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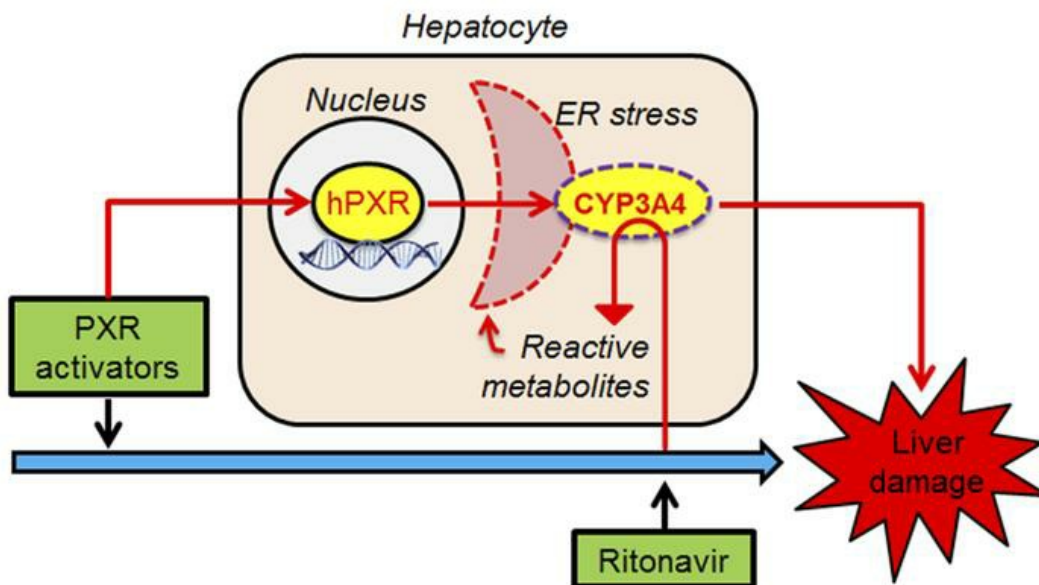
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Concise Communication

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Pregnane X receptor activation potentiates ritonavir hepatotoxicity

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Ritonavir (RTV) is on the World Health Organization's list of essential medicines for antiretroviral therapy, but can cause hepatotoxicity by unknown mechanisms. Multiple clinical studies found that hepatotoxicity occurred in 100% of participants who were pretreated with rifampicin or efavirenz followed by RTV-containing regimens. Both rifampicin and efavirenz are activators of the pregnane X receptor (PXR), a transcription factor with marked interspecies differences in ligand-dependent activation. Using PXR-humanized mouse models, we recapitulated the RTV hepatotoxicity observed in the clinic. PXR was found to modulate RTV hepatotoxicity through CYP3A4-dependent pathways involved in RTV bioactivation, oxidative stress, and endoplasmic reticulum stress. In summary, the current work demonstrated the essential roles of human PXR and CYP3A4 in RTV hepatotoxicity, which can be applied to guide the safe use of RTV-containing regimens in the clinic.

Introduction

With the advent of antiretroviral therapy (ART), the overall mortality and morbidity in HIV/AIDS patients has decreased substantially. However, the toxicity of ART is one of the major concerns of the HIV/AIDS community. A considerable number of patients have poor adherence to ART, in part due to drug toxicity (1), consequently leading to the emergence of drug resistance and/or virologic failure. Ritonavir (RTV) is the backbone of boosted protease inhibitor-based regimens in ART. In early clinical studies, treatment with a full dose of RTV frequently caused liver damage (2, 3). The use of low-dose RTV as a pharmacoenhancer for RTV-containing regimens decreased the overall rate of liver injury. However, RTV is still considered the cause of liver damage during treatment with RTV-containing regimens (4, 5). Because the mechanisms of RTV hepatotoxicity remain elusive, no approach is currently available to predict and prevent such toxicity.

Remarkably, multiple clinical studies found that hepatotoxicity occurred in 100% of participants who were pretreated with rifampicin (RIF) (an antibiotic for treating tuberculosis) or efavirenz (EFV) (a nonnucleoside reverse transcriptase inhibitor for treating HIV) followed by RTV-containing regimens (6–9). We noted that both RIF and EFV are activators of the human pregnane X receptor (PXR), a ligand-dependent transcription factor that is highly expressed in the liver and upregulates drug-metabolizing enzymes, including CYP3A4 (10–14). In addition, CYP3A4 plays a critical role in RTV metabolism and bioactivation (15–20). These data led us to hypothesize that human PXR modulates RTV

hepatotoxicity through CYP3A4-dependent pathways. Genetically engineered PXR- and CYP3A4-humanized mouse models were developed and used to test our hypothesis.

Results and Discussion

This project was initiated from the clinical observations showing hepatotoxicity in subjects pretreated with RIF followed by RTV-containing regimens (Figure 1A) (6–8). We first used WT mice to mimic the hepatotoxicity that occurred in the clinical studies. However, no significant liver injury was found in WT mice pretreated with RIF for 7 days followed by RTV (Figure 1, B and C, and Supplemental Figure 1; supplemental material available online with this article; <https://doi.org/10.1172/JCI128274DS1>). These data suggest interspecies differences between mice and humans in response to RIF and/or RTV. RIF is a human-specific activator of PXR, a ligand-dependent transcription factor highly expressed in the liver, which regulates a gene network including the major hepatic drug-metabolizing enzyme CYP3A4 (11, 14). To overcome the interspecies differences in RIF-mediated PXR activation, we generated a double-transgenic mouse model expressing human PXR and CYP3A4 (hPXR/CYP3A4) on the background of both mouse *Pxr* and *Cyp3a* knockout (Figure 1D). As expected, treatment with RIF induced a panel of PXR target genes, including CYP3A4, in the livers of hPXR/CYP3A4 mice (Supplemental Figure 2), indicating that human PXR is functional in these mice.

