Pregnane X Receptor Activator Rifampin Increases Blood Pressure and Stimulates Plasma Renin Activity

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We conducted a clinical trial with 22 healthy volunteers to investigate the effects of pregnane X receptor (PXR) agonist rifampin on blood pressure (BP). The study was randomized, crossover, single-blind, and placebo-controlled. Rifampin 600 mg or placebo once daily was administered for a week and the 24-hour ambulatory BP was monitored at the end of each arm on the eighth day. Rifampin elevated the mean systolic and diastolic 24-hour BP (4.7 mmHg, \( P < 0.0001 \), and 3.0 mmHg, \( P < 0.001 \), respectively) as well as the mean heart rate (3.5 bpm, \( P = 0.038 \)). The serum renin concentration and the plasma renin activity were increased. Although rifampin increased circulating \( 4\beta \)-hydroxycholesterol (4\( \beta \)HC) as expected, the plasma 4\( \beta \)HC concentration strongly negatively correlated with 24-hour BP, especially systolic, in both rifampin and placebo arms (rifampin systolic BP, \( r = −0.69, P < 0.001 \); placebo systolic BP, \( r = −0.70, P < 0.001 \)). The 4\( \beta \)HC, an agonist for liver X receptor (LXR), induced renin expression modestly in LXR-\( \alpha \) expressing Calu-6 cells but only at unphysiologically high 4\( \beta \)HC concentrations. In conclusion, rifampin stimulates renin activity and has a hypertensive effect. This finding should be considered when designing interaction studies involving rifampin or other PXR agonists. Furthermore, PXR may represent a putative therapeutic target for the treatment of hypertension.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Pregnane X receptor (PXR) is a major regulator of drug and bile acid metabolism. Preclinical studies have suggested that PXR is involved in the regulation of blood pressure (BP).

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ We investigated the effects of rifampin, a prototypical PXR agonist, on 24-hour ambulatory BP without interfering diseases and other medications. No previous trial has reported the effects of PXR agonism on BP regulation in humans.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Rifampin elevated the 24-hour ambulatory systolic and diastolic BP and heart rate in healthy volunteers. Serum renin concentration and plasma renin activity were increased. Plasma 4\( \beta \)-hydroxycholesterol, an index of CYP3A activity and a liver X receptor agonist, was strongly negatively correlated with 24-hour BP.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ Increase of BP is a novel adverse effect of rifampin. This should be considered when designing interaction studies with rifampin. If the future studies confirm the role of PXR in human BP regulation, therapies targeting PXR-mediated pathways may provide new treatment options for hypertension.

Pregnane X receptor (PXR; NR1I2) is a ligand-activated transcription factor belonging to the nuclear hormone receptor superfamily. PXR was originally identified as a xenobiotic sensor that regulates xenobiotic metabolism, including enzymes in the cytochrome P450 (CYP) family, various conjugating enzymes, as well as transporters in the ATP-binding cassette family.1–3 It is predominantly expressed in the liver and intestine.1 Due to the structural properties of its ligand-binding domain, it accepts a wide variety of compounds as ligands, including clinically used medications, such as glucocorticoids, anti-epileptics, and statins, as well as various environmental contaminants and pesticides.4,5 Accumulating evidence during the last decade has revealed much

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The induction of CYP3A4, the most important target gene of PXR, can be assessed by analyzing the serum/plasma concentration of 4β-hydroxycholesterol (4βHC), an oxysterol metabolite of cholesterol. 

The concentration of 4βHC can be increased by up to 20-fold by PXR activators, such as enzyme-inducing anti-epileptics. 

As many as other oxysterols, 4βHC is a ligand for liver X receptors (LXRs). Both LXR-α (NR1H3) and LXR-β (NR1H2) are activated by 4βHC to a similar degree. LXR-α is expressed in the liver, intestine, kidneys, adipose tissue, adrenals, and macrophages, whereas LXR-β is expressed ubiquitously. 

The activation of LXR leads to upregulation of lipogenesis in the liver, which may result in hepatic steatosis, whereas the induction of cholesterol efflux transporters, such as ATP-binding cassette A1, in peripheral tissues may reduce atherosclerosis. 

The role of 4βHC in the regulation of metabolism in general, and in the regulation of LXRs targets specifically, is yet to be explored.

