Pharmacological Characterization of A Novel Liver X Receptor Agonist with Partial LXRα Activity and a Favorable Window in Non-Human Primates

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Non-standard abbreviations: NHR, nuclear hormone receptor; RCT, reverse cholesterol transport; LXR, liver X receptor; ABCA1, ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette transporter G1; ABCG5, ATP-binding cassette transporter G5; ABCG8, ATP-binding cassette transporter G8; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglyceride; apo B, apolipoprotein B; CETP, cholesteryl ester transfer protein; FAS, fatty acid synthase; SCD1, stearoyl-CoA desaturase 1; IDOL, inducible degrader of LDL receptor; Angptl3, angiopoietin like 3 protein; LPL, lipoprotein lipase; CYP7A1, cytochrome P450 7A1; PK, pharmacokinetics; PD, pharmacodynamics; FPLC, fast protein liquid chromatography; MR, magnetic resonance; CSI, cholesterol saturation index; FGF21, fibroblast growth factor 21; LLQ, lower limit of quantitation; mg/dl, milligrams per deciliter; FOV, field of view; TR, repetition time; TE, echo time.

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Abstract

Liver X Receptors (LXRs) \(\alpha\) and \(\beta\) are nuclear hormone receptors that regulate multiple genes involved in reverse cholesterol transport (RCT) and are potential drug targets for atherosclerosis. However, full pan agonists also activate lipogenic genes resulting in elevated plasma and hepatic lipids. We report the pharmacology of BMS-779788, a potent partial LXR agonist with LXR\(\beta\) selectivity, which has an improved therapeutic window in the cynomolgus monkey compared to a full pan agonist. BMS-779788 induced LXR target genes in blood \textit{in vivo} with an EC\(_{50}\) = 610 nM, a value similar to its \textit{in vitro} blood gene induction potency. BMS-779788 was 29 and 12 fold less potent than the full agonist T0901317 in elevating plasma triglyceride and LDL cholesterol, respectively, with similar results for plasma CETP and apolipoprotein B. However, ABCA1 & ABCG1 mRNA inductions in blood, which are critical for RCT, were comparable. Increased liver triglyceride was observed following 7d treatment with BMS-779788 at the highest dose tested and was nearly identical to the dose-response for plasma triglyceride, consistent with the central role of liver LXR in these lipogenic effects. Dose-dependent increases in biliary cholesterol and decreases in phospholipid & bile acid occurred in BMS-779788 treated animals, similar to LXR agonist effects reported in mouse. In summary, BMS-779788, a partial LXR\(\beta\) selective agonist, has decreased lipogenic potential compared to a full pan agonist in cynomolgus monkeys, with similar potency in the induction of genes known to stimulate RCT. This provides support in non-human primates for improving LXR agonist therapeutic windows by limiting LXR\(\alpha\) activity.
Introduction

Cellular cholesterol levels are normally maintained within narrow limits by a variety of homeostatic processes. However, some cells, most notably macrophages in atherosclerotic lesions, can accumulate excess cholesterol through uptake of modified lipoproteins. The resultant foam cells are key players in the pathophysiology of the disease. Most extra-hepatic cells are unable to catabolize cholesterol and, since excess unesterified cholesterol is toxic, several mechanisms have evolved to protect cells against elevated levels. In one mechanism, reverse cholesterol transport (RCT), cholesterol is removed from peripheral tissues and returned to the liver for elimination (Rosenson et al., 2012). Stimulation of this process in foam cells is likely to be an effective therapy for the treatment of atherosclerosis. The liver X receptors, LXRα (NR1H3) and LXRβ (NR1H2), are nuclear hormone receptors that control the transcription of genes involved in all major phases of RCT, including efflux of excess cholesterol out of cells, trafficking in the circulation, uptake and metabolism in the liver, and excretion in feces (Tontonoz and Mangelsdorf, 2003). LXRαs sense cholesterol status by binding to and being transactivated by specific oxysterol cholesterol metabolites (Janowski et al., 1999). Upon activation, they induce the expression of the key cholesterol efflux transporters ABCA1 (Venkateswaran et al., 2000a) and ABCG1 (Venkateswaran et al., 2000b), which transport cholesterol out of cells on to HDL particles. HDL can deliver the cholesterol to liver either directly, through uptake by the scavenger receptor SR-B1, or via the transfer of cholesteryl ester to LDL by CETP, also induced by LXR (Luo and Tall, 2000), with subsequent uptake by hepatic LDL receptors. In addition, LXRαs induce apolipoprotein E in macrophages (Laffitte et al., 2001), which also facilitates cholesterol efflux from these cells. Two additional transporters induced in liver and intestine by LXR, ABCG5 and ABCG8 (Repa et al., 2002), promote the elimination of cholesterol by enhancing secretion into bile and excretion in the feces. LXRαs also have profound effects on the immune system, regulating both innate and acquired immunity (Zelcer and Tontonoz, 2006), including anti-inflammatory activities. Due to this regulation of RCT and the
immune system, LXR agonists have robust anti-atherosclerotic activity in mouse (Joseph et al., 2002a; Terasaka et al., 2003) and rabbit models (Giannarelli et al., 2012; Vucic et al., 2012).