Using hPXR/CYP3A4 mice, we recapitulated the RTV hepatotoxicity observed in clinical studies (6–8), as the biomarkers of liver damage were significantly increased in hPXR/CYP3A4 mice pretreated with RIF for 7 days followed by RTV (Figure 1, E and F). In addition, histological analysis revealed massive hepatocyte degeneration in hPXR/CYP3A4 mice pretreated with RIF followed by RTV (Supplemental Figure 3). These data indicate that human PXR is the key mediator of hepatotoxicity caused by lead-in treat-

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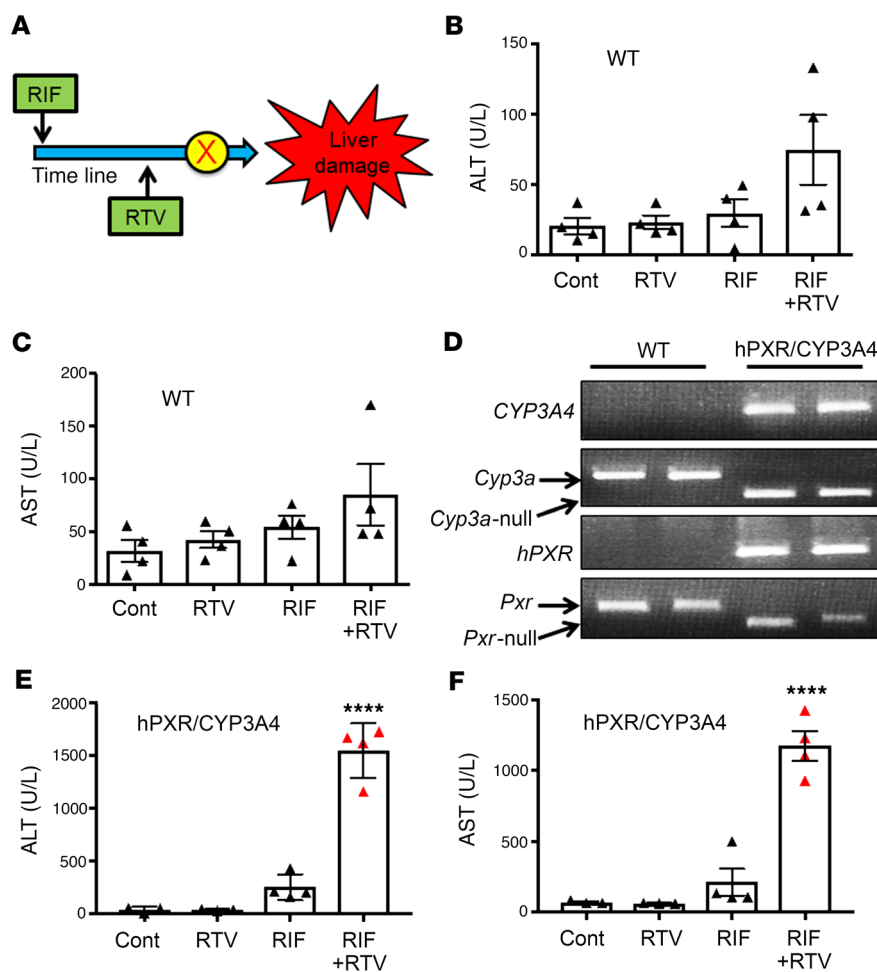


Figure 1. Role of human PXR in the hepatotoxicity cause by pretreatment with RIF followed by RTV. (A) Schematic showing the adverse drug interactions between RIF and RTV in humans that led to the early termination of clinical studies.

(B–F) Evaluation of liver damage in WT and hPXR/CYP3A4 mice pretreated with RIF for 7 days followed by RTV. (B and C) Activities of ALT and AST in the serum of WT mice. (D) Genotyping results of hPXR/CYP3A4 mice, which are positive for human PXR and CYP3A4, but negative for mouse Pxr and Cyp3a. (E and F) Activities of ALT and AST in the serum of hPXR/CYP3A4 mice. All data are shown as mean \pm SEM ($n = 3$ –4). Statistical significance was determined by 1-way ANOVA with Tukey's post hoc test. **** $P < 0.0001$ for RIF+RTV group vs. control, RTV, and RIF groups.

al supplements (26, 27). Hence, clinicians should be mindful of both PXR and CAR activators before starting RTV-containing regimens in HIV/AIDS patients, as they could induce CYP3A4 and predispose patients to the risk of liver injury.