Rifampin, an antibiotic used in the treatment of tuberculosis, is a well-established ligand for human PXR and commonly used as a prototypical PXR-activating compound. We have previously reported that 1-week rifampin dosing leads to the elevation of glucose levels during oral glucose tolerance test in a randomized, crossover, placebo-controlled study in healthy volunteers, perhaps via the suppression of glucose transporter 2 expression. 

Office blood pressure (BP) measurements were performed during the study as a safety measure, and we noticed that rifampin seemed to increase systolic and diastolic BP as well as the heart rate. After 1 week of rifampin dosing, the systolic BP (mean 117.2 ± 7.8 vs. 112.4 ± 7.7 mmHg; P = 0.027), diastolic BP (65.5 ± 5.2 vs. 62.0 ± 5.6 mmHg; P = 0.020), and heart rate (67.7 ± 9.3 vs. 62.7 ± 9.3 mmHg; P = 0.042) were higher than after the placebo arm.

Although rifampin has been reported to compromise the effect of antihypertensive drugs, no study has so far reported BP elevating effects of rifampin treatment as such without concomitant medications. Based on our preliminary finding, we hypothesized that PXR activation by rifampin could have a BP elevating effect. Because LXR is known to regulate renin expression in mice, we also hypothesized that PXR activation and the resultant increased circulating 4βHC may modulate renin expression through LXR activation. Renin is secreted from the juxtaglomerular cells in the vicinity of the afferent arterioles of the kidneys, and it is an integral part of the renin-angiotensin-aldosterone system regulating BP and fluid and electrolyte balance. Both the transcription and the secretion are important steps in the regulation of circulating active renin.

In the current study, we set out to investigate the effect of rifampin on human ambulatory BP and BP-regulating hormones with a randomized, single-blind, placebo-controlled, crossover design in healthy volunteers. Furthermore, the effect of 4βHC on renin expression was studied in vitro.

**METHODS**

**Subjects**

Healthy volunteers, aged 18–40 years, were recruited for the study. Inclusion criteria were systolic BP between 95 and 140 mmHg and body mass index of 19–30 kg/m². The exclusion criteria included diastolic pressure > 90 mmHg, any continuous medication (including oral contraceptives), history of significant disease, including any liver disease (as judged by the study physician on the basis of history, physical examination, and basic laboratory values), insensitivity to rifampin, pregnancy, and breast feeding, continuous use of soft contact lenses (rifampin may color), history of difficult venipuncture, drug and alcohol abuse, and participation in any other pharmaceutical trial within 1 month.

Written, informed consent was obtained from each subject. The study was approved by the Ethics Committee of the Northern Ostrobotnia Hospital District (Oulu, Finland) and the Finnish Medicines Agency. The study procedures performed were in accordance with the ethical standards of the Helsinki Declaration. The subjects were compensated financially for their participation in the study. The number of subjects (n = 22) was based on a power analysis to detect an effect of 4.079 mmHg in the 24-hour systolic BP based on the assumption that the SD of the difference in the response variable is 6.5 with α = 0.05 and β = 0.2. This trial was registered at ClinicalTrials.gov under the name “The Effect of PXR Activation on Blood Pressure Regulation” as NCT01690104.

**Experimental protocol of the volunteer study**

This study had a randomized single-blind (study personnel were blinded), placebo-controlled, and cross-over design. Subjects of the study were not aware of the hypothesis (BP was expected to rise) but they were not blinded because rifampin colors urine red rendering blinding impossible. The sequence (i.e., rifampin or placebo period first) of the two arms was randomized by the pharmacy of the Oulu University Hospital. After randomization was performed in a block of 22 and, due to miscommunication with the pharmacy, the replacement of 2 withdrawn subjects was unbalanced leading to 10 subjects in the rifampin arm first and 12 subjects in the placebo arm first. Subjects received 600 mg rifampin (RIMAPEN; Orion, Espoo, Finland) or placebo daily for a week with at least a 4-week washout period. The study was conducted on an outpatient basis, and each subject visited the Research Unit of Internal Medicine in the Oulu University Hospital two times in each arm. At the start of each arm, the first rifampin or placebo tablet was administered under the supervision of a study nurse ensuring that the blinding of the nurse was not broken; the subsequent daily doses were taken by the subjects at home between 4 and 8 pm at least 1 hour before a meal or 2 hours after a meal, at the subjects’ convenience. The subjects were instructed to not reveal the assigned treatment to study personnel. To monitor the compliance to drug regimen, the volunteers wrote the date and time of each dose taken in a medication diary, and the participants were required to return the used medication containers. In addition, plasma 4βHC was measured to monitor the compliance to rifampin dosing. The participants were asked to abstain from the use of alcohol, licorice-containing sweets and other products, salty snacks, such as potato chips and salted nuts, energy drinks, over-the-counter-medications, and dietary and herbal supplements during the study arms. The subjects were asked to continue their regular coffee drinking and smoking habits with no significant changes during the study to avoid withdrawal symptoms. Otherwise the subjects consumed their regular diets. The dietary instructions were given by a registered diettian.