LXRs also stimulate fatty acid synthesis and metabolism through the induction of SREBP1c, FAS, SCD1, and other genes (Schultz et al., 2000). As a consequence, LXR activation can cause increased triglyceride (TG) synthesis and elevated VLDL secretion in liver, with resultant hypertriglyceridemia and hepatic steatosis. Other LXR target genes are also modulated in liver that result in inhibition of triglyceride lipolysis in the circulation, including angiopoietin-like 3 protein (Inaba et al., 2003) and apoAV (Jakel et al., 2004). In addition, LXRs upregulate the expression of IDOL, an E3 ubiquitin ligase, which ubiquitinates the LDL receptor causing its degradation (Zelcer et al., 2009). This mechanism has the potential to downregulate hepatic LDL receptors resulting in elevated circulating LDL. Increased production of VLDL and the induction of CETP mentioned above can also contribute to elevated LDL. Thus, multiple pathways are activated by LXR in liver which have the potential to cause hepatic steatosis and dyslipidemia and, indeed, LXR agonists, including GW3965 and T0901317, have been shown to increase liver and plasma TG and LDL in mouse and hamster models (Groot et al., 2005; Joseph et al., 2002b; Schultz et al., 2000). More limited results of LXR lipid effects have been reported in primates with variable responses, depending on the compound and study (Groot et al., 2005; Quinet et al., 2009).

These undesirable effects on plasma lipids have been the major impediment for the development of LXR agonist therapeutics. It is likely that LXRα is the isoform that mediates most of the hepatic effects, at least in mice, since LXRα null mice have reduced lipogenic responses to pan agonists (Lund et al., 2006; Quinet et al., 2006). Furthermore, LXR pan agonists inhibit atherosclerosis in these mice, indicating that LXRβ is sufficient to bring about the beneficial effects (Bradley et al., 2007). Thus, one approach to limit lipogenesis is to selectively target LXRβ. However, this approach has not been adequately tested in primates. We report here the in vivo pharmacology in cynomolgus monkeys of BMS-779788, a novel
partial, LXRβ selective LXR agonist. This compound has decreased lipogenic potential compared to a full pan agonist while maintaining similar induction of genes known to enhance RCT.
Materials and Methods

Compounds. BMS-779788 (2-(2-(1-(2-chlorophenyl)-1-methylethyl)-1-(3'-(methylsulfonyl)-4-biphenyl)-1H-imidazol-4-yl)-2-propanol) was synthesized at Exelixis, Inc., San Diego, CA. The reference ligand, T0901317, was synthesized at Bristol-Myers Squibb, Princeton, NJ.

Animals. Animal studies were performed according to guidelines established by the American Association for Accreditation of Laboratory Animal Care and protocols were approved by the Bristol-Myers Squibb-Hopewell Animal Care and Use Committee. Male cynomolgus monkeys (Macaca fascicularis) were obtained from the Bioculture Group (Mauritius) and were pair-housed for 3 weeks to allow acclimation prior to the start of the study. The animals were then transferred to single housing in standard non-human primate cages at the start of the study. Water and standard primate chow (Harlan Teklad Global 2050; 20% protein diet, Harlan Laboratories, Frederick, MD) were provided ad libitum. Water was provided through an automatic UV purified and chlorinated watering system. Food was provided daily in the amount of 120 grams. When fasting was required, food was removed at 3 PM and returned on the following day at 9 AM, 2 hours after dosing. For a single-dose PK-PD study, 2 animals each were treated either with vehicle (0.5% carboxymethyl cellulose and 2% Tween 80 (Sigma, St. Louis, MO) in purified water) or 1 mg/kg BMS-779788. For the 7 day PD study, 18 animals were randomized into 6 treatment groups (N=3/group; 3-6 kg) and received the following treatments at 7 am, q.d. for 7 days by oral gavage: vehicle, 10 mg/kg/d T0901317, and 0.3, 1, 3, or 10 mg/kg/d BMS-779788. A similar protocol was used for the 7 day liver TG MRS study with the following modifications: 16 animals were randomized into 4 treatment groups (N=4/group) including vehicle, and 0.3, 1, and 10 mg/kg/d BMS-779788. In each of the above studies, an initial venous blood sample was obtained on the day prior to the start of dosing (day -1) for baseline RNA and lipid measurements, followed by samples taken a) at 0.5, 0.75, 1, 2, 4, 6, 8, 24, & 48 hrs for PK and RNA in the single-dose PK-PD study, b) on days 1, 4, and 7 of dosing for the PD study, and c) on days 1 and 7 for the liver TG.
MRS study. In the 2 multi-dosing studies, blood samples for RNA and compound exposure determinations were collected at 5-6 hours post-dose, and those for plasma lipids were collected 24 hours post-dose. In a liver mRNA and bile composition study conducted as part of a larger toxicology study, 12 animals, 2-3 kg each were randomized into 4 treatment groups (N=3/group) and dosed orally, q.d. for 14 days with vehicle, and 1, 10, or 30 mg/kg/d BMS-779788. Liver samples were taken at 24 hours after the final dose for compound level and mRNA determinations.