To further verify the role of human PXR and CYP3A4 in RTV hepatotoxicity, the adverse interactions between EFV and RTV were investigated in hPXR/CYP3A4 and hPXR/Cyp3a-null mice. EFV is also a PXR activator (Supplemental Figure 5A). We found that lead-in treatment with EFV for 7 days potentiated RTV hepatotoxicity in hPXR/CYP3A4 mice (Supplemental Figure 5, B–F), which mimicked EFV- and RTV-induced liver injury

observed in a previous clinical study (9). In addition, lead-in treatment with EFV for 7 days followed by RTV, resulted in hepatocyte degeneration (Supplemental Figure 5E), exhibiting the same phenotype as RIF- and RTV-induced liver damage (Supplemental Figure 3). We also noted that RIF had a more significant impact than EFV on RTV hepatotoxicity, as revealed by alanine transaminase (ALT) and aspartate transaminase (AST) values (Figure 1, E and F, and Supplemental Figure 5, B and C), which could be the result of enterohepatic circulation of RIF leading to long-term exposure of liver to RIF (28). Furthermore, we found that the hepatotoxicity associated with lead-in treatment with EFV followed by RTV was CYP3A4 dependent (Supplemental Figure 5, B–F). These data further confirmed the roles of human PXR and CYP3A4 in RTV hepatotoxicity.

ment with RIF followed by RTV. This information is extremely important for the HIV/AIDS community because many prescription drugs and herbal supplements are potent PXR activators that individuals may encounter in daily life (21–24). Therefore, we suggest reviewing whether HIV/AIDS patients are under treatment with PXR activators before starting RTV-containing regimens.

We next explored the pathways downstream of PXR that lead to RTV hepatotoxicity. We hypothesized that PXR modulates RTV hepatotoxicity through CYP3A4-dependent pathways because CYP3A4 is a primary PXR target gene (11, 13, 14) and CYP3A4 plays an important role in RTV metabolism and bioactivation (15–20). To test this hypothesis, we generated a humanized PXR mouse model deficient in *Cyp3a* (hPXR/Cyp3a-null) (Figure 2A). Treatment with RIF for 7 days significantly induced PXR target genes other than *Cyp3a* in the liver of hPXR/Cyp3a-null mice (Figure 2B and Supplemental Figure 4), indicating that PXR is functionally intact in these mice. Compared with hPXR/CYP3A4 mice, no liver injury was observed in hPXR/Cyp3a-null mice pretreated with RIF for 7 days followed by RTV (Figure 2, C–F), indicating that PXR modulates RTV hepatotoxicity through CYP3A4-dependent pathways. These data suggest that CYP3A4 induction should be considered as a risk factor for RTV hepatotoxicity. Apart from PXR, other nuclear receptors, such as constitutive androstane receptor (CAR), also upregulate CYP3A4 expression (25) and many CAR activators are found among prescription drugs (such as phenobarbital) and herb-

We next investigated the pharmacokinetic interactions between RIF and RTV in the liver of hPXR/CYP3A4 and hPXR/Cyp3a-null mice. RTV had no effect on RIF metabolism and disposition (Supplemental Figure 6). However, pretreatment with RIF for 7 days significantly increased the metabolism and bioactivation pathways of RTV in the livers of hPXR/CYP3A4 mice, but not in hPXR/Cyp3a-null mice (Supplemental Figure 7). The major metabolism and bioactivation pathways of RTV were M1 followed by M13 (Supplemental Figure 7, A and B), and both metabolites are formed by CYP3A (20). Compared with the control group, the production of M1 and M13 increased 19- and 7-fold, respectively, in liver microsomes of hPXR/CYP3A4 mice pretreated with RIF (Supplemental Figure 7, C and D).

These data further confirmed the roles of human PXR and CYP3A4 in RTV hepatotoxicity.