Ambulatory BP was measured with a 24-hour BP monitoring device Oscar2 (AccuWin PRO version 3 software; SunTech Medical, Morrisville, NC) at the end of each arm on the eighth day for about 24 hours ending on the morning of day 9. The subjects were instructed...
to start the measurements between 7 and 8 AM and end them the same time the next morning. The measurements were automatically performed with 30-minute intervals during daytime and with 60 minutes interval at night (23 PM to 7 AM). The BP values measured by the device and reported by the software were included in the analysis; the software omitted few values with reported reasons such as “Airway obstructed” and “Measurement timeout.” One measurement with unrealistically high BP values was excluded by the study staff (BP 411/162 measured during “a really hectic removal of a tick” from a pet dog). Blood samples were collected for analysis on the morning of the ninth day of each arm when the subjects visited the Research Unit. The office BP was measured twice with 5-minute intervals and the average of the two measurements was calculated. The participants were advised to avoid strenuous exercise during the 2 days before and on the day of the ambulatory BP measurement (between day 6 and the morning of day 9).

**Analytic methods**

The clinical laboratory analyses were performed by the Clinical Laboratory of Oulu University Hospital (NordLab, Oulu, Finland) and were validated for clinical use. Plasma renin activity was measured with radioimmunoassay and serum aldosterone was measured with chemiluminescent immunoassay. The photometric enzymatic method was used for plasma glucose and creatinine, and the indirect ion selective electrode method for plasma sodium and potassium measurements.

The following enzyme-linked immunosorbent assay (ELISA) kits were utilized according to the instructions of the their manufacturers: serum renin (Human Renin ELISA Kit RIS004R; BioVendor, Brno, Czech Republic), serum prorenin (Human Prorenin ELISA Kit RM1001R; Biovendor), 20-HETE (20 HETE/ beta glucuronidase ELISA Kit ab175818; Abcam PLC, Cambridge, UK), and endoglin (Human Endoglin/CD105 Quantikine ELISA Kit DNDG00; R&D Systems, Minneapolis, MN). The concentrations of 4HC in plasma and 4βHC and 4αHC in plasma were quantified simultaneously with the ultrahigh performance liquid chromatography coupled with high resolution mass spectrometry method by Admescope (Oulu, Finland).

**Establishment of LXR-α overexpressing Calu-6 cell line (Calu-6-LXR-α)**

The Calu-6 (human pulmonary adenocarcinoma cell line; ATCC; LGC Standards, Teddington, UK), was utilized in the study because it is the only human cell line known to express renin. To produce LXR-α overexpressing Calu-6 cell line, Calu-6 cells were transfected with human LXR-α expression plasmid constructed in pCMV6-Entry plasmid (Origene No: RC223767; Rockville, MD). Transfection was performed with FuGENE HD reagent according to the manufacturer’s protocol (Promega, Madison, WI). After this, the cells were maintained under geneticin (Gibco, ThermoFisher Scientific, Waltham, MA) selection. After 2 weeks, the surviving cells were shorted with the BD FACs Aria III (BD Biosciences, San Jose, CA) to individual wells to form uniform clones. The best clone based on the human LXR-α mRNA expression was selected for further experiments and named Calu-6-LXR-α.

**Treatment of cells with LXR ligands**

Calu-6 and Calu-6-LXR-α cells were cultured in MEM Eagle medium (Sigma-Aldrich, St. Louis, MO) supplemented with L-glutamine (Sigma) and sodium pyruvate (Sigma) at standard conditions at 37°C and 5% CO₂. The cells were treated with ligands 20 and 40 µM 4βHC and 20 µM 22(R)-HC (both from Avanti Polar Lipids, Alabaster, AL) dissolved in ethanol; 10 µM T0901317 and 5 µM GW966 (both from Sigma) dissolved in dimethyl sulfoxide in serum-free medium for 48 hours. At the end of the experiment, the cells were collected and subjected to RNA extraction.

