**Response to comment on “Nuclear receptor PXR targets AKR1B7 to protect mitochondrial metabolism and renal function in AKI”**

Xiaowen Yu1,2,3, Yue Zhang1,2,3*, Zhanjun Jia1,2,3*, Aihua Zhang1,2,3*

The nuclear pregnane X receptor protects against bilateral renal ischemia/reperfusion-induced acute kidney injury in mice.

Recently, we reported that the nuclear pregnane X receptor (PXR) protected against acute kidney injury (AKI) induced by cisplatin or ischemia/reperfusion (I/R) renal injury in mice and rats (1). In their Technical Comment, Luan et al. (2) confirmed our finding of a protective role of PXR in cisplatin-induced kidney injury, but they failed to find a beneficial effect of treatment with the PXR agonist pregnane 16a-carbonitrile (PCN) or rifampicin on ischemic AKI in wild-type mice or mice carrying human PXR (hPXR). They also claimed that PXR was not expressed in the human kidney, which may diminish the potential of a PXR agonist in treating human AKI. We carefully reviewed their results and found obvious differences in the animal models used and the drug treatment.

First, Luan et al. (2) stressed the absence or extremely low expression of PXR in the human kidney based on three reports published in 1998 and 2004 using Northern blot and RT-PCR (3–5). It is well known that Northern blot and RT-PCR have limitations in the sensitivity of mRNA detection. According to a number of microarray and single-cell sequencing datasets, PXR mRNA is detectable in the human kidney, although expression is low in the human kidney compared to liver and intestine (https://www.nephroseq.org/; http://humphreyslab.com/SingleCell). We also examined the mRNA and protein expression of PXR using human para-carcinoma kidney tissues and human kidney cells and found the existence of PXR mRNA and protein (Fig. 1, A to E). Most proteins and genes exhibit differential expression in different types of cells and organs (6), and lower expression of an mRNA or protein does not mean low activity or no function. In fact, the expression profile of PXR in murine kidney was much lower than that in other organs (7). However, both our group and Luan’s group found the same protective effect of PXR against cisplatin-induced AKI. Moreover, mRNA expression does not always correlate with protein abundance, which can be regulated by post-transcriptional mechanisms.

Second, in their experiments, Luan et al. (2) performed the unilateral nephrectomy in mice, followed by 35 min of ischemia. This could alter renal blood flow dynamics and enhance the working load of the injured kidney compared to the bilateral I/R renal injury mouse model that we used. It is known that any protection by drug is limited within a reasonable range of disease severity. Luan et al. (2) used a lower dose of PCN (40 mg/kg per day) administered by oral gavage than we did (50 mg/kg per day, ip) to treat more severe kidney disease. To better explain the different findings regarding the effect of PXR activation on I/R-induced AKI between our study and Luan et al.’s study (2), we re-examined the role of PCN using bilateral I/R and uninephrectomy I/R renal injury models. In the bilateral I/R renal injury model, mice were administered PCN (50 mg/kg per day) intraperitoneally or intragastrically for 2 days before bilateral I/R surgery. In agreement with the results shown in our previous study (1), both intraperitoneal and intragastric administration of PCN (50 mg/kg per day) resulted in reduced serum creatinine (sCr) and blood urea nitrogen (BUN), along with improved kidney pathology in mice subjected to bilateral I/R renal injury (Fig. 1, F to H). In the uninephrectomy I/R renal injury model of Luan et al. (2), mice were intragastrically administered PCN (40 mg/kg per day) for 4 days and then subjected to unilateral nephrectomy and I/R renal injury. Consistent with the data of Luan et al. (2), PCN did not protect renal function and tubular morphology in this uninephrectomy I/R renal injury mouse model (fig. S1). Indeed, the uninephrectomy I/R mouse model showed more severe tubular injury as indicated by the extensive necrosis of the proximal tubular cells, denuded tubules, and protein cast in the tubular lumens (fig. S1C). However, in the bilateral I/R renal injury mouse model, the renal pathology was mainly tubular dilation and loss of the tubular brush border (Fig. 1H). Moreover, the uninephrectomy I/R mouse model displayed a greater inflammatory response than did the bilateral I/R mouse model (Fig. 1G and fig. S1B). It is well recognized that uninephrectomy could enhance the glomerular filtration rate (GFR) of single nephrons in the remaining kidney due to a compensatory response and the alteration of local blood flow dynamics, which could result in higher blood perfusion, a heavier working load on kidney tubules, and subsequently more severe structural damage to tubules. In theory, the enhanced GFR in single nephrons in the early stages of I/R renal injury (within 24 hours) could boost the output of metabolic products such as urea and creatinine. Indeed, our bilateral renal I/R mouse model and Luan et al.’s (2) uninephrectomy I/R mouse model showed comparable BUN and sCr concentrations, although uninephrectomized I/R mice had more severe tubular damage and inflammation. However, cases of renal I/R with uninephrectomy are rare in the clinic so the disease model of Luan et al. (2) does not represent a common clinical situation.