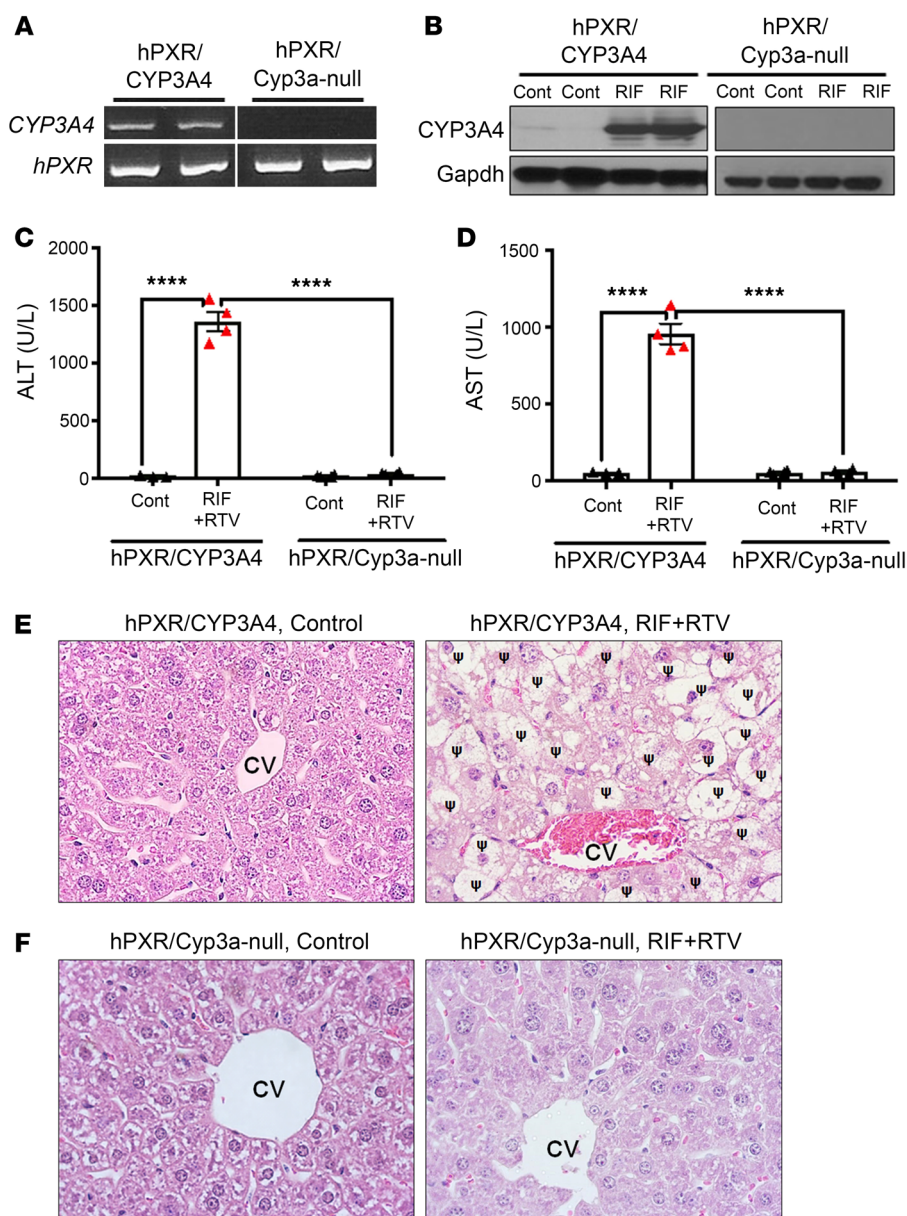


Figure 2. Role of CYP3A4 in the hepatotoxicity caused by pretreatment with RIF followed by RTV. hPXR/CYP3A4 and hPXR/Cyp3a-null mice were pretreated with RIF for 7 days followed by RTV. **(A)** Genotyping results of hPXR/Cyp3a-null mice, which are positive for human PXR, but negative for human CYP3A4. **(B)** Expression of CYP3A4 in the liver of hPXR/CYP3A4 and hPXR/Cyp3a-null mice pretreated with or without PXR ligand RIF for 7 days. Gapdh was used as a loading control. **(C and D)** Activities of ALT and AST in the serum. All data are shown as mean ± SEM. (n = 3-4). Statistical significance was determined by 2-way ANOVA with Tukey's post hoc test. ****P < 0.0001. **(E and F)** Histological analysis of liver samples from control and RIF+RTV groups of hPXR/CYP3A4 and hPXR/Cyp3a-null mice. H&E staining. CV, central vein; ψ indicates hepatocyte degeneration. Original magnification: ×400.

tase 3 (*Cbr3*), and solute carrier family 25 member 24 (*Slc25a24*) (Figure 3, E-G). Similarly to the adverse drug interactions between RIF and RTV, lead-in treatment with the PXR activator EFV followed by RTV also caused oxidative stress in the livers of hPXR/CYP3A4 mice, but not in hPXR/Cyp3a-null mice (Supplemental Figure 8). These data indicate that pretreatment with PXR activators followed by RTV causes oxidative stress in the liver, which is dependent on CYP3A4.

Oxidative stress can lead to endoplasmic reticulum (ER) stress (34). Indeed, we found that the ER is a target organelle of RTV hepatotoxicity, as electron-microscopic analysis revealed massive ER dilation in hepatocytes of hPXR/CYP3A4 mice pretreated with RIF for 7 days followed by RTV (Figure 4A). In addition, lead-in treatment with RIF

Accompanied with M1, 2-isopropylthiazole-4-carbaldehyde (M1-1) was produced, which can be further metabolized to form an adduct with glutathione (GSH) (20). In addition, M13 and 2-methylpropanethioamide (M13-1) were isopropylthiazole ring-open metabolites of RTV. The ring-open metabolites of thiazole derivatives can be further oxidized and result in liver injury (29, 30). These data indicate that PXR-mediated CYP3A4 induction increases RTV bioactivation in the liver, which can potentially lead to liver damage.

CYP-mediated drug bioactivation can cause cellular stress (31, 32). Using a metabolomic approach, we found a dramatic increase in ophthalmic acid (OA), a biomarker of oxidative stress (33), in the livers of hPXR/CYP3A4 mice pretreated with RIF for 7 days followed by RTV, but not in hPXR/Cyp3a-null mice with the same treatment (Figure 3, A-D). In addition, pretreatment with RIF followed by RTV in hPXR/CYP3A4 mice caused upregulation of mRNAs encoded by genes that are involved in cellular responses to oxidative stress in the liver, including glutathione peroxidase 2 (*Gpx2*), carbonyl reduc-