**RNA extraction and quantitative real-time polymerase chain reaction**

Total RNA was isolated using RNAzol RT (Sigma) according to the manufacturer’s protocol. RNA was reverse transcribed using p(dN)6 random primers (Roche Diagnostics, Mannheim, Germany) and M-MLV reverse transcriptase (Promega) to produce complementary DNA. The quantitative real-time polymerase chain reaction (qRT-PCR) reactions were performed using FastStart Universal SYBRGreen Master Mix (Roche) or TaqMan Gene Expression Master Mix (Applied Biosystems, Foster City, CA). The primer sequences and Taqman assays are presented in Table S1. Fluorescence values of the qRT-PCR products were corrected with the fluorescence signals of the passive reference dye (ROX; Roche). The RNA levels of target genes were normalized against the 18S control levels using the comparative CT method.

**Statistical analysis**

The BP indexes were compared across treatments by two-tailed paired Student’s t-test and laboratory values by Wilcoxon signed-rank test. The Pearson correlation coefficients were calculated to analyze the correlations between BP and oxysterol concentrations. \( P < 0.05 \) was considered statistically significant. The statistics for the cell culture experiments was calculated using one-way analysis of variance and Tukey’s post hoc test.

**RESULTS**

**Subjects**

Twenty-two healthy volunteers (14 men and 8 women) completed the trial. The mean age of the subjects was 24.3 years (SD ± 3.5; range 20–37 years), the mean weight 71.5 kg (SD ± 10.9; range 52–92 kg), and the mean body mass index 23.8 kg/m² (SD ± 2.2; range 20.7–29.2 kg/m²). All the participants were white. There were two dropouts; one withdrew due to pregnancy between the study arms and another had heartburn and flatulence during the rifampin arm.

**The effect of rifampin on the ambulatory BP**

We administered healthy volunteers with rifampin 600 mg a day or placebo for a week, and 24-hour ambulatory and office BPs were monitored at the end of each arm on the eighth day. The mean of successful BP measurements performed per subject was 39 on both arms (rifampin arm SD ± 1.9, range 32–41; placebo arm SD ± 2.6, range 33–43).

The mean 24-hour systolic BP was elevated by 4.7 mmHg \((P < 0.0001)\) by rifampin (Figure 1 and Table 1). The mean 24-hour diastolic BP was increased by 3.0 mmHg \((P < 0.001)\) and the mean 24-hour heart rate was increased by 3.5 bpm \((P = 0.038; \text{Figure 1 and Table 1})\). The individual mean 24-hour systolic and diastolic BP values are presented in Figure 2. Furthermore, the mean of awake systolic and awake diastolic BPs were elevated by 4.8 and 3.5 mmHg, respectively, as was the asleep systolic BP by 3.7 mmHg. The asleep diastolic BP and the night-time dipping of BP were not affected by rifampin. In addition, the mean of 24-hour mean arterial pressure (MAP) was increased by 3.6 mmHg. The mean of awake MAP was elevated by 4 mmHg, whereas asleep MAP was not affected (Table 1). We also observed an increase in 24-hour pulse pressure by 1.6 mmHg. The asleep pulse pressure was raised by 2.6 mmHg, whereas awake pulse pressure was not increased by rifampin. Furthermore, office systolic BP was elevated significantly by 3.4 mmHg.
The results of 21 sample pairs are reported here. Rifampin increased activity (data not shown). One heart rate were not correlated with renin concentration or renin treatment. The 24-hour systolic and diastolic BP and (range 1.3–4.8) as expected. Thus, all the subjects analyzed for renin, aldosterone concentrations were not affected by rifampin dosing. In addition, the plasma concentration of \( \beta \)-hydroxycholesterol (\( \beta \)-HC) did not correlate with 24-hour diastolic BP (rifampin \( r = -0.60 \), \( P < 0.001 \); placebo, \( r = -0.16 \), \( P = 0.19 \)).