**Plasma and biliary lipid and protein analyses.** Plasma lipids were determined using a Hitachi H917 chemistry analyzer and kits for total cholesterol, 2nd generation direct LDL-C, 2nd generation direct HDL-C, and triglycerides from Roche Diagnostics (Indianapolis, IN). All plasma lipids were confirmed by FPLC analysis. Plasma apo B (ALerCHECK, Inc., Portland, ME) and CETP mass (Wako Diagnostics, Richmond, VA) were quantitated by ELISA according to manufacturer’s protocols. For the analysis of biliary lipids, 0.10 ml was sampled from each gall bladder using a 27G needle attached to an insulin syringe and diluted 1:20 with PBS without calcium and magnesium. This dilution was used for the cholesterol and phospholipids assays. Samples were further diluted with PBS to make a final 1:10,000 dilution for the analysis of bile acids. A 100 µl aliquot of each diluted sample was analyzed using reagent kits for cholesterol (Roche Diagnostics), phospholipid (Wako Diagnostics, Richmond, VA), and total bile acids (BQ Kits, Inc., San Diego, CA) using the COBAS MIRA instrument. The lithogenic index based on cholesterol, phospholipid, and bile acid content was calculated according to the method of Carey (Carey, 1978).

**RNA preparation and analysis.** Blood samples (1 ml) obtained by femoral venipuncture were mixed with 1 ml of PBS (without calcium and magnesium) and 2 mL of 2x RNA Lysis Solution (Applied Biosystems, Foster City, CA.). Samples were mixed and stored frozen until RNA preparation. Total RNA was prepared using ABI RNA Purification plates on an ABI 6100 using a pre-loaded protocol according to the manufacturer. For quantitation of RNAs, reverse transcriptase (RT) reactions were
performed in an MJ Thermocycler in 40 µl reaction volumes using a Bio-Rad iScript™ cDNA Synthesis Kit and then added to Sybr-Green reactions using iTaq™ SYBR® Green Supermix with ROX (Bio-Rad, Hercules, CA) according to the supplier. Forward and reverse PCR primers for each gene are included (Supplemental Table 1). The relative amount of each LXR target gene mRNA was calculated using the second derivative comparative Ct method ($2^{-\Delta\Delta Ct}$). Quantification was obtained after normalization to the internal standard ribosomal protein L30 mRNA and confirmed with a second standard mRNA for β2 microglobulin. Each sample was tested in duplicate wells and the average Ct was used for calculations.