tionase 3 (*Cbr3*), and solute carrier family 25 member 24 (*Slc25a24*) (Figure 3, E-G). Similarly to the adverse drug interactions between RIF and RTV, lead-in treatment with the PXR activator EFV followed by RTV also caused oxidative stress in the livers of hPXR/CYP3A4 mice, but not in hPXR/Cyp3a-null mice (Supplemental Figure 8). These data indicate that pretreatment with PXR activators followed by RTV causes oxidative stress in the liver, which is dependent on CYP3A4. Oxidative stress can lead to endoplasmic reticulum (ER) stress (34). Indeed, we found that the ER is a target organelle of RTV hepatotoxicity, as electron-microscopic analysis revealed massive ER dilation in hepatocytes of hPXR/CYP3A4 mice pretreated with RIF for 7 days followed by RTV (Figure 4A). In addition, lead-in treatment with RIF followed by RTV caused severe ER stress in the livers of hPXR/CYP3A4 mice, as indicated by increased expression of ER stress biomarker mRNAs, including C/EBP homologous protein (*Chop*), binding immunoglobulin protein (*Bip*), and cyclic AMP-dependent transcription factor 3 (*Atf3*) (Figure 4, B and C). ER stress also occurred in the livers of hPXR/CYP3A4 mice pretreated with EFV for 7 days followed by RTV, but not in hPXR/Cyp3a-null mice with the same treatment (Supplemental Figure 9). Persistent ER stress can lead to cell death (34). Concordantly, we observed a significant increase in the expression of mRNAs encoded by genes associated with cell death and tissue injury, including death receptor 5 (*Dr5*), BCL2-associated X (*Bax*), and monocyte chemoattractant protein 1 (*Mcp1*) in the liver of hPXR/CYP3A4 mice pretreated with RIF for 7 days followed by RTV, but not in hPXR/Cyp3a-null mice (Figure 4, D-F). These data suggest that lead-in treatment with PXR activators followed by RTV causes ER stress and hepatocellular injury and that it is CYP3A4 dependent.