Perhaps surprisingly, also the plasma concentration of \( \alpha \)-HC had a significant negative correlation with 24-hour systolic BP (rifampin \( r = -0.68 \), \( P < 0.001 \); placebo, \( r = -0.64 \), \( P < 0.01 \)) but not with the 24-hour diastolic BP (rifampin \( r = -0.30 \), \( P = 0.19 \); placebo, \( r = -0.16 \), \( P = 0.49 \)).

The serum level of endoglin, a factor mediating 22(R)-hydroxycholesterol-elicited hypertension in rodents, and 20-hydroxyicosatetraenoic acid, an eicosanoid metabolite of arachidonic acid suggested to be regulated by PXR, were not affected by rifampin dosing (Table 2).

The effect of \( \beta \)-HC on renin mRNA expression in vitro
As \( \beta \)-HC has been reported to be an LXR-\( \alpha \)-ligand, and the LXR activation has been reported to activate renin transcription, we hypothesized that elevated \( \beta \)-HC could mediate the increased plasma renin activity observed in the rifampin-treated study subjects (Table 2). We utilized the Calu-6 cell line constitutively expressing renin. The Calu-6 cells were treated for 48 hours with \( \beta \)-HC and 3 well-established LXR ligands, a naturally occurring oxysterol 22(R)-hydroxycholesterol (22(R)-HC) and 2 synthetic ligands, T0901317 and GW3965. The renin mRNA expression was measured with qRT-PCR. Furthermore, we measured LXR-\( \alpha \) expression because LXR-\( \alpha \) is known to autoregulate its own expression.

All the positive control compounds 22(R)-HC, T0901317, and GW3965 induced LXR-\( \alpha \) expression. The synthetic ligands T0901317 and GW3965 were more efficient and upregulated LXR-\( \alpha \) mRNA 4.7-fold and 4.9-fold, respectively, whereas 22(R)-HC induced LXR-\( \alpha \) mRNA 1.7-fold (Figure 4). In contrast, the renin expression was upregulated only by GW3965 and surprisingly 22(R)-HC repressed renin expression (Figure 4). High concentration of \( \beta \)-HC (40 \( \mu \)M) increased LXR-\( \alpha \) mRNA expression 1.4-fold (\( P < 0.01 \)) but had no effect on renin expression (Figure 4).

To further investigate the role of LXR-\( \alpha \) in renin regulation, we established a Calu-6 cell line with stable LXR-\( \alpha \) overexpression (Calu-6-LXR-\( \alpha \)). In this cell line, the synthetic ligands T0901317 and GW3965 induced renin expression 9.2-fold and 7.9-fold, respectively, and the oxysterol ligand 22(R)-HC 1.6-fold (Figure 5). The 40 \( \mu \)M concentration of \( \beta \)-HC induced renin expression 1.3-fold (\( P < 0.05 \)) in the Calu-6-LXR-\( \alpha \) cell line indicating that \( \beta \)-HC can induce renin expression through LXR-\( \alpha \).
DISCUSSION

This study demonstrates that the administration of the prototypical PXR agonist rifampin leads to elevated BP. In healthy young normotensive adults with no interacting medications, 1-week rifampin dosing elevated the mean 24-hour systolic BP 4.7 mmHg and diastolic BP 3.0 mmHg. The degree of the BP-elevating effect of rifampin is about half of the BP-lowering effect of common antihypertensive medications at the standard dose and about two-thirds of the BP-lowering effect of half-dose medications.²⁵ Naturally the clinical and mechanistic aspects of BP effects in normotensive subjects and patients with hypertension are fundamentally different. In addition, MAP and pulse pressure were elevated by rifampin. Rifampin did not affect the extent of the night-time dipping of BP. Furthermore, the mean 24-hour heart rate was elevated by 3.5 bpm. We also demonstrated that rifampin elevated serum renin concentration and plasma renin activity.

Prior publications have reported the hypertensive effect of tuberculosis treatment with rifampin, but all the studies involved patients with existing antihypertensive medications.²⁶–³⁰ Thus, the BP-elevating effect is mostly explained by drug-drug interactions involving the PXR-mediated induction of drug metabolism and transport by rifampin. A study with patients requiring maintenance hemodialysis demonstrated dramatically lower concentrations of amlodipine, metoprolol, and prazosin after the start of rifampin-based antitubercular therapy with 46% of the 24 patients experiencing hypertensive crisis.²⁹ Especially calcium-channel blockers are susceptible to the rifampin-elicited loss of BP control as CYP3A4 plays a major role in their metabolism.³¹ Our study in normotensive healthy volunteers without concomitant medications indicates a direct, drug-drug interaction independent, BP-regulating effect by rifampin. This direct rifampin effect may also contribute to the elevated BP in the patients with antihypertensive medications, in addition to the pharmacokinetic drug-drug interactions.

