Rixinoid Bexarotene Modulates Triglyceride but not Cholesterol Metabolism via Gene-Specific Permissivity of the RXR/LXR Heterodimer in the Liver

Fanny Lalloyer, Thomas Åskov Pedersen, Barbara Gross, Sophie Lestavel, Saïd Yous, Emmanuelle Vallez, Jan-Åke Gustafsson, Susanne Mandrup, Catherine Fievet, Bart Staels, Anne Tailleux

Objective—Bexarotene (Targretin) is a clinically used antitumoral agent which exerts its action through binding to and activation of the retinoid-X-receptor (RXR). The most frequent side-effect of bexarotene administration is an increase in plasma triglycerides, an independent risk factor of cardiovascular disease. The molecular mechanism behind this hypertriglyceridemia remains poorly understood.

Methods and Results—Using wild-type and LXRα/β-deficient mice, we show here that bexarotene induces hypertriglyceridemia and activates hepatic LXR-target genes of lipogenesis in an LXR-dependent manner, hence exerting a permissive effect on RXR/LXR heterodimers. Interestingly, RNA analysis and Chromatin Immunoprecipitation assays performed in the liver reveal that the in vivo permissive effect of bexarotene on the RXR/LXR heterodimer is restricted to lipogenic genes without modulation of genes controlling cholesterol homeostasis.

Conclusion—These findings demonstrate that the hypertriglyceridemic action of bexarotene occurs via the RXR/LXR heterodimer and show that RXR heterodimers can act with a selective permissivity on target genes of specific metabolic pathways in the liver. (Arterioscler Thromb Vasc Biol. 2009;29:1488-1495.)

Key Words: retinoid-X-receptor ■ rexinoid ■ hypertriglyceridemia ■ liver-X-receptor ■ murine model ■ ChIP

Received May 28, 2008; revision accepted June 21, 2009.
Correspondence to Bart Staels, Inserm U545, Institut Pasteur de Lille, 1 rue du Professeur Calmette, F-59019 Lille, France. E-mail bart.staels@pasteur-lille.fr

© 2009 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol is available at http://atvb.ahajournals.org DOI: 10.1161/ATVBAHA.109.189506

1488
implicated in triglyceride metabolism, permissive ligand activation of RXR by rexinoids could elicit responses of the dimerization partner and as such modulate triglyceridemia. However, whereas PPARα and FXR activation leads to decreased plasma triglyceride levels, LXR activation increases triglycerides, presenting the permissive RXR/LXR heterodimer as a potential mediator of bexarotene-induced hypertriglyceridemia.

LXRs (LXRα and LXRβ) are nuclear receptors that are activated by oxysterols or synthetic ligands such as T0901317, and bind DNA as obligate heterodimers with RXR at specific LXR response elements (LXREs) composed of a direct repeat of 5′-AGGTCA-3′ spaced by 4 bases. LXR activation regulates triglyceride metabolism and induces hepatic steatosis and hypertriglyceridemia attributable, in part, to increased hepatic fatty acid synthesis and VLDL secretion by upregulation of both sterol regulatory element-binding protein 1c (SREBP1c) and carbohydrate response element-binding protein (ChREBP) expression. Moreover, LXRs play an important role in the regulation of cholesterol homeostasis, and their activation leads to the induction of several genes implicated in reverse cholesterol transport and mobilization of cholesterol, such as the ATP binding cassette (ABC) transporters ABCA1, ABCG1, ABCG5, ABCG8, and apolipoprotein E.

Using wild-type mice and mice deficient for both isoforms of LXR (LXRα/β-deficient mice), we show that the permissive heterodimer RXR/LXR mediates bexarotene-induced hypertriglyceridemia and hepatic lipogenesis in vivo. Surprisingly, RNA levels and chromatin immunoprecipitation (ChIP) assays reveal that this LXR-mediated effect of bexarotene in the liver is restricted to genes implicated in triglyceride homeostasis and lipogenesis, without modulation of genes controlling cholesterol homeostasis. These findings demonstrate that RXR heterodimers can act with a selective permissivity on target genes of specific metabolic pathways.

Materials and Methods

Animal Experiments

All experiments were performed with the approval of the Pasteur Institute review board, Lille France.

Bexarotene was synthesized in the Laboratoire de Chimie Thérapeutique (Faculté des Sciences Pharmaceutiques, Université Lille Nord de France, France).

