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Liver X receptor agonist inhibits HIV-1 replication and prevents HIV-induced reduction of plasma HDL in humanized mouse model of HIV infection

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ABSTRACT

HIV-infected subjects are at high risk of developing atherosclerosis, in part due to virus-induced impairment of HDL metabolism. Here, using as a model of HIV infection the NOD.Cg-*Prkdc*^{scid}IL2rg^{tm1Wjl}/SzJ (NSG) mice humanized by human stem cell transplantation, we demonstrate that LXR agonist TO901317 potently reduces viral replication and prevents HIV-induced reduction of plasma HDL. These results establish that humanized mice can be used to investigate the mechanisms of HIV-induced impairment of HDL formation, a major feature of dyslipidemia associated with HIV-1 infection, and show potential benefits of developing LXR agonists for treatment of HIV-associated cardio-vascular disease.

1. Introduction

HIV-infected patients are at increased risk of development of atherosclerosis, in large part due to the suppressive effect of HIV-1 Nef on the cellular cholesterol transporter ABCA1 [1–3]. Therefore, agents that can reverse Nef-mediated impairment of ABCA1 function would be highly beneficial for HIV patients. Agonists of the liver X receptor (LXR) are an example of such agents. LXR α and β isoforms [4] are members of the type II nuclear receptor family that also includes peroxisome proliferator activated receptors (PPAR), farnesoid X receptors (FXR) and the pregnane X receptor (PXR). LXRα is expressed in the liver, intestine, kidney, spleen, and adipose tissue, whereas LXRβ is ubiquitously expressed at a lower level [5]. Both isoforms share almost 80% identity of their aminoacid sequences. The identification of oxidized derivatives of cholesterol as natural ligands for the LXRs indicated the role of these receptors in the regulation of lipid metabolism [6]. Indeed, LXRs activate transcription of cholesterol transporters ABCA1 and ABCG1 leading to enhanced cholesterol efflux from various cell types [7], increase the expression of Niemann-Pick C1 (NPC1) and C2 (NPC2) proteins thus stimulating cholesterol trafficking from lysosomes [8], stimulate fatty acid synthesis in hepatocytes by activating transcription of sterol response element binding protein 1c (SREBP-1c) [9], and regulate expression of lipoprotein remodeling enzymes CETP and PLTP [10]. In addition, LXRs can negatively regulate transcription of some inflammatory genes by a mechanism termed *trans*-repression [11,12].

Both cholesterol-related and *trans*-repression activities of LXR contribute to reported suppression of HIV infection by LXR agonist TO901317: stimulation of cholesterol efflux results in depletion of cholesterol in lipid rafts and assembling virions, leading to reduced viral production and decreased infectivity of nascent virions [3,13], while the *trans*-inhibitory effect is likely responsible for attenuated transcription of the integrated provirus [14]. However, these anti-HIV effects of TO901317 were demonstrated mostly *in vitro*, except of our small study in humanized Rag-hu mice [13]. To extend these studies and determine whether the mouse model can reproduce the adversary effects of HIV-1 infection on reverse cholesterol transport and HDL formation, which put HIV-infected subjects at increased risk of developing atherosclerosis [1,2,15,16], and whether these effects of HIV infection on HDL metabolism can be prevented by LXR agonist, we have chosen to utilize the NOD.Cg-*Prkdc*^{scid}IL2rg^{tm1Wjl}/SzJ (NSG) mice humanized by human stem cell transplantation. This model has been shown to improve the efficiency of xenotransplantation, provide increased levels of engraftment and allow studies of chronic HIV-1 infection in immune grafts that are sustained for the lifetime of the animal [17,18].

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2. Methods

2.1. Ethics statement

All mouse experiments were approved by the George Mason University Institutional Animal Care and Use Committee (IACUC).

2.2. Preparation and infection of humanized mice

The NSG mice were humanized as described previously [17,19]. Briefly, neonates were injected intraperitoneally (i.p.) with 1×10^6 fetal liver derived CD34+ hematopoietic stem cells and were maintained for no less than 16 weeks to allow cell differentiation. Animals exhibiting significant levels of engraftment, as measured by CD45+ and CD4+ cells in the peripheral blood, were then infected i.p. with 100 μl of HIV-1 89.6 at 5 ng p24/μl. The animals were treated or not with 10 mg/kg of TO901317 administered i.p. every second day. This dose proved effective in stimulating reverse cholesterol transport in mice [20]. Given that TO901317 reduces virion-associated cholesterol thus impairing viral fusogenic ability [13], we used fusion inhibitor T20 (3 mg/kg every second day, a practical compromise with the 2 mg/kg dose used to treat HIV-infected patients [21]) as a positive control. Two treatment regimens were tested. In the first, treatment was initiated at the time of infection and continued for two weeks. In the second, we started treating mice two weeks after infection, when viral replication had been established, and continued treatment for one week. At the end of experiment, mice were euthanized and blood was collected for further analysis.