No earlier study has demonstrated the effect of PXR activation on BP. However, indirect evidence supports the role of PXR in BP regulation. The activation of PXR has been implicated in

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Table 1 Effect of the treatment with 600 mg rifampin or placebo once daily for 1 week on BP indexes in healthy volunteers

|                      | Rifampin       | Placebo       | P value | Ratio and 95% CI of difference |
|----------------------|----------------|---------------|---------|---------------------------------|
| 24-hour systolic BP, mmHg | 132.1 ± 10.5   | 127.5 ± 10.1  | < 0.0001 | 1.04 (3.09, 6.27)               |
| 24-hour diastolic BP, mmHg | 74.0 ± 6.6     | 71.0 ± 6.0    | < 0.001  | 1.04 (1.59, 4.42)               |
| Awake systolic BP, mmHg | 135.8 ± 10.3   | 131 ± 10.3    | < 0.0001 | 1.04 (2.78, 6.77)               |
| Awake diastolic BP, mmHg | 77.6 ± 6.9     | 74.2 ± 6.6    | < 0.001  | 1.05 (1.78, 5.13)               |
| Asleep systolic BP, mmHg | 119 ± 13.1     | 116 ± 11.3    | 0.029    | 1.03 (0.40, 7.05)               |
| Asleep diastolic BP, mmHg | 61.8 ± 6.5     | 60.7 ± 5.9    | 0.278    | 1.02 (−0.95, 3.13)              |
| Asleep dip systolic BP, % | 11.9 ± 6.1     | 11.5 ± 5.0    | 0.814    | 1.03 (−2.66, 3.36)              |
| Asleep dip diastolic BP, % | 20.3 ± 5.5     | 18.2 ± 5.8    | 0.160    | 1.12 (−0.92, 5.24)              |
| 24-hour heart rate, bpm | 66.5 ± 8.4     | 63.0 ± 8.3    | 0.038    | 1.05 (0.21, 6.70)               |
| Awake heart rate, bpm  | 69.4 ± 9.4     | 65.5 ± 9.0    | 0.054    | 1.06 (−0.07, 7.71)              |
| Asleep heart rate, bpm | 56.9 ± 7.2     | 54.4 ± 6.5    | 0.058    | 1.05 (−0.09, 5.00)              |
| 24-hour mean arterial pressure, mmHg | 93.4 ± 7.1 | 89.8 ± 6.6 | < 0.0001 | 1.04 (2.31, 4.87)               |
| Awake mean arterial pressure, mmHg | 97.0 ± 7.4 | 93.0 ± 7.0 | < 0.0001 | 1.04 (2.42, 5.67)               |
| Asleep mean arterial pressure, mmHg | 81.0 ± 8.0 | 79.2 ± 6.9 | 0.105    | 1.02 (−0.44, 4.26)              |
| 24-hour pulse pressure, mmHg | 58.1 ± 8.5     | 56.5 ± 8.3    | 0.049    | 1.03 (0.00, 3.26)               |
| Awake pulse pressure, mmHg | 58.2 ± 8.5     | 56.8 ± 8.2    | 0.115    | 1.02 (−0.36, 3.09)              |
| Asleep pulse pressure, mmHg | 58.0 ± 9.6     | 55.4 ± 9.1    | 0.040    | 1.05 (0.13, 5.15)               |
| Office systolic BP, mmHg | 117.9 ± 8.8     | 114.5 ± 9.3   | 0.039    | 1.03 (0.19, 6.63)               |
| Office diastolic BP, mmHg | 68.1 ± 7.7     | 67.3 ± 7.3    | 0.337    | 1.01 (−0.89, 2.48)              |
| Office heart rate, bpm | 69.3 ± 11.1    | 70.3 ± 9.8    | 0.689    | 0.99 (−5.84, 3.93)              |