Female C57BL6 (13 weeks of age) wild-type (n=28) or LXRα/β-deficient (n=28) mice were obtained from Jan-Åke Gustafsson’s laboratory were divided into 4 groups (n=7/group) and were treated

with the RXR agonist bexarotene (suspended in 1% carboxymethylcellulose at the doses of 30 or 100 mg/kg body weight [mpk]), the LXR agonist (T0901317, 30 mpk), or vehicle alone by oral gavage once daily for 14 days. At the end of the treatment, blood was collected by retro-orbital venipuncture under isoflurane anesthesia after 4 hours of fasting (9 AM to 1 PM). Plasma was separated by centrifugation (15 minutes, 4°C, 4000 rpm) and used within 3 days for biochemical analysis. Animals were weighed and euthanized by cervical dislocation. Livers were removed immediately, frozen in nitrogen, and stored at −80°C for further analysis.

For expanded Material and Methods, please see supplemental information (available online at http://atvb.ahajournals.org).

Results

Bexarotene Increases Plasma and Hepatic Triglyceride Levels in an LXRα/β-Dependent Manner

To determine whether LXRs are implicated in the bexarotene-induced hypertriglyceridemia, wild-type mice and mice lacking both LXRα and LXRβ (LXRα/β-deficient mice) were treated with the RXR agonist (bexarotene, 30 and 100 mpk), the LXR agonist (T0901317, 30 mpk), or vehicle alone for 14 days (Figure 1A). In wild-type mice, the LXR agonist induced a hypertriglyceridemia, an effect which was not observed in LXRα/β-deficient mice. Interestingly, the RXR agonist bexarotene also induced a robust dose-dependent hypertriglyceridemia, which was strongly blunted in LXRα/β-deficient mice. Thus, the RXR/LXR heterodimer appears to be the principal mediator of the bexarotene-induced hypertriglyceridemia.

Because LXR is a lipogenic transcription factor in the liver, we analyzed whether bexarotene influences triglyceride metabolism in the liver and whether these effects are mediated by LXR (Figure 1B). As previously shown, the LXR agonist T0901317 increased hepatic triglyceride levels in wild-type but not in LXRα/β-deficient mice. Interestingly, bexarotene induced a dose-dependent increase in hepatic triglyceride concentration in wild-type but not in LXRα/β-deficient mice. Thus, the bexarotene-induced hypertriglyceridemia and hepatic triglyceride accumulation are dependent on the RXR/LXR pathway.

Bexarotene Induces Hepatic Lipogenic Genes in an LXRα/β-Dependent Manner

To further explore the role of LXR in the effects of bexarotene on hepatic triglyceride metabolism, the expression of LXR-target genes implicated in lipogenesis (SREBP1c, FAS, and SCD1) was measured by quantitative PCR (Figure 2). As expected, LXR agonist administration robustly increased the

Lalloyer et al Permissive Effects of Bexarotene on RXR/LXR 1489

Figure 1. RXR or LXR agonist treatment increases plasma and hepatic triglyceride levels in vivo in an LXRα/β-dependent manner. Plasma (A) and hepatic (B) triglyceride concentrations were measured in LXRα/β-deficient mice (■) and wild-type mice (●) gavaged with the RXR agonist (bexarotene, 30 and 100 mpk), the LXR agonist (T0901317, 30 mpk), or vehicle alone for 14 days (n=7 per group). **P<0.01, ***P<0.001 vs wild-type mice treated with vehicle; §P<0.05, §§P<0.01 vs LXRα/β-deficient mice treated with vehicle.
bexarotene treatment in wild-type but not in LXR-deficient mice. Interestingly, the mRNA levels of SREBP1c, FAS, and SCD1 were measured in LXR-deficient mice. Hepatic mRNA levels of SREBP1c, SCD1, and FAS were decreased in the LXR-deficient mice (supplemental Figure IA) as well as Cyp3A11, CD36 and FAE mRNA both in wild-type and LXR-deficient mice, suggesting that the lipogenic enzyme FAE could be implicated in bexarotene-induced lipogenesis, similar as SREBP-1c, SCD1, and FAS.

These data show that bexarotene upregulates specific hepatic LXR-target lipogenic genes via the permissive heterodimer RXR/LXR in vivo.

**Bexarotene Increases RXR Binding to the LXREs of Hepatic Lipogenic Genes**

To investigate whether the activation of hepatic lipogenic LXR-target genes was associated with an increased ability of RXR complexes to bind LXREs in the promoters of these genes, ChIP assays were performed on livers of mice treated with the highest dose of the RXR agonist, the LXR agonist, or vehicle alone (Figure 3). Antibodies directed against RXR (Figure 3A) and RNA polymerase II (Figure 3B) were used, and input as well as immunoprecipitated DNA was quantified by real-time PCR using primers positioned around the LXREs of these genes (Figure 3A) or within the gene (Figure 3B), respectively. The antibody against RNA polymerase II recognizes both the nonphosphorylated and the phosphorylated extending forms of RNA polymerase II, and occupancy within the gene can be used as a measure of transcriptional activity.