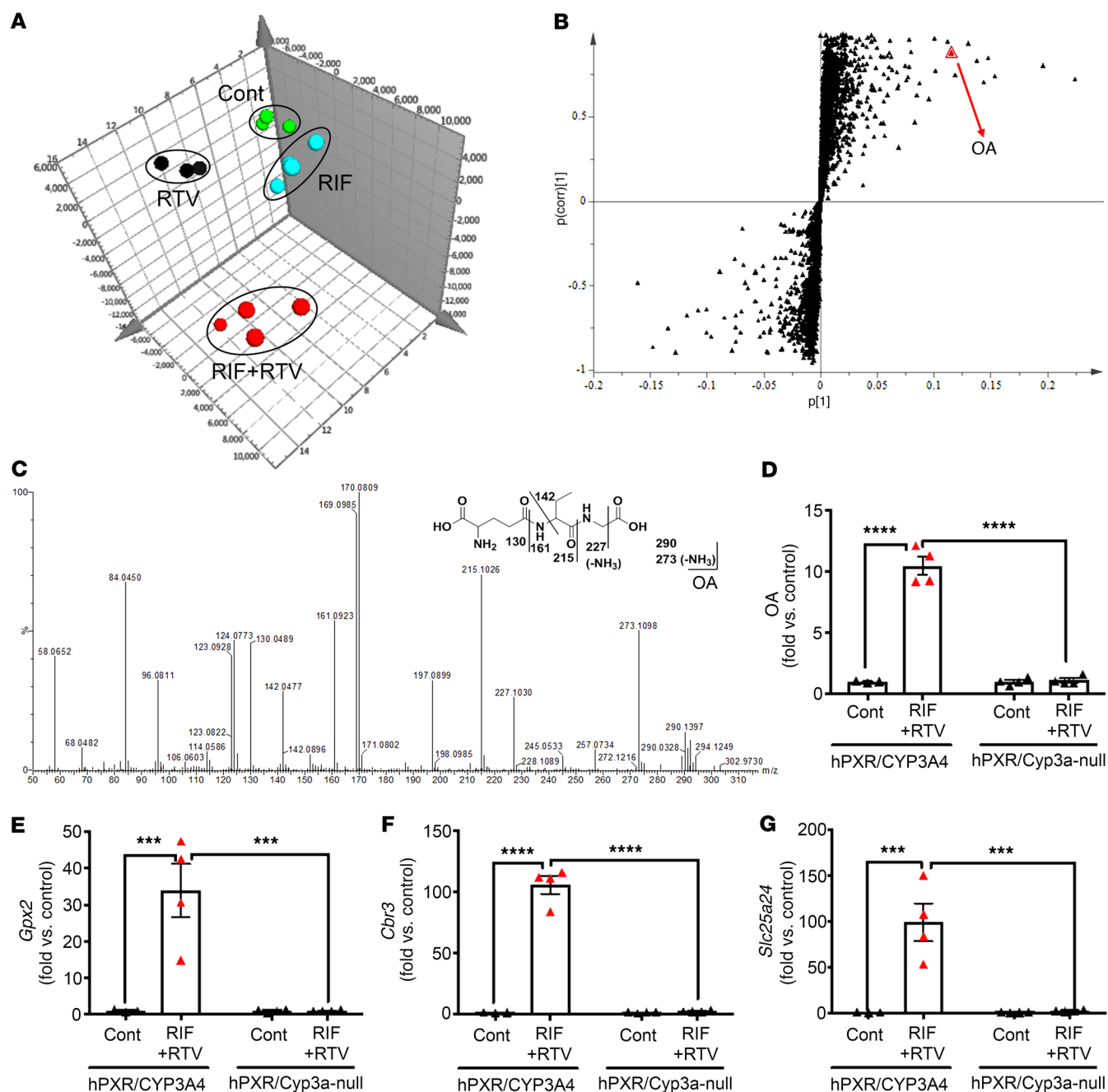


Figure 3. Metabolomics reveals oxidative stress in the liver of hPXR/CYP3A4 mice pretreated with RIF for 7 days followed by RTV. Liver samples were analyzed by UPLC-QTOFMS. **(A)** Principal component analysis (PCA) of liver samples from control, RIF, RTV, and RIF/RTV groups of hPXR/CYP3A4 mice. **(B)** Loading S plots generated by orthogonal projections to latent structures discriminant analysis (OPLS-DA) analysis of liver samples. The x axis is a measure of the relative abundance of ions, and the y axis is a measure of the correlation of each ion to the model. OA, a biomarker of oxidative stress, was identified as a top-ranking ion in the RIF+RTV group. **(C)** Structural illustration of OA by tandem mass spectrometry (MS/MS) fragmental analysis. **(D)** Relative quantification of OA in the liver of hPXR/CYP3A4 and hPXR/Cyp3a-null mice. **(E–G)** The expressions of genes related to oxidative stress. *Gpx2* **(E)**, *Cbr3* **(F)**, and *Slc25a24* **(G)** mRNAs were analyzed by quantitative PCR (qPCR). All data are shown as mean \pm SEM. ($n = 3-4$). Statistical significance was determined by 2-way ANOVA with Tukey's post hoc test. The data in the control group of hPXR/CYP3A4 mice were set as 1. **** $P < 0.0001$; *** $P < 0.001$.

The ER is critical for protein maturation, including post-translational modification and proper folding (34). The activation of PXR, being a transcription factor, by ligands such as RIF and EFV upregulates a network of genes, including CYP3A4, and thus increases the workload of the ER for protein maturation and processing. On the other hand, CYP3A4 is located in the ER, and

PXR-mediated CYP3A4 induction increases the production of RTV-reactive metabolites that can directly target the ER, leading to ER stress and hepatocellular injury (Figure 4G).

In summary, the current study demonstrated the essential roles of human PXR and CYP3A4 in RTV hepatotoxicity. These results can be used to develop novel strategies based upon PXR,

