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

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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 16α-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

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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.
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
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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

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