Values are represented as means ± SD, the ratio of arithmetic means, and the 95% CI of differences between mean of arms. Two-tailed paired Student’s t-test was used as the statistical test.
BP regulation in rodent studies. In PXR-humanized mice, rifampin treatment for 5 weeks led to elevated plasma aldosterone, the adrenal mineralocorticoid regulating BP. In spontaneously hypertensive rats, CYP3A activity measured as corticosterone 6β-hydroxylation correlated with BP, and both 6β-hydroxylation and BP were lowered with troleandomycin, a macrolide antibiotic and inhibitor of CYP3A.33 PXR is expressed in human and mice aorta and mice mesenteric arteries, and direct vascular PXR-mediated effects have been described.34–36 The treatment of mice with 5β-dihydroprogesterone, a PXR agonist, enhanced endothelium-dependent relaxation of mouse mesenteric arteries.35

Figure 3 The Pearson correlation of plasma 4β-hydroxycholesterol (4βHC) concentration with the 24-hour systolic and diastolic ambulatory blood pressure (BP) after the treatment with 600 mg rifampin or placebo once daily for 7 days in healthy volunteers. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 2 Effect of the treatment with 600 mg rifampicin or placebo once daily for 1 week on BP regulating factors and 4βHC

|                      | Rifampin               | Placebo               | P value  | Ratio and 95% CI of difference |
|----------------------|------------------------|-----------------------|----------|-------------------------------|
| Plasma renin activity, µg/l/hour | 1.8 ± 0.7              | 1.3 ± 0.9             | 0.014    | 1.35 (−0.10, 0.80)             |
| Serum renin, pg/mL    | 9.1 ± 4.7              | 6.7 ± 5.2             | 0.028    | 1.37 (0.05, 3.76)              |
| Serum prorenin, ng/mL | 1.1 ± 0.7              | 1.1 ± 0.7             | 0.610    | 1.03 (−0.06, 0.06)             |
| Plasma 4βHC, ng/mL    | 54.1 ± 18.1            | 16.1 ± 5.8            | < 0.0001 | 3.36 (30.10, 46)               |
| Plasma 4αHC, ng/mL    | 3.33 ± 0.6             | 3.32 ± 0.7            | 0.668    | 1.00 (−0.34, 0.25)             |
| 20-HETE, ng/mL        | 26.0 ± 20.8            | 29.0 ± 24.2           | 0.679    | 0.90 (−8.70, 8.20)             |
| Serum endoglin, ng/mL | 5.1 ± 0.6              | 5.1 ± 0.7             | 0.924    | 1.00 (−0.19, 0.240)            |
| Serum aldosterone, pmol/L | 430.8 ± 254.1        | 443.9 ± 298           | 0.881    | 0.97 (−118, 190)               |
| Plasma glucose, mmol/L | 5.0 ± 0.3              | 5.1 ± 0.4             | 0.625    | 0.99 (−0.20, 0.20)             |
| Plasma creatinine, µmol/L | 70.5 ± 12.9            | 71.7 ± 12.3           | 0.466    | 0.98 (−3.00, 2.00)             |
| Plasma K, mmol/L      | 3.9 ± 0.2              | 3.9 ± 0.2             | 0.755    | 1.00 (−0.20, 0.20)             |
| Plasma Na, mmol/L     | 141.1 ± 1.8            | 141.1 ± 1.5           | 0.911    | 1.00 (−1.00, 2.00)             |

Values are represented as means ± SD, the ratio of arithmetic means, and the 95% CI of differences between median of arms. Wilcoxon matched pairs test was used as the statistical test.

4αHC, 4α-hydroxycholesterol; 4βHC, 4β-hydroxycholesterol; 20-HETE, 20-hydroxy-5, 8, 11, 14-eicosatetraenoic acid; BP, blood pressure; CI, confidence interval.
Figure 4 Effect of natural and synthetic liver X receptor (LXR) agonists on the expression of LXR-α (a, b) and renin (c, d) in wild-type Calu-6 cells \( (n = 4 / \text{group}) \). Values are represented as means ± SD. **\( P < 0.01 \), ****\( P < 0.0001 \). 22(R)-HC, 22(R)-hydroxycholesterol; 4βHC, 4β-hydroxycholesterol; NT, no treatment. [Colour figure can be viewed at wileyonlinelibrary.com]

