

Reviewer #1:

1. For a better understanding for a not so familiar reader it would be helpful to include a small Table indicating the effect of BDL on the three transporters in the three organs.

Answer:

Thank for the reviewer's suggestion. A new table (Table 3) has been added in the manuscript.

2. As a clinician, I would like to ask for potential clinical implications of this interesting study.

Answer:

In physiological condition, the main route of elimination of digoxin is renal excretion which is closely correlated with glomerular filtration rate. Biliary excretion is the major non-renal route. Enterohepatic cycle has minor importance (Clin Pharmacokinet 2(1): 1-16, 1977). Our finding demonstrated that under pathological condition, cholestasis in the current study, cell membrane digoxin transporters are regulated which is in favor of an increase in digoxin excretion in renal tubules and decrease in its absorption from intestine. These changes compensate the reduced digoxin clearance due to cholestasis. This finding could have clinical application by modifying transporters' activities through pharmaceutical approaches for improving digoxin clearance during cholestasis (This is mentioned in the "Discussion").

3. Is digoxin clearance affected by cholestatic human liver diseases? Which other drugs are affected in cholestasis?

Answer:

Drugs with biliary elimination decrease clearance in patients with cholestasis as reviewed by Delcò et al (reference 2 in the manuscript, Drug Saf 2005; 28(6): 529–45).

4. The three transporters are not specific only for digoxin but surely for other drugs, too.

Answer:

The drugs transported by the three transporters are different in different tissues. MDR1 has been reported to act on dozens of pharmaceutical compounds including digoxin (reviewed in Pharmacogenet Genomics. 2011; 21(3): 152–161).

Beside digoxin, OATP1A4 also transports fexofenadine, statins, glibenclamide, estrone 3-sulfate (E3S), dehydroepiandrosterone 3-sulfate (DHEAS), prostaglandin E2, and taurocholate etc. (reviewed in Drug Metab Dispos, 2004. 32(3): p. 291-4).

OATP4C1, which is localized in the basolateral membrane of the proximal tubule, plays a major role in the urinary secretion of cardiac glycosides (digoxin and ouabain), thyroid hormones (triiodothyronine[T3] and thyroxine), cAMP, methotrexate, sitagliptin, estrone 3-sulfate, chenodeoxycholic acid, and glycocholic acid (J Pharmacol Exp Ther 362:271–277, 2017).

5. A small inaccuracy: Digitalis glycosides were the mainstay of therapy in congestive heart failure, but this is history.

Answer:

This sentence has been deleted.

Reviewer #2:

If authors would like to evaluate digoxin clearance according to each transporter expression, authors should examine cells with knock-in or knock-out experiments with each transporter genes.

Answer:

The current study was designed as an exploratory research for providing clues for future study in this field. Previous studies on the transporters in kidney and intestine were done only by in vitro experiments. To the best of our knowledge, the current report is the first study to investigate the regulation of the digoxin transporters in kidney and intestine in animal model of cholestasis. Our results does demonstrate that the cell membrane transporters were regulated which is in favor of digoxin excretion during cholestasis. This finding opens a new window for future studies.

We absolutely agree on the reviewer's comment. Examining cells with knock-in or knock-out experiments with each transporter genes will be a powerful tool to confirm our finding. Knock-out animal, especially tissue-specific KO, can also be considered.

Reviewer #3:

This study only observed the changes in the expression of several transporters in the BDL model. The changes of these proteins may have an impact on the metabolism of digoxin, but the expression sites, uptake and excretion functions of these proteins are different.

Theoretically, it is still difficult to infer the specific mechanism affecting the metabolism of digoxin. Therefore, as the author said, It is also necessary to understand the specific indicators such as tissue distribution and concentration of digoxin

Answer:

This research project was designed as an exploratory study. Previous reports of the transporters in kidney and intestine are based on in vitro experiments only. The current report is the first study to investigate the regulation of the digoxin transporters in kidney and intestine in animal model of cholestasis. Our results does demonstrate that the cell membrane transporters were regulated which is in favor of digoxin excretion during cholestasis. This finding opens a new window for future studies.

To further confirm the relationship between the regulations of the transporters and changes in digoxin metabolism and clearance, more detailed pharmacokinetic studies need to be done. Tissue distributions of digoxin, as the reviewer suggested, renal and intestine clearance of digoxin (we mentioned these in "Discussion" as the limitation of the study) will help for better understanding of the mechanisms.

Re-reviewer:

The revised version of the manuscript can be accepted.

Answer: Thanks for your comments.