Liver TG measurements by MRS. The effect of BMS-779788 on liver TG was determined at baseline and 24 hrs following the 7th dose (day 7) by MR spectroscopy according to the method of Szczepaniak et al. (Szczepaniak et al., 2005). All in vivo MR spectroscopic measurements were made using the following procedure with a Bruker 4.7T/40cm MRI system (Bruker BioSpin Corp., Billerica, MA). After prepping the animal for MR imaging by a veterinary anesthesiologist, the animal was placed in the magnet. Scout images were collected using RARE tripilot pulse sequence of the abdominal area including the liver using coronal and axial orientations. Using the scout images, subsequent axial and coronal images were obtained using the following instrument settings: For axial scout images: FOV=12cm; 15 slices; 3mm slice thickness; TR/TE = 500/15ms and a matrix of 256x256. For coronal scout images: FOV=20cm; 17 slices; 3mm slice thickness; TR/TE = 1200/18ms and a matrix of 256x256. Localized MR spectroscopy of a 12mm voxel in the center of the liver was performed using a PRESS pulse sequence and the data acquisition was carried out with and without water suppression. Respiratory gating was used during data acquisition. Respiratory gated MR spectra of the 12mm liver voxel were collected using a TR/TE of 3000/15ms with water suppression for 256 and 512 averages. In addition to the water suppressed acquisition, an unsuppressed spectrum was also acquired using 256 averages.
Quantitation of BMS-779788 in plasma and liver samples. The mass spectrometer used for quantitative analysis was a MDS Sciex API-3000 triple quadrupole equipped with a turbo ion spray source (Toronto, Canada). Plasma samples were treated with 3 volumes of methanol containing an internal standard. After centrifugation, the supernatant was further diluted with water 5 fold prior to analysis using LC/MS/MS. The LC system used was an Aria TX-2 TurboFlow® (Cohesive Technologies, Franklin, MA, USA) with a Waters Atlantis C18 column using a five-minute gradient. All standard curves were fitted using linear regression weighted by the reciprocal of concentration squared. Liver homogenate samples were first diluted with blank plasma and then quantified together with plasma samples against plasma standard curves. BMS-779788 was quantified in positive ESI mode. The MRM transition monitored was m/z 515.4 > 497.2 for BMS-779788 and 526.2 > 176.2 for the internal standard (BMS-692080).

Statistical analysis. Values are shown as means ± S.E.M. unless otherwise indicated. Statistical significance was determined by one-way ANOVA and Dunnett’s post test (GraphPad Prism 5.0 software, LaJolla, CA).
Results

The structure and in vitro properties of BMS-779788 have been described elsewhere (Kick et al., 2014). Briefly, it has modest human LXRβ binding selectivity (IC$_{50}$ = 68 and 14 nM, for LXRα and LXRβ, respectively) with 38% LXRα and 72% LXRβ activity in whole receptor functional transactivation assays, compared to a full agonist. The EC$_{50}$ for endogenous ABCA1 & ABCG1 gene induction in an in vitro human whole blood assay (WBA) is 1 µM for each, and its efficacy is 45% and 55%, respectively. In an equivalent cynomolgus monkey assay, EC$_{50}$s are 140 nM and 340 nM, with efficacies of 41% and 73% respectively. Consistent with its partial activity, BMS-779788 has 50% of the activity compared to T0901317 in a human THP1 macrophage cholesterol efflux assay. T0901317, the full LXRα/β dual reference agonist used in these studies, has equal binding potency for both human receptors (IC$_{50}$ = 45 nM for each), and is a full agonist in both LXRα and LXRβ transactivation assays. It is a full agonist with an EC$_{50}$ = 305 nM in the cynomolgus monkey whole blood assay.

In an initial study to characterize the relationship between BMS-779788 plasma exposure and ABCG1 mRNA induction in blood cells, we performed a single dose PK-PD study in male cynomolgus monkeys. Following a 1 mg/kg oral dose, the time course of plasma compound concentration and ABCG1 mRNA induction mirrored each other out to 24 hrs (Fig 1). The Tmax for BMS-779788 and gene induction was 2 hrs with a Cmax of 2227 nM and a maximal ABCG1 induction of 4.7 fold. ABCG1 mRNA levels were back to near baseline by 24 hrs and the BMS-779788 plasma concentration was 154 nM by 48 hrs. ABCA1 mRNA levels are not shown for reasons described below.

A repeat dosing study of BMS-779788 was then performed to determine the exposure-response for the induction of ABCG1 in blood vs. the effect on plasma lipids, apo B, and CETP. This was compared to the effects of a 10 mg/kg/d dose of T0901317. BMS-779788 was given orally at 0.3, 1, 3, and 10 mg/kg/d, along with T0901317 and vehicle control, qd for a total of 7 days. Table 1 shows the plasma
levels for both compounds at 5 hrs post-dose on days 1, 4, and 7, in nM concentration as well as fold over the cynomolgus monkey WBA EC\textsubscript{50}. BMS-779788 exposure was dose-proportional, ranging from 1.8 to 28.4 fold over its EC\textsubscript{50} on day 1, and 0.8 to 20.2 fold on day 7. T0901317 exposure was 1.0 and 0.7 fold over its EC\textsubscript{50} on days 1 and 7, respectively. Therefore, plasma levels of both compounds were sufficient for partial to full LXR target engagement in this repeat dosing study.