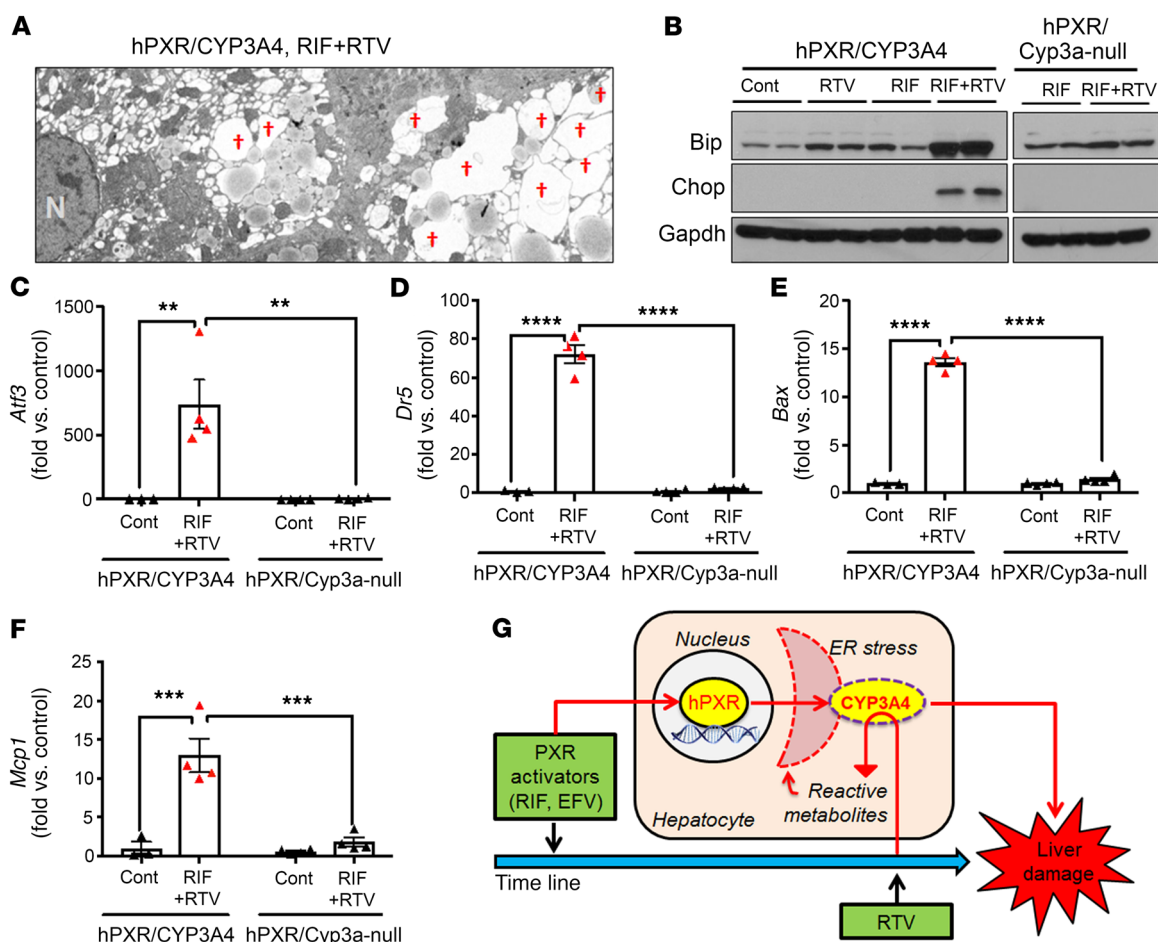


Figure 4. ER is a target organelle in the hepatotoxicity associated with RIF pretreatment for 7 days followed by RTV. (A) Electron microscopic examination of the liver in hPXR/CYP3A4 mice pretreated with RIF followed by RTV. N, nucleus; †, dilated ER. Original magnification, ×4000. (B and C) ER stress in the liver of hPXR/CYP3A4 and hPXR/Cyp3a-null mice. Bip, Chop, and Atf3 were used as biomarkers of ER stress. Bip and Chop were analyzed by Western blotting. Gapdh was used as a loading control. Atf3 mRNA was analyzed by qPCR. (D–F) Expression of genes related to cell death and tissue injury in the liver of hPXR/CYP3A4 and hPXR/Cyp3a-null mice. Dr5 (D), Bax (E), and Mcp1 (F) mRNAs were analyzed by qPCR. All data are shown as mean ± SEM. (n = 3–4). The data in control groups of hPXR/CYP3A4 mice were set as 1. Statistical significance was determined by 2-way ANOVA with Tukey’s post hoc test. **P < 0.01; ***P < 0.001; ****P < 0.0001. (G) Schematic representation for the roles of human PXR and CYP3A4 in modulating RTV hepatotoxicity: (i) ligand-dependent activation of PXR upregulates CYP3A4 expression; (ii) overexpressed CYP3A4 is located in the ER; (iii) CYP3A4 catalyzes RTV to produce reactive metabolites; and (iv) ER is exposed to a high level of reactive metabolites of RTV, which leads to ER stress and hepatocellular injury.

CYP3A4, and their downstream pathways to improve the safety profile of RTV-containing regimens in the clinic.

Methods

Details of experimental procedures are provided in the Supplemental Methods. Primers are listed in Supplemental Table 1.

Statistics. GraphPad Prism 7.0 (GraphPad Software) was used for statistical analysis. All data are shown as mean ± SEM. One-way or 2-way ANOVA was used with Tukey’s post hoc test to compare the differences among multiple groups. Two-tailed unpaired Student’s *t* test was performed for statistical analysis between 2 groups. *P* value < 0.05 was considered statistically significant.

Study approval. All mice were handled in accordance with study protocols approved by the University of Pittsburgh Animal Care and Use Committee.

Author contributions

XM and AIS conceived the project and wrote the manuscript. AIS,

JL, PW, JZ, YW, and XM performed the experiments. DY, WX, FJG, and XM contributed to the new reagents, analytic tools, and animal models. XM, WX, FJG, and DM contributed to the scientific discussion and experimental design.