Figure 5 Effect of (a) natural and (b) synthetic liver X receptor (LXR) agonists on the renin expression in LXR-α overexpressing Calu-6 cells \( (n = 4 / \text{group}) \). Values are represented as means ± SD. *\( P < 0.05 \), ****\( P < 0.0001 \). 22(R)-HC, 22(R)-hydroxycholesterol; 4βHC, 4β-hydroxycholesterol; NT, no treatment. [Colour figure can be viewed at wileyonlinelibrary.com]
endothelial nitric oxide synthase in the aorta and endothelium-derived hyperpolarizing factors in mesenteric arteries. The PXR-regulated 4βHC is an LXR agonist and LXR is implicated in the BP regulation. The activation of LXR-α by synthetic LXR agonists T0901317 and GW3965 have been shown to reduce the experimentally stimulated RAAS and/or BP. In contrast, both T0901317 and 22(R)-HC treatment elevated BP in mice with no prior hypertension-inducing procedures or treatments. The interpretation of the T0901317 results is complicated by the finding that T0901317 has also some affinity for mouse PXR. The experiments provided evidence that the elevated BP was mediated by an LXR-regulated circulating soluble endoglin. In our study, serum soluble endoglin was not affected by rifampin. Both LXR-α and LXR-β have been shown to regulate transcriptionally the expression of renin in vitro and single administration of synthetic LXR agonists increased renin expression in mice kidneys in vivo. Thus, LXR activation by synthetic agonists seems to regulate positively renin expression in the kidneys at least in acute settings but the experimentally stimulated activity of RAAS and elevated BP may be blunted by LXR activation.

The BP-elevating effect of rifampin detected in our study may theoretically be mediated by direct PXR agonism (perhaps through an effect on vasculature), through the LXR agonism by elevated 4βHC, or by unknown rifampin action. The impact of antibiotic action of rifampin cannot be fully excluded. Antibiotics are generally not considered to affect human BP, although some antibiotics have demonstrated hypotensive and hypertensive effects in preclinical models of hypertension and dysbiosis of the gut has been associated with human hypertension. A limitation of our study was the single-blind design due to rifampin coloring the urine red. However, the study subjects were not aware of the hypothesis that BP was expected to rise. In addition, we did not measure markers of sympathetic nervous system activation. This would have been relevant as the central sympathetic activation regulates BP and heart rate as well as renin secretion via β-adrenoceptors.

Thus, rifampin-elicited sympathetic activation by unknown mechanism may also be involved in BP and heart rate as well as renin effects.

We demonstrated that 4βHC is able to induce renin expression in vitro but only modestly with unphysiologically high 4βHC concentration in the LXR-α overexpressing cells, suggesting that the rifampin-elevated circulating 4βHC is not behind the increased renin concentration. Importantly, the plasma 4βHC concentration was strongly negatively associated with BP arguing that higher 4βHC concentration than CYP3A5 nonexpressors, this would be consistent with our finding that higher 4βHC is associated with lower 24-hour BP. To conclude, we demonstrate that rifampin increases 24-hour systolic and diastolic BP and heart rate as well as renin concentration and activity in normotensive healthy volunteers. Future studies are needed to establish if rifampin is also able to increase BP in rifampin-treated patients with tuberculosis. Especially elderly patients and patients with pre-existing hypertension may be at risk. Our findings should be considered when designing interaction studies with rifampin. The plasma concentration of 4βHC was strongly negatively associated with BP arguing against the role of 4βHC in the rifampin-elevated BP. The exact mechanisms mediating the rifampin effects on BP and renin regulation require further study. As PXR ligands include a multitude of medications, herbal remedies, and environmental toxicants, the potential hypertensive effect of PXR could have implications for the etiology of hypertension. If the future studies confirm the role of PXR in human BP regulation, therapeutics targeting PXR-mediated pathways may provide new treatment options for hypertension.

SUPPLEMENTARY INFORMATION
Supplementary information accompanies this paper on the Clinical Pharmacology & Therapeutics website (www.cpt-journal.com).

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CONFLICTS OF INTEREST
H.H. was employed by Admescope and is a current shareholder. All other authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS
F.H.-N.-G., J.Ha., and J.Hu. wrote the manuscript. F.H.-N.-G., T.S., J.R., J.Ha., and J.Hu. designed the research. F.H.-N.-G., T.S., and J.Hu. performed the research. F.H.-N.-G., and J.Hu. analyzed the data. H.H. contributed new analytical tools.
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