In wild-type mice, the RXR ChIP-PCR revealed that both LXR and RXR agonist treatment increased the recruitment of RXR on the LXREs of SREBP1c, SCD1, and FAS genes (Figure 3A). RNA polymerase II ChIP-PCR showed that this bexarotene and T0901317-induced RXR recruitment was associated with increased RNA polymerase II occupancy in the genes encoding SREBP1c, SCD1, and FAS in wild-type mice (Figure 3B). By contrast, bexarotene-induced RXR recruitment to the FAS and SREBP1c LXREs was abolished in the LXRα/β-deficient mice, on bexarotene and T0901317 treatment, whereas a small albeit significant increase was still observed on the SCD1 LXRE (Figure 3A). These data suggest that RXR can bind independent of LXR in the vicinity of this LXRE. Moreover, in LXRα/β-deficient mice, neither bexarotene nor T0901317 led to a significant increase of RNA polymerase II occupancy on the SREBP1c and FAS genes. In keeping with the modest LXR independent binding of RXR to the SCD1 gene, a modest bexarotene-induced recruitment of RNA polymerase II was observed after bexarotene treatment.
Altogether, both LXR and RXR agonist treatment increased RXR binding to LXREs and increased RNA polymerase II occupancy on hepatic lipogenic genes in wild-type mice but not in LXRα/β-deficient mice.

**Plasma Cholesterol Concentrations Are Increased After LXR but not RXR Agonist Treatment**

Because LXR is a major regulator of cholesterol homeostasis, plasma cholesterol concentrations were also measured in wild-type and LXRα/β-deficient mice treated with the LXR and RXR agonists, respectively (Figure 4A). The LXR agonist T0901317 increased plasma total cholesterol by increasing both HDL-C and non–HDL-C in wild-type but not in LXRα/β-deficient mice (Figure 4B and 4C). Hepatic cholesterol content was not modified after T0901317 treatment (data not shown). Interestingly, plasma total cholesterol, HDL-C, and non–HDL-C did not change on treatment of wild-type and LXRα/β-deficient mice with RXR agonist treatment.

**Figure 4.** LXR but not RXR agonist treatment increases plasma cholesterol levels in vivo. TC (A), HDL-C (B), and non-HDL-C (C) were measured in LXRα/β-deficient mice and wild-type mice (n=7 per group). **P<0.01, ***P<0.001 vs wild-type mice treated with vehicle.
the RXR agonist bexarotene. Moreover, bexarotene treatment did not influence hepatic cholesterol content (data not shown). Thus, bexarotene-mediated RXR activation of the RXR/LXR heterodimer does not influence plasma cholesterol concentrations, whereas T0901317-induced LXR activation of RXR/LXR increases plasma cholesterol concentrations.

Hepatic LXR-Target Genes Implicated in Cholesterol Homeostasis Are Induced by LXR but not RXR Agonist Treatment

LXR influences cholesterol homeostasis in the liver by regulating genes encoding proteins of the ATP binding cassette transporter family. Therefore, the expressions of these LXR-target genes were measured in the liver. In wild-type mice, LXR agonist treatment induced a strong increase in the expression of ABCG1, ABCA1, ABCG5, and ABCG8, an effect which was totally absent in LXRα/β-deficient mice (Figure 5). Notably, in contrast to lipogenic LXR target genes, the hepatic mRNA levels of these LXR target genes of cholesterol metabolism only displayed minor or no responsiveness to RXR agonist treatment. Indeed, bexarotene treatment only slightly influenced the mRNA level of these genes and these effects were comparable between wild-type and LXRα/β-deficient mice. These data show that, in contrast to hepatic lipogenic genes, only T0901317 induces hepatic LXR-target genes implicated in cholesterol homeostasis via RXR/LXR in vivo, demonstrating that RXR/LXR heterodimers can act with a selective permissivity on target genes of specific pathways in the liver. To analyze whether bexarotene can modulate these LXR-target genes of cholesterol homeostasis in another organ such as the intestine, the expression of ABCG5 and ABCG8 as well as NPC1L1 was measured in the jejunum. In contrast to T091317, the bexarotene-induced decrease of NPC1L1 expression was not LXR-dependent. However, both bexarotene and T091317 increased intestinal ABCG5 and ABCG8 mRNA levels in an LXR-dependent manner (supplemental Figure 11). These LXR-dependent increases of ABCG5 and ABCG8 expression by bexarotene in the intestine differ from the absence of LXR-dependent effects of bexarotene on these genes in the liver, identifying tissue-specific mechanisms of RXR/LXR heterodimer permissivity.