2.3. Triglyceride and HDL analysis

Triglycerides and HDL in the plasma were measured using a colorimetric assay from Wako Diagnostics (Richmond, VA) following manufacturer's instructions.

3. Results and discussion

The use of immunodeficient mice for transplantation of human CD34+ hematopoietic stem cells has been well established and shown to produce mature human lymphoid organs populated with lymphoid and myeloid cells [22,23]. These humanized mouse models produce human CD4+ T-cells which are readily infected by HIV-1 and can exhibit similar phenotypes of T-cell depletion as seen in humans [18,24]. We infected humanized NSG mice with HIV-1 89.6 (a dual-tropic strain using CXCR4 and CCR5 co-receptors [25]) and treated them with LXR agonist TO901317. Treatment was initiated either at the time of infection or two weeks later, when infection had been established.

First, we measured the RT activity in the plasma. As shown in Fig. 1A, treatment with TO901317, when initiated at the time of infection, dramatically reduced the RT activity in the plasma, indicating a potent anti-HIV effect of the drug. The anti-viral effect of TO901317 was even more pronounced than of T20. A similar reduction of HIV replication was observed when treatment was initiated 2 weeks after infection, when viral replication had been established, and continued for 1 week. To get a more quantitative measurement of the drugs' effect on HIV replication, we analyzed viral load in the plasma of infected animals. Consistent with results of the RT analysis, TO901317 reduced the viral load in infected animals by over 1 log, demonstrating a slightly better anti-HIV activity than T20 (Fig. 1B). When treatment was initiated 2 weeks after infection and continued for 1 week, both drugs reduced viral load by about 0.6 log, similar to the effect of RT and integrase inhibitors tested over the same time in humanized mouse model [24]. Taken together, these results confirm potent anti-HIV activity of TO901317 by extending previous observations to a new humanized mouse model.

We next tested the effect of the drugs on lipid metabolism and HDL formation. Despite a previously shown stimulating effect of TO901317 on triglyceride levels in the blood [26], no increase in plasma triglycerides was observed in our experiment (results not shown). This may be due either to rapid clearance of VLDL-TG particles, as proposed by Grefhorst and colleagues who also did not observe increased plasma triglycerides in TO901317-treated mice [20], or to insufficient time for establishing this metabolic change. Interestingly, we also did not detect any triglyceride increase in HIV-infected animals, despite demonstrated raise of triglycerides in HIV-infected subjects [27]. Our failure to observe the HIV-induced increase in triglycerides, a hallmark of the HIV-related dyslipidemia [28], may suggest that hypertriglyceridemia is not induced in this mouse model. Indeed, elevation of plasma TGs is due to a combination of hepatic very low-density lipoprotein (VLDL) overproduction and reduced TG clearance [20,28,29]. Specific mechanisms responsible for these effects of HIV have not been identified, but it is feasible to speculate that these mechanisms might not operate in mice in general or specifically in HIV-infected humanized mice. Alternatively, it is also possible that the short duration of infection in our experiments did not provide sufficient time for development of this metabolic complication.

Analysis of HDL cholesterol demonstrated an expected rise in plasma HDL in uninfected TO901317-treated mice, although this increase did not reach a statistically significant value (Table 1). HDL levels in plasma of T20-treated uninfected mice were insignificantly reduced. A significant decrease of HDL concentration in the plasma was observed in HIV-infected animals. This reduction was not seen in mice treated with T20. Mice treated with TO901317 showed levels of plasma HDL exceeding those in untreated uninfected animals. These results are consistent with the anti-HIV effect of these drugs (see Fig. 1) and with the known activity of TO901317 to stimulate expression of ABCA1, a critical participant in HDL formation [30]. In a 1-week treatment setting, no reversion of HDL levels in the plasma by either of the two drugs was detected (results not shown).

Taken together, these results indicate that the NSG mouse model of HIV infection recapitulates the key effect of HIV-1 infection on lipid metabolism. Specifically, we observed the HIV-induced reduction of plasma HDL concentration, a phenomenon associated with HIV infection in humans and believed to play the key role in putting HIV-infected patients at high risk for developing atherosclerosis and cardio-vascular disease [15,16]. Biogenesis of HDL is a multi-step process initiated by secretion from liver and intestine of cholesterol free apoA-I which is then lipidated to form mature HDL particles. ABCA1 plays the major role in initial steps of apoA-I particle lipidation by transferring cholesterol from peripheral cells to apoA-I. Our previous studies demonstrated that Nef exerts potent inhibitory activity against ABCA1 [1,3]. Using an SIV model of HIV disease, we also showed that Nef released into the bloodstream from infected cells can inhibit ABCA1 activity in uninfected cells, thus explaining the systemic effect of Nef on HDL [1]. Results of the current study provide further support for this model. Indeed, HDL production in humanized mice is mostly determined by mouse cells which are resistant to HIV infection, but are sensitive to Nef-mediated inhibition of ABCA1 activity [3]. Therefore, the proposed mechanism of plasma HDL level reduction in HIV-infected NSG mice likely involves inhibition of ABCA1 activity in peripheral cells by Nef released into the bloodstream.