We next determined ABCG1 mRNA induction in blood at 5 hours post-dose on days 1 and 7 (Fig 2A). BMS-779788 treatment caused a dose-dependent induction from 6.7 to 12.8 fold vs. baseline on day 1, and 4 to 14.3 fold on day 7. T0901317 induced ABCG1 7.6 and 6.3 fold on days 1 and 7, respectively. At comparable exposures plotted as a function of fold over their respective WBA EC\textsubscript{50} values, both compounds showed similar blood ABCG1 induction (Fig. 2B). In order to explore the effects of a broader dose range on ABCG1 mRNA and to determine the potency of the compound \textit{in vivo}, animals were dosed for 5 hours with vehicle or 0.1 to 10 mg/kg BMS-779788, and blood ABCG1 mRNA levels and plasma compound levels were determined. The \textit{in vivo} EC\textsubscript{50} for ABCG1 induction was 630 nM (Supplemental Figure 1), a value similar to the 340 nM potency seen in the \textit{in vitro} WBA. The effect of the LXR agonists on blood ABCA1, the other major cholesterol efflux transporter, was also determined in the 7 day repeat dosing study above (Supplemental Figure 2). In all studies that we have performed in cynomolgus monkeys including those reported here, we observe an induction of ABCA1 mRNA in vehicle treated animals (2 to 3 fold in this study). This appears to be a procedural effect of unknown mechanism due to gavaging, since similar effects were observed when dosing water (data not shown). It is possible that an interaction occurs between LXR agonists and this procedural effect since we often do not see clear compound dose-responses in ABCA1 induction. Thus, we have used blood ABCG1, and not ABCA1 mRNA as our PD biomarker in this model.

Next we determined the effect of BMS-779788 on plasma lipids on days 4 and 7 and compared it to T0901317. Exposure-dependent increases in TG above baseline (11 to 298\%) and LDL-C (10\% to
101%) were observed in BMS-779788 treated animals on day 7 (Fig. 3). By comparison, the full pan agonist T0901317 caused marked increases in TG (293%) and LDL-C (51%) at much lower exposures than those that resulted in similar changes with BMS-779788 (29 and 12 fold lower for TG and LDL-C, respectively). Similar patterns were observed for plasma apo B and CETP mass (Fig. 4). Effects on HDL-C were similar for both compounds (Fig. 3). The difference in the plasma lipid therapeutic indices between these two compounds is evident in Fig. 5 in which ABCG1 mRNA and plasma lipids on day 7 are plotted. T090137 exposures of 0.7 x WBA EC$_{50}$ resulted in 6.3 fold ABCG1 induction and 293% and 51% increase from baseline for plasma TG and LDL-C, respectively. By comparison, BMS-779788 at approximately 4 fold higher exposure (2.7 x WBA EC$_{50}$) resulted in similar ABCG1 induction (7.2 fold) with little effect on TG (12% increase) and LDL-C (3% increase). The time course of effects on plasma TG, LDL-C, total cholesterol, and HDL-C were measured on days 2, 4, and 7 (Supplemental Figure 3), and for apo B and CETP mass (Supplemental Figure 4). There was little evidence of time-dependent effects on plasma lipids, with the exception of TG, LDL-C, and total cholesterol responses in the highest dose group (30 mg/kg/d), where progressively larger elevations above baseline occurred on days 2, 4, and 7.

Due to their induction of hepatic lipogenic pathways, increased liver TG has been observed in LXR agonist treated mice, hamsters, and cynomolgus monkeys. In a separate 7 day cynomolgus monkey study, the change from baseline in hepatic TG following treatment with vehicle or 0.3, 1, and 10 mg/kg/d BMS-779788 was determined using magnetic resonance spectroscopy, and compared with changes in plasma TG (Fig. 6). Nearly identical liver and plasma TG dose-responses were observed; TG was increased 18% to 20% and 75-82% in the 1 and 10 mg/kg/d treated groups, respectively. Modest decreases from baseline in liver and plasma TG occurred in vehicle and 0.3 mg/kg/d treated animals. As part of a 2 week toxicology study in cynomolgus monkeys, the response in liver of LXR target lipid metabolism genes following 14 days of dosing with vehicle and 1, 10, and 30 mg/kg/d BMS-779788
was also determined (Fig. 7). Two doses, 1 and 10 mg/kg/d, were the same as the top 2 doses in the previous study. The hepatic drug exposure at 24 hrs post-dose was <LLQ, 3.4 µM (14 fold WBA EC₅₀), and 40.9 µM (170 fold WBA EC₅₀) at these 3 doses. Dose-dependent increases in the lipogenic genes SREBP1c, FAS, SCD1, and Angptl3 were apparent, consistent with the dose-dependent increase in liver and plasma TG in the above magnetic resonance spectroscopy study. Furthermore, the response of these genes at 1 and 10 mg/kg/d is consistent with the relative TG and LDL-C response at these doses in the 7 day PD study. The cholesterol efflux transporters, ABCG5 and ABCG8, were also modestly induced in liver, a result similar to the reported inductions in mouse by full pan agonists (Repa et al., 2002).