RXR Recruitment on the LXREs of Hepatic Genes Implicated in Cholesterol Homeostasis Is Induced by LXR but not RXR Agonist Treatment

To determine how T0901317 and bexarotene affect RXR recruitment to the LXREs of genes implicated in cholesterol homeostasis, RXR ChIP-PCR was performed on livers of mice treated with the highest dose of the RXR agonist, the LXR agonist or vehicle alone (Figure 6A). LXR agonist treatment increased RXR occupancy on a putative LXRE sequence adjacent to the ABCG1 gene in wild-type mice but not in LXRα/β-deficient mice. Even though ABCA1 has a high basal LXRE occupancy by RXR, RXR recruitment on the reported LXRE in the proximal promoter of ABCA1 also tended to increase in wild-type mice but not in LXRα/β-deficient mice. Because the mouse LXREs in ABCG5 and ABCG8 genes have not yet been characterized, we used computational analysis to identify the DR4 elements in the vicinity of these genes, and we identified 2 putative LXREs in the first intron of the ABCG8 gene.
Interestingly, LXR agonist treatment increased the recruitment of RXR to these putative LXREs only in wild-type mice, indicating that these sequences are indeed functional LXREs. In keeping with ABCG1, ABCA1, ABCG5, and ABCG8 being LXR target genes in the liver, RNA polymerase II ChIP-PCR demonstrated LXR agonist dependent polymerase II occupancy in wild-type but not in LXRα/β-deficient livers (Figure 6B). Interestingly, in contrast to the LXR agonist, RXR agonist did not induce major changes in RXR binding to the examined LXREs adjacent to genes involved in cholesterol homeostasis neither in wild-type nor in LXRα/β-deficient liver. Moreover, recruitment of RNA polymerase II to the ABCG1, ABCA1, ABCG5, and ABCG8 genes was not influenced by bexarotene in wild-type and in LXRα/β-deficient mice (Figure 6B). These data demonstrate that LXR and RXR agonists differently regulate RXR binding on hepatic genes implicated in cholesterol homeostasis, an effect that may contribute to their distinct effects on plasma and hepatic lipid levels.

**Discussion**

In the present study, we show that bexarotene, a rexinoid used clinically for the treatment of certain cancers and dermatologic disorders, induces hypertriglyceridemia in an LXR-dependent manner in vivo. This observation is of particular importance because rexinoids have been proposed for the treatment of metabolic diseases and insulin-resistance syndromes. However, side effects such as hypertriglyceridemia, which is now widely recognized to be an independent risk factor of CVD,7,8 have limited their use. Until now, the mechanism underlying this effect was not understood. In a study performed in ZDF rats treated for 14 days with a broad range of bexarotene doses, the authors proposed that the bexarotene-induced dose-dependent hypertriglyceridemia was attributable to elevated VLDL caused by a primary defect in LPL activity mainly in the muscle.26 Another study performed in wild-type and PPARα-deficient mice suggested 2 separate pathways to explain bexarotene action on plasma triglyceride concentrations, a PPARα-dependent pathway by activation of the RXR/PPARα heterodimer for a TG-decreasing effect of bexarotene and a PPARα-independent pathway for the TG-raising effect of bexarotene but without identifying the heterodimer implicated.27 As a previous study performed in our laboratory in the apolipoprotein E2 knock-in mouse model showed a bexarotene-induced hypertriglyceridemia associated with an increase in the hepatic expression of FAS and SCD1, 2 direct LXR-target genes implicated in lipogenesis,6 we assessed whether hypertriglyceridemia induced by the RXR agonist bexarotene could involve the RXR/LXR pathway. Indeed, the heterodimer RXR/LXR is known to be permissive, and the activation of this heterodimer by LXR agonists induces hepatic steatosis and hypertriglyceridemia.13,14 By measuring plasma triglyceride concentrations in wild-type and LXRα/β-deficient mice, we demonstrated that bexarotene-induced hypertriglyceridemia occurred in a dose-dependent manner through the
RXR/LXR pathway. This effect was associated with an upregulation of hepatic lipogenic genes (SREBP1c, FAS, FAE, and SCID1) in a dose- and LXR-dependent manner. The implication of other heterodimers as mediators of the effects of bexarotene in the liver was also analyzed. Bexarotene activation of the permissive heterodimers RXR/PPAR or RXR/FXR was not affected by LXR deficiency because both ACO (RXR/PPAR target gene) and BSEP (RXR/FXR target gene) expression was strongly increased by bexarotene in wild-type and LXRαβ-deficient mice. The potential role of the thyroid hormone axis in the triglyceride response to bexarotene was also dismissed because of the fact that bexarotene decreased DIO1 (RXR/TR target gene) expression to a similar extent in wild-type and LXRαβ-deficient mice. The fact that the RXR/LXR heterodimer mediates bexarotene-induced hypertriglyceridemia suggests that it could also be implicated in hypertriglyceridemia reported in vivo with many other RXR agonists.26–28 It is, however, difficult to extrapolate to other rexinoids because each rexinoid selectively activates its own pattern of heterodimers.29,30 However, LG10506, a new rexinoid shown to selectively activate RXR/PPAR but not RXR/LXR, RXR/RAR, and RXR/FXR heterodimers, has beneficial metabolic effects without inducing hypertriglyceridemia,31 reinforcing the hypothesis that, in case of RXR agonist-induced hypertriglyceridemia, LXR seems to be the main partner implicated.