The most important finding of the current study is that LXR agonist TO901317 potently inhibited HIV-1 replication and restored normal HDL levels. In a short-term (1 week) treatment modality tested here, TO901317 decreased HIV-1 viral load in HIV-infected humanized mice by about 0.6 log, similar to the effect of an

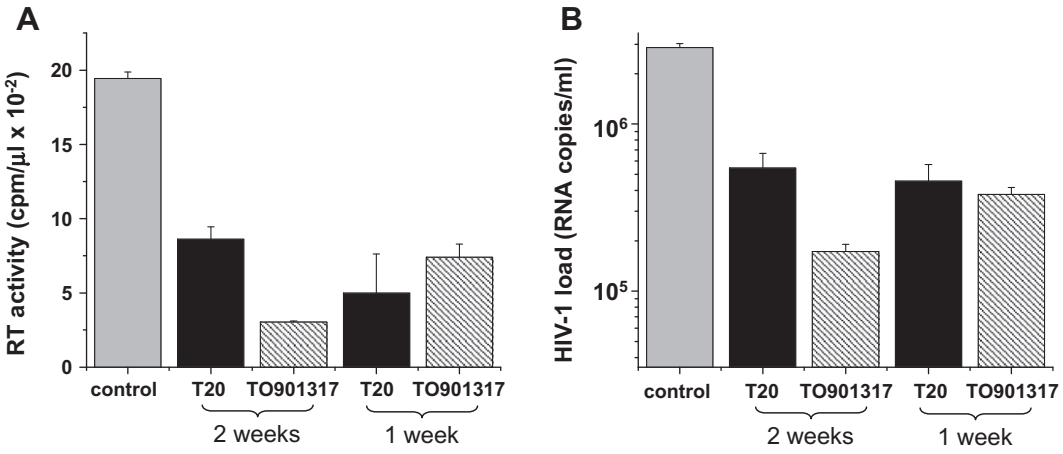

Fig. 1. Analysis of viral replication in humanized NSG mice. Ten NSG mice were infected with HIV-1 89.6 as described in the text. Two were left untreated (control), two were treated with T20 and four treated with TO901317 for 2 weeks starting at the time of infection, two were treated with T20 and two treated with TO901317 for 1 week starting 2 weeks after infection. Virus replication was analyzed 2 weeks (animals treated for 2 weeks) or 3 weeks after infection (animals treated for 1 week) by measuring RT activity (A) or viral load (B) in the plasma.

Table 1
Analysis of HDL in plasma of NSG mice/.

Treatment	Mean ± SD (mg/dl)	*P* value (relative to uninfected, untreated)	*P* value (relative to HIV-infected, untreated)
Uninfected, untreated	31.26 ± 2.06		
Uninfected, TO901317	54.03 ± 8.09	0.061	
Uninfected, T20	26.48 ± 4.78	0.324	
HIV-1, untreated	20.70 ± 2.40	0.042 ↓	
HIV-1, TO901317	40.04 ± 3.12	0.024 ↑	0.002 ↑
HIV-1, T20	32.15 ± 0.71	0.622	0.023 ↑

Statistical analysis was performed using Student's 2-sample *t*-test, and significant differences are marked with an arrow. Six uninfected (two animals per group) and ten HIV-infected (four animals in TO901317-treated group, and two animals per two other groups) NSG mice were treated with indicated drugs for two weeks (treatment of HIV-infected animals was initiated at the time of infection). Each sample was assayed three times.

FDA-approved fusion inhibitor T20. Together with previous findings in a different humanized mouse model (Rag-hu [13]), this result indicates a great potential of LXR agonists as anti-HIV agents. The primary indication for LXR agonists is treatment or management of dyslipidemia and atherosclerosis [31]. Much effort in pre-clinical development of LXR agonists is devoted to identifying compounds with reduced negative side-effects, such as stimulation of lipogenesis and triglyceride production. New generation of LXR agonists, such as *N*,*N*-dimethyl-3β-hydroxy-cholenamide, was shown to exert potent anti-atherogenic activity without increasing hepatic triglyceride levels [32]. Such drugs would be highly beneficial to HIV-positive patients who exhibit altered cholesterol profiles, including significantly reduced HDL levels [28], and are at increased risk of developing atherosclerosis [33]. Treatment of systemic HIV infection with LXR agonists could not only inhibit the progression of infection, but could also have the added benefit of reversing or inhibiting the development of atherosclerosis linked to HIV infection.

An important limitation of our study is the small sample size. Given that variations within the groups were small (in most cases less than 10%), and the effect on HIV replication was similar to the previous study [13], the obtained results should be reproducible. Future studies using this humanized NSG mouse model with larger groups of animals would be needed to validate these findings and provide detailed characterization of the effects of LXR agonists on lipid metabolism.

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