Previous studies in mouse have shown an induction of LPL in liver that could account for a blunting of elevated plasma TGs due to increased clearance of TG rich lipoprotein particles (Peng et al., 2010; Zhang et al., 2001). In these studies, no effect of BMS-779788 on cynomolgus monkey hepatic LPL mRNA was observed, suggesting that this effect may not translate to higher species.

LXR agonists have the potential to change biliary lipid composition, due to their effects on hepatic biliary cholesterol secretion via hepatic ABCG5 and ABCG8 mRNA induction. Bile composition in vehicle and BMS-779788 treated male monkeys was determined following 2 weeks of treatment as part of the same toxicology study described above (Fig. 7). A dose-dependent increase in biliary cholesterol and a decrease in total phospholipids and bile acids were observed in response to treatment. Similar effects on bile composition have been reported in the mouse. The effect of the compound on the cholesterol saturation index (CSI) of bile, expressed as a percentage of the maximum possible soluble cholesterol, was calculated. Phospholipid and bile acids act to solubilize cholesterol in bile and, thus, the CSI increases as the ratio of cholesterol to total lipids increases. BMS-779788 caused a dose-dependent increase in CSI. The CSI in the vehicle, 1, 10, and 30 mg/kg/d treatment groups was 49%, 62%, 130%, and 310%, respectively. The difference from vehicle in the 30 mg/kg/d dose group was statistically significant. The increased CSI was due to both increased cholesterol content and decreased
phospholipid and bile acids. An increase in CSI above 100% could potentiate gallstone formation, however this was not investigated in the present study.
Discussion

The LXRβ selectivity strategy for avoiding undesirable lipid effects is based on mouse studies. Dual LXR agonists including T0901317, GW3965, and others cause triglyceridemia and liver steatosis in wild type animals but this response is largely absent in mice lacking LXRα. However, the mouse differs significantly from human in several key aspects of lipoprotein metabolism, whereas the cynomolgus monkey is more human-like. Unlike the mouse, cynomolgus monkeys express CETP and, therefore, should better predict LXR agonist effects on cholesterol trafficking between HDL and LDL in humans. In addition, mouse and rat CYP7A1, the rate limiting enzyme for bile acid synthesis by the classical pathway, is stimulated by LXR(Lehmann et al., 1997; Peet et al., 1998), whereas in humans(Chiang et al., 2001) and, likely primates, it is not. Furthermore, rodent whole body sterol metabolism is substantially different from humans(Turley et al., 1995). The majority of rodent whole body cholesterol synthesis occurs in the liver whereas in humans and cynomolgus monkeys most of it takes place in extra-hepatic tissues. Finally, the overall rate of cholesterol synthesis and LDL turnover is much higher in rodents than in monkeys and humans. All of these differences in sterol synthesis and trafficking could result in markedly different LXR agonist responses in humans compared to mice. Therefore, the cynomolgus monkey, with more human-like cholesterol metabolism, is likely a better predictor of clinical LXR agonist plasma cholesterol responses. Dual LXR agonists elevate LDL-C and TG in this species(Groot et al., 2005). In the current work, we show that the LXRβ selective agonist BMS-779788 has a substantially improved lipid therapeutic index in cynomolgus monkeys compared to the full dual agonist T0901317. On a potency adjusted exposure basis, BMS-779788 was approximately 40 fold less potent than T0901317 in raising plasma TG, and 17 fold less potent in LDL-C and apo B elevation following 7 days of treatment. In an earlier report, T0901317 has been shown to increase plasma CETP activity in cynomolgus monkeys(Honzumi et al., 2010), consistent with CETP being an LXR target.
gene, and an activity that is likely to play a significant role in LXR-dependent LDL-C elevations. As was the case with TG, LDL-C, apo B, BMS-779788 was also less potent in CETP mass elevation. While this improved profile of BMS-779788 is consistent with the LXRα hypothesis of lipogenesis, other properties of the compound including its overall partial activity in whole blood target gene induction may also contribute to the favorable profile in non-human primates. Partial agonists can display tissue-specific activation or repression of nuclear receptors, as has been shown for the estrogen receptor partial agonist raloxifen (Delmas et al., 1997). The basis for tissue selectivity of NHR partial agonists is incompletely understood but probably relates to differential competitive recruitment of coactivators and corepressors (Albers et al., 2006). Hepatic exposure differences between T0901317 and BMS-779788 could also potentially contribute to differences in lipogenic responses.