Notably, the results of this study support a new interesting concept concerning the molecular mechanisms of RXR heterodimer action. Indeed, we identify the existence of heterodimer-specific selective permissivity on target genes of specific metabolic pathways in one given tissue, ie, the liver. The concept of selective permissivity linked to tissue-specific activity has previously been introduced with respect to RXR heterodimers.32 The heterodimer RXR/TR, which was shown to be nonpermissive, displays a permissive activity depending on the cell type.33–35 Conversely, the heterodimer RXR/FXR, shown to be permissive in different assays in vitro, can be antagonized by RXR agonists.32 Taken together, these studies have demonstrated tissue-specific differences in permissivity of certain RXR heterodimers. This concept has been extended in our study to the LXR/RXR heterodimer, because bexarotene treatment increased ABCG5 and ABCG8 mRNA levels in an LXR-dependent manner in the jejunum, but not in the liver, suggesting the existence of distinct molecular mechanisms of LXR target gene regulation in the intestine versus the liver. Moreover, we show that, in liver, RXR heterodimers can also act with a selective permissivity on specific metabolic pathways. Indeed, similar to the LXR agonist T0901317, bexarotene increased the recruitment of RXR on the LXREs of specific LXR-target genes implicated in hepatic lipogenesis (SREBP1c, SCID1, and FAS), leading to their upregulation and hepatic accumulation of triglycerides. Thus, the RXR/LXR heterodimer is permissive for lipogenesis in the liver. By contrast, whereas the LXR agonist induces the recruitment of RXR on the LXREs of hepatic genes encoding proteins implicated in cholesterol homeostasis, leading to a strong increase of their expression in an LXR-dependent manner, the RXR agonist bexarotene only slightly increased the expression of these genes and did not influence liver and plasma cholesterol homeostasis, either in wild-type or in LXRαβ-deficient mice. Taken together, these results demonstrate that the heterodimer RXR/LXR is nonpermissive for cholesterol homeostasis in the liver. Thus, these data show that, in the same organ (ie, the liver in our study), an RXR heterodimer (in this case RXR/LXR) may be permissive for target genes of specific metabolic pathways. These observations reinforce the concept that rexinoids can have molecule-, intertissue-, and intratissue specific effects, which may find application in drug development by synthesizing RXR modulators with appropriate tissue and gene selective profiles for a potential clinical use.

In conclusion, this study shows that the molecular mechanism of bexarotene-induced hypertriglyceridemia is dependent on the RXR/LXR pathway and that in liver the in vivo permissive effect of bexarotene on the RXR/LXR heterodimer is restricted to specific lipogenic LXR-target genes but does not affect LXR-target genes implicated in cholesterol homeostasis. In addition, our study supports the notion of selective permissivity on target genes of specific metabolic pathways for the mode of action of RXR heterodimers.

Acknowledgments
We thank Véronique Touche for excellent technical assistance.

Sources of Funding
This work was supported by grants from the Swedish Research Council and European FP6 STREP project X-TRA-NET (018882), the Foundation Coeur et Artères, the Danish Natural Science Research Council, Conseil Régional Nord-Pas de Calais, and Fond Européen de Développement.

Disclosures
None.

References
Lalloyer et al

Permissive Effects of Bexarotene on RXR/LXR


18. Cha JY, Repa JJ. The liver X receptor (LXR) and hepatic lipogenesis. The


