Peer Review File
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Reviewer Comments

This article performed the retro-construction of plasma drug concentration-time curves from using urine excretion data and single-point plasma concentrations. Since the difficulty of blood sampling due to ethical restrictions is a serious problem in pediatric PK study, this study is intriguing. Thank you for the opportunity to review this manuscript. Major comments are provided below.

Major comments
Discussion

Comment 1: ・Page 16, Line 511
As one of the limitations, author maintained that Xu∞ was dependent on the precision of the excretion rates of the last two sampling points because of its estimation method and could come to a bias plasma curve, especially in the terminal phase.
So how should urine sampling be done? Should we continue sampling until the urinary excretion rate plateaus?

Reply 1:
Thanks for the comment. This is a great question and we should address this aspect more in the manuscript. First, urinary excretion data at the terminal phase are the key factors for accurate modelling. It requests precisely quantification because of the low urinary concentration at the terminal phase. Second, for drug with half-life lower than 8 h, blood sampling until 24h or 48h after dosing is long enough to construct the plasma C-t curve, and the total excretion would not change significantly from our experience. Accordingly, we, in general, stop urine sampling at 24h or 48h in parallel with the plasma C-t curve, since at that time the cumulative urinary excretion has reached the plateaus. In summary, the time span of urine sampling is depended on the target compound plasma C-t curve, a longer urine sampling plan is unnecessary.

Changes in the text:
Regarding the clinical urine sampling ending point, we revised fig 5 to comparison figures with different sampling ending points (fig 7 A and B). And, we added in Page 22, Line 542, "Meanwhile, as compared between fig 7A and B, a longer urine sampling time did not come to a better fitting, so clinically, urine sampling could be sufficient in parallel with the plasma C-t curve."

Comment 2: ・Page 16, Line 517
Author showed the ratio of CLr,t/CLr.T in Fig.6. After all, when of sampling time points should be used for the calculation of CLr in clinical practice? Sampling time points after 8 hours, which seem to be the elimination phase, there seems to be relatively little inter-individual variability.

Reply 2:
Selection of blood sampling time points to calculate CLr was not to decrease the inter-individual variability, but to promote the accuracy of intra-individual modeling. The results

showed the best blood sampling time points are model depended, which are 1 h for Scenario 3, 4 h for Scenario 4, 1 h for Scenario 5, and 1 h for Scenario 6. Clinically, for a certain drug, a preliminary study on modelling should be taken to determine the best blood sampling time point for CLr calculation.

Changes in the text:

Thank the reviewer, this advice is very important for clinical application of this model. So, a sentence was added in Page 23, Line 565, "Nonetheless, regarding the modeling method presented in this paper, preliminary studies are suggested to determine the best blood sampling time point for CLr calculation when a different drug or a different scenario is confronted clinically."

Comment 3: ・Page 17, Line 543

Author maintained that the larger the CLr, the more renal excretion contributes to the total elimination pathway, the larger the amount of the parent drug in urine, the smaller the variability of renal excretion clearance at each time point, and the more accurate the model will be. However, the urinary excretion rate of desloratadine and busulfan used in this study is very low at less than 2%. (Table S1) In order to confirm the accuracy of the model in this study, shouldn't we first have tested drugs with a high urinary excretion rate (such as water-soluble antibacterial drugs that contribute significantly to renal excretion)? Although both were selected as the first choice in pediatric field, they are not very suitable for confirming the predictability of the model constructed using urine data.

Reply 3:

Thanks for the comment and the authors totally agree with the opinion that a high urinary excretion rate drug would be easier for model concept proving. To exhibit better performance of the model, drugs with high urinary excretion (CLr) are considered more suitable for modeling. However, drugs in this category only account 30 percent of total market drugs (*Bo Feng, Jennnifer L LaPerle, George Chang, Manthena V S Varma. Renal clearance in drug discovery and development: molecular descriptors, drug transporters and disease state Expert Opin Drug Metab Toxicol. 2010 Aug;6(8):939-52.*). As clinical application is the ultimate golden standard, also for a broader application of this model, we targeted the drugs extensively applied in clinical practices, which also represent the majority of market drugs, from beginning of this project, even it brought in more challenges for modeling with their low urinary excretion.

Changes in the text: We added the text in Page 24, Line 574 "Although drugs with high renal excretion clearance are considered more suitable for better performance of the model, two low urinary excretion drugs, representing the majority of market drugs, were selected in this study with aim to fulfill the clinical demands, and broader the application of this model."

Comment 4: ・Discussion of scenario 6

Scenario 6 examined with busulfan in both rats and humans. Please consider the comparison and add the discussion.

In the clinical trial, it should be stated as a limitation that only 3 cases were performed.

Reply 4:

Thanks for the comments. We did not include the comparison in initial manuscript based on the consideration that this is not a pharmacokinetic study, but a report for a new technology. However, since more information on pediatric medication should be benefit for future research, we extend the discussion with the comparison and related references.

The limitation that only 3 cases were included in clinical verification is needed to emphasis.

Changes in the text:

We added the text in Page 20, Line 476, "The mean values of total clearance (CL) and volume of distribution of busulfan in this study were 0.21 L/h/kg and 0.69 L/kg, respectively, which are consistent with the reported pediatric median typical values[19], lower than the mean values we obtained in rats (0.27 L/h/kg and 0.95 L/kg), but higher than the reported values from adults[20]." with 2 references (19, 20) added.

And in Page 24, Line 585, "3 cases clinical data were insufficient to verify the practicability."