The effects of another LXR agonist, WAY-252623, in cynomolgus monkeys has also been reported (Quinet et al., 2009). This compound has an in vitro profile similar to BMS-779788 with 7 fold LXRβ binding selectivity and partial LXRα activity (Wrobel et al., 2008). However, instead of elevating lipids, WAY-252623 decreased plasma LDL-C and only transiently increased plasma TG. The mechanism for this result is unclear, however it seems unlikely that it is related to the LXRα partial activity of the compound. The authors suggest that it may be due to its induction of intestinal FGF19 expression. Then again, GW3965, which elevates LDL-C and TG in cynomolgus monkeys, also induced FGF19. The molecular pharmacology underlying the unexpected plasma lipid lowering by this agonist requires more investigation.

The effect of LXR agonists on liver and circulating TG is multi-facettted with regulation in liver of both fatty acid and TG synthesis and lipolysis pathways. The TG synthesis genes SREBP1c, FAS, and SCD1 are induced while angptl3, which blocks lipolysis, is upregulated. BMS-779788 treatment increased liver TG in a dose-response that mirrored plasma TG changes, and that was consistent with the dose-response of the lipogenic genes SREBP1c, FAS, and SCD1 in liver. In the mouse, LXR also has pro-
lipolytic effects through the induction of LPL in liver, an organ that doesn’t ordinarily express LPL. This has the potential to limit LXR-dependent hypertriglyceridemia but also to potentiate liver TG accumulation through re-directing LPL-derived fatty acids back into liver. However, this does not appear to be the case in cynomolgus monkeys since no hepatic induction of LPL by BMS-779788 was observed. Therefore, primates may lack this regulation of TG metabolism by LXR.

In bile, cholesterol is maintained in soluble form by bile acids and phospholipids. When the ratio of biliary cholesterol to these solubilizing agents increases, the maximal soluble cholesterol concentration decreases and, if it rises above the saturation limit, gall stone formation can occur. Treatment of mice with T0901317 caused increased biliary cholesterol and decreased phospholipid and bile acids(Yu et al., 2003), however effects on cholesterol saturation was not explored in this study. BMS-779788 treatment also elevated biliary cholesterol and lowered phospholipid and bile acids in our primate studies. When the cholesterol saturation index, a measure of lithogenic potential, was calculated using published critical tables, a dose-dependent increase in CSI was observed following 2 weeks of treatment, reaching 310% of maximal soluble biliary cholesterol at the top dose. In other words, biliary cholesterol was 3 fold higher than its solubility limit. While we did not examine gallstone formation in this study, this suggests that LXR agonists could be lithogenic at high doses in the clinical setting.

In summary, BMS-779788, a novel LXRβ selective agonist, has a markedly improved plasma lipid profile compared to a dual agonist in a cynomolgus monkey model. This data in non-human primates lends further support to the concept of restricting LXRα activity in order to avoid the undesirable lipid effects of LXR agonists.

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References


Figure legends.

**Figure 1.** The relationship between BMS-779788 plasma exposure and ABCG1 mRNA induction in blood cells following a single dose in cynomolgus monkeys. A 1 mg/kg oral dose was administered to 2 male cynomolgus monkeys by gavage. Blood samples were drawn at the indicated times and plasma BMS-779788 concentrations and ABCG1 mRNA in blood cells were determined. The Tmax for both gene induction and compound levels was 2 hours, reaching 4.7 fold and 2227 nM, respectively. mRNA and compound levels returned to near baseline by 24 and 48 hours, respectively. Values are plotted as mean and range at each time point.

**Figure 2.** BMS-779788 dose and exposure-response of blood ABCG1 mRNA in 7 day study. Male cynomolgus monkeys (N=3/treatment group) were dosed q.d. orally with vehicle, 10 mg/kg/d T0901317, or 0.3, 1, 3, and 10 mg/kg/d BMS-779788. ABCG1 mRNA was quantitated by RT-PCR in blood samples drawn on day -1 (baseline) and 5 hours post-dose on days 1 and 7. A. The mean ± SEM fold induction of mRNA vs. baseline in each treatment group on days 1 and 7 after normalization to ribosomal protein L30 mRNA. B. Data in panel A plotted as plasma exposure, expressed as fold over the WBA EC50 for each compound (BMS-779788, 340 nM; T0901317, 305 nM), vs. fold induction of ABCG1 mRNA. Note that T0901317 and BMS-779788 show similar potency adjusted exposure-mRNA responses. *, p<0.05; **, p<0.01; ***, p<0.001 vs. baseline.