Finally, Luan et al. (2) generated hPXR mice and observed the role of rifampicin in the activation of hPXR in a uninephrectomy I/R model in this mouse strain. After reviewing the literature, we found that others have generated hPXR mice that also failed to...
express hPXR in mouse kidney (8). Moreover, hPXR shares only 77.4% sequence similarity with mouse PXR such that the correct hPXR gene expression cannot be guaranteed with a mouse promoter. If hPXR in mouse kidney cannot drive expression of the target genes of mouse PXR, then hPXR mice would have a negative phenotype following rifampicin treatment.

**Fig. 1.** Expression of human PXR in human kidney and a protective role for PCN in bilateral I/R-induced AKI in wildtype C57BL/6 mice. (A) Total RNA was extracted from human para-carcinoma kidney tissues (n = 2) by TRIzol. Then, total RNA (5 µg) was reverse transcribed into cDNA using a SuperScript III First-Strand Synthesis System (Invitrogen, #18080051). Human PXR mRNA expression in human para-carcinoma kidney tissues was determined by qRT-PCR analysis using the PXR primers: 5’-ACCAAGCGACCAAGGAT-3’ (forward) and 5’-GGGAAGTGGGAGACAGG-3’ (reverse). The PCR was run in triplicate for each sample, and the PCR product was analyzed on an agarose gel. (B) Human PXR protein expression in human para-carcinoma kidney tissues was examined by Western blotting using an antibody (Abcam, catalog no. ab85451) against human PXR (n = 3). (C) Shown is a Western blot for PXR expression in human kidney podocytes transfected with PXR plasmids to confirm the specificity of this antibody against human PXR (n = 2). (D) Shown is in situ hybridization of human PXR using a Cy3-labeled probe in human para-carcinoma kidney tissues (n = 3). Scale bars, 20 µm. (E) Shown is qRT-PCR analysis of human PXR expression in human kidney podocytes and human renal tubular epithelial cells (n = 2). (F) sCr and BUN concentrations were measured in mice after I/R-induced AKI with or without intraperitoneal or intragastric administration of PCN. (G) qRT-PCR analyses of renal Il-1β and Il-6 mRNA expression is shown. (H) Left: Representative images of mouse kidney sections stained with periodic acid–Schiff stain are shown. Scale bars, 20 and 50 µm. Right: Tubular injury scores in mice were analyzed. Six random fields were examined in each mouse kidney. Data are expressed as means ± SD (n = 6). Statistically significant differences were determined by one-way analysis of variance (ANOVA). ####P < 0.0001, **P < 0.01, ***P < 0.001, and ****P < 0.0001.
In summary, through additional experiments and by analyzing datasets from other groups, our results suggest that there is an expression of PXR in the human kidney. Meanwhile, we found that the protective effect of the PXR agonist PCN on ischemic AKI in mice may depend on the severity of renal tubular damage. Further investigation is required to determine whether a higher dose of PCN or other types of specific PXR agonists could protect against advanced ischemic AKI.

**SUPPLEMENTARY MATERIALS**

stm.sciencemag.org/cgi/content/full/13/593/eabf9849/DC1

Fig. S1. PCN gavage did not protect against uninephrectomy I/R-induced AKI in wild-type C57BL/6 mice.

Data file S1. Primary data.

**REFERENCES AND NOTES**


**Funding:** This work was supported by grants from the National Natural Science Foundation of China (82090022, 81325004, 81830020, 81530023, and 81625004 to A.Z.; 81700604 to X.Y.; 81570616 to Y.Z.; and 81207001, 81670647, and 81873599 to Z.J.), the National Key Research and Development Program (2019YFA0802702 and 2016YFC0906103 to A.Z.), and the Natural Science Foundation of Jiangsu Province (BL2014007 to A.Z. and BK20170148 to X.Y.).

**Author contributions:** X.Y., Z.J., and A.Z. designed the study. X.Y. performed the experiments and analyzed the data. X.Y., Z.J., and A.Z. interpreted the results. X.Y., Z.J., and A.Z. wrote the manuscript. Competing interests: The authors declare that they have no competing interests.

**Data and materials availability:** All data associated with this study are present in the paper or the Supplementary Materials.

Submitted 4 December 2020
Accepted 23 April 2021
Published 12 May 2021
10.1126/scitranslmed.abf9849

**Citation:** X. Yu, Y. Zhang, Z. Jia, A. Zhang, Response to comment on “Nuclear receptor PXR targets AKR1B7 to protect mitochondrial metabolism and renal function in AKI”. *Sci. Transl. Med.* **13**, eabf9849 (2021).
Response to comment on "Nuclear receptor PXR targets AKR1B7 to protect mitochondrial metabolism and renal function in AKI"
Xiaowen Yu, Yue Zhang, Zhanjun Jia and Aihua Zhang

Sci Transl Med 13, eabf9849,
DOI: 10.1126/scitranslmed.abf9849