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1. d'Arminio Monforte A, et al. Insights into the reasons for discontinuation of the first highly active antiretroviral therapy (HAART) regimen in a cohort of antiretroviral naïve patients. I.CO.N.A. Study Group. Italian Cohort of Antiretroviral-Naïve Patients. *AIDS*. 2000;14(5):499–507.
2. Sulkowski MS. Hepatotoxicity associated with antiretroviral therapy containing HIV-1 protease inhibitors. *Semin Liver Dis*. 2003;23(2):183–194.
3. Sulkowski MS, Thomas DL, Chaisson RE, Moore RD. Hepatotoxicity associated with antiretroviral therapy in adults infected with human immunodeficiency virus and the role of hepatitis C or B virus infection. *JAMA*. 2000;283(1):74–80.
4. Duval X, et al. Incidence of and risk factors for adverse drug reactions in a prospective cohort of HIV-infected adults initiating protease inhibitor-containing therapy. *Clin Infect Dis*. 2004;39(2):248–255.
5. Cooper CL, van Heeswijk RP, Gallicano K, Cameron DW. A review of low-dose ritonavir in protease inhibitor combination therapy. *Clin Infect Dis*. 2003;36(12):1585–1592.
6. Nijland HM, et al. High incidence of adverse events in healthy volunteers receiving rifampicin and adjusted doses of lopinavir/ritonavir tablets. *AIDS*. 2008;22(8):931–935.
7. Haas DW, et al. Hepatotoxicity and gastrointestinal intolerance when healthy volunteers taking rifampin add twice-daily atazanavir and ritonavir. *J Acquir Immune Defic Syndr*. 2009;50(3):290–293.
8. Schmitt C, Riek M, Winters K, Schutz M, Grange S. Unexpected hepatotoxicity of rifampin and saquinavir/ritonavir in healthy male volunteers. *Arch Drug Inf*. 2009;2(1):8–16.
9. Jamois C, Riek M, Schmitt C. Potential hepatotoxicity of efavirenz and saquinavir/ritonavir coadministration in healthy volunteers. *Arch Drug Inf*. 2009;2(1):1–7.
10. Sharma D, Lau AJ, Sherman MA, Chang TK. Agonism of human pregnane X receptor by rilpivirine and etravirine: comparison with first generation non-nucleoside reverse transcriptase inhibitors. *Biochem Pharmacol*. 2013;85(11):1700–1711.
11. Lehmann JM, McKee DD, Watson MA, Willson TM, Moore JT, Kliewer SA. The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J Clin Invest*. 1998;102(5):1016–1023.
12. Kliewer SA, et al. An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell*. 1998;92(1):73–82.
13. Blumberg B, Evans RM. Orphan nuclear receptors--new ligands and new possibilities. *Genes Dev*. 1998;12(20):3149–3155.
14. Bertilsson G, et al. Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. *Proc Natl Acad Sci USA*. 1998;95(21):12208–12213.
15. Kumar GN, Rodrigues AD, Buko AM, Denissen JF. Cytochrome P450-mediated metabolism of the HIV-1 protease inhibitor ritonavir (ABT-538) in human liver microsomes. *J Pharmacol Exp Ther*. 1996;277(1):423–431.
16. Denissen JF, et al. Metabolism and disposition of the HIV-1 protease inhibitor ritonavir (ABT-538) in rats, dogs, and humans. *Drug Metab Dispos*. 1997;25(4):489–501.
17. Koudriakova T, et al. Metabolism of the human immunodeficiency virus protease inhibitors indinavir and ritonavir by human intestinal microsomes and expressed cytochrome P4503A4/3A5: mechanism-based inactivation of cytochrome P4503A by ritonavir. *Drug Metab Dispos*. 1998;26(6):552–561.
18. Gangl E, Utkin I, Gerber N, Vouros P. Structural elucidation of metabolites of ritonavir and indinavir by liquid chromatography-mass spectrometry. *J Chromatogr A*. 2002;974(1-2):91–101.
19. Yao M, Ma L, Humphreys WG, Zhu M. Rapid screening and characterization of drug metabolites using a multiple ion monitoring-dependent MS/MS acquisition method on a hybrid triple quadrupole-linear ion trap mass spectrometer. *J Mass Spectrom*. 2008;43(10):1364–1375.
20. Li F, Lu J, Ma X. Metabolomic screening and identification of the bioactivation pathways of ritonavir. *Chem Res Toxicol*. 2011;24(12):2109–2114.
21. Honkakoski P, Sueyoshi T, Negishi M. Drug-activated nuclear receptors CAR and PXR. *Ann Med*. 2003;35(3):172–182.
22. Wang L, et al. The Chinese herbal medicine *Sophora flavescens* activates pregnane X receptor. *Drug Metab Dispos*. 2010;38(12):2226–2231.
23. Satsu H, Hiura Y, Mochizuki K, Hamada M, Shimizu M. Activation of pregnane X receptor and induction of MDR1 by dietary phytochemicals. *J Agric Food Chem*. 2008;56(13):5366–5373.
24. Mills E, Montori V, Perri D, Phillips E, Koren G. Natural health product-HIV drug interactions: a systematic review. *Int J STD AIDS*. 2005;16(3):181–186.
25. Pascucci JM, Gerbal-Chaloin S, Drocourt L, Marel P, Vilarem MJ. The expression of CYP2B6, CYP2C9 and CYP3A4 genes: a tangle of networks of nuclear and steroid receptors. *Biochim Biophys Acta*. 2003;1619(3):243–253.
26. Qatanani M, Moore DD. CAR, the continuously advancing receptor, in drug metabolism and disease. *Curr Drug Metab*. 2005;6(4):329–339.
27. Huang W, Zhang J, Moore DD. A traditional herbal medicine enhances bilirubin clearance by activating the nuclear receptor CAR. *J Clin Invest*. 2004;113(1):137–143.
28. Malik MY, Jaiswal S, Sharma A, Shukla M, Lal J. Role of enterohepatic recirculation in drug disposition: cooperation and complications. *Drug Metab Rev*. 2016;48(2):281–327.
29. Ji T, Ikehata K, Koen YM, Esch SW, Williams TD, Hanzlik RP. Covalent modification of microsomal lipids by thiobenzamide metabolites in vivo. *Chem Res Toxicol*. 2007;20(4):701–708.
30. Mizutani T, Suzuki K. Relative hepatotoxicity of 2-(substituted phenyl)thiazoles and substituted thiobenzamides in mice: evidence for the involvement of thiobenzamides as ring cleavage metabolites in the hepatotoxicity of 2-phenylthiazoles. *Toxicol Lett*. 1996;85(2):101–105.
31. Park BK, Kitteringham NR, Maggs JL, Pirmohamed M, Williams DP. The role of metabolic activation in drug-induced hepatotoxicity. *Annu Rev Pharmacol Toxicol*. 2005;45:177–202.
32. Stepan AF, et al. Structural alert/reactive metabolite concept as applied in medicinal chemistry to mitigate the risk of idiosyncratic drug toxicity: a perspective based on the critical examination of trends in the top 200 drugs marketed in the United States. *Chem Res Toxicol*. 2011;24(9):1345–1410.
33. Soga T, et al. Differential metabolomics reveals ophthalmic acid as an oxidative stress biomarker indicating hepatic glutathione consumption. *J Biol Chem*. 2006;281(24):16768–16776.
34. Malhotra JD, Kaufman RJ. Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double-edged sword? *Antioxid Redox Signal*. 2007;9(12):2277–2293.