCA1 \(^3\) or anti-GAPDH (Chemicon) antibodies.

**Plasma lipid analysis**

Plasma cholesterol, HDL and TG assays were performed as previously described \(^2\). Equal volumes of plasma were pooled and used for lipoprotein separation by fast protein liquid chromatography (FPLC) on a Superose 6B 10/30 column (Amersham Bioscience), as previously described \(^2\).

**Statistical Analysis**

Statistical analysis was performed using Graphpad software, using a 2-tailed Student's t-test to compare two groups, and a oneway ANOVA with a Newmann-Keuls post-test to compare three groups. Data are expressed as mean ± standard error.
Supplemental Figure Legend

Supplemental Figure 1. Plasma HDL cholesterol levels in control (fl/fl), liver (-L/-L), and intestinal (-i/-i) specific ABCA1 knock-out mice at baseline. * $P<0.01$, **$P<0.001$. n=5-11 per group.

Reference List


Supplemental Figure 1

HDL cholesterol (mg/dL)

Abca1 genotype

fl/fl  -L/-L  -i/-i

**  *