**Figure 3.** Effect of BMS-779788 and T0901317 on plasma lipids in 7 day study. Animals were treated with LXR agonists as described in Figure 2. Plasma TG, LDL-C, and HDL-C were measured at 24 hours post-dose. Percent change from baseline of each of the lipids is plotted vs. plasma exposure expressed as fold over the WBA EC50 for each compound. Note the lower potency of BMS-779788 for plasma TG and LDL-C elevation compared to T0901317, and the comparable apoAI responses.
Figure 4. Effect of BMS-779788 and T0901317 on plasma apo B and CETP in 7 day study. Treatment of animals and plotting of apo B and CETP responses, measured at 24 hours post-dose, are as described in Figures 2 and 3.

Figure 5. Comparison of BMS-779788 and T0901317 effects on blood ABCG1 mRNA induction vs. plasma TG and LDL-C elevation. Day 7 percent change plasma TG from baseline and fold ABCG1 mRNA induction (panel A) and LDL-C and ABCG1 mRNA (panel B) vs. compound exposure, expressed as fold over the WBA EC50 for each compound.

Figure 6. Effect of BMS-779788 on hepatic TG and comparison with plasma TG responses. Percent change from baseline in liver TG following 7 days of treatment with vehicle or 0.3, 1, and 10 mg/kg/d BMS-779788 was determined using magnetic resonance spectroscopy, and correlated with changes in plasma TG. ***, p<0.001 vs. vehicle treated.

Figure 7. Effect of BMS-779788 on lipid metabolism gene expression in liver. As part of a 2 week toxicology study, male cynomolgus monkeys were dosed orally, q.d. for 14 days with vehicle or 1, 10, and 30 mg/kg/d BMS-779788. Total RNA was prepared from liver samples harvested at 24 hours post-final dose and the indicated mRNAs were quantitated by RT-PCR and expressed as fold induction vs. vehicle. Liver compound exposure at this time was <LLQ, 3.4 µM, and 40.9 µM for the 3 doses. *, p<0.05; **, p<0.01; ***, p<0.001 vs. vehicle treated.

Figure 8. Effect of treatment with BMS-779788 for 2 weeks on biliary lipids. Bile was harvested 24 hours post-final dose from animals in the study described in Fig. 7. Biliary concentrations of cholesterol (panel A), phospholipid (panel B), total bile acids (panel C), and % maximum soluble cholesterol, or lithogenic index (panel D) were measured as described in the Materials and Methods section. *, p<0.05; **, p<0.01 vs. vehicle treated. Note the dose-dependent increase in cholesterol and decrease in phospholipid and bile acids, resulting in an increased lithogenic index.
Table 1. Mean (SD) plasma compound levels on days 1, 4 and 7 at 5 hours post-dose in 7 day cynomolgus PD study

<table>
<thead>
<tr>
<th>Day</th>
<th>BMS-779788</th>
<th>T0901317</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.3 mg/kg/d</td>
<td>1 mg/kg/d</td>
</tr>
<tr>
<td>1</td>
<td>613 (82)</td>
<td>2.6 (0.34)</td>
</tr>
<tr>
<td>4</td>
<td>461 (29)</td>
<td>1.9 (0.12)</td>
</tr>
<tr>
<td>7</td>
<td>267 (10)</td>
<td>1.1 (0.04)</td>
</tr>
</tbody>
</table>
Figure 4

Day 4

Apo B

% change from baseline

plasma exposure fold WBA EC50

Day 7

CETP

% change from baseline

plasma exposure fold WBA EC50

BMS-779788

T0901317
Figure 5

A

TG (% change from baseline)

fold mRNA induction

compound plasma exposure (fold WBA EC<sub>50</sub>)

- ■ plasma TG, BMS-779788
- ▲ plasma TG, T090137
- ▼ ABCG1 mRNA, BMS-779788
- ▽ ABCG1 mRNA, T0901317

B

LDL-C (% change from baseline)

fold mRNA induction

compound plasma exposure (fold WBA EC<sub>50</sub>)

- ■ LDL-C, BMS-779788
- ▲ LDL-C, T0901317
- ▼ ABCG1 mRNA, BMS-779788
- ▽ ABCG1 mRNA, T0901317
Figure 6

% change from baseline vs. mg/kg/d BMS-779788

- Liver
- Plasma

*** indicates statistically significant difference.
Figure 7

SREBP1c

FAS

mg/kg/d BMS-779788

mg/kg/d BMS-779788

SCD1

Angptl3

mg/kg/d BMS-779788

mg/kg/d BMS-779788

ABCG5

ABCG8

mg/kg/d BMS-779788

mg/kg/d BMS-779788

LPL

mg/kg/d BMS-779788