Target-Mediated Drug Disposition—A Class Effect of Soluble Epoxide Hydrolase Inhibitors

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Abstract

Pharmacological target-mediated drug disposition (TMDD) represents a special source of nonlinear pharmacokinetics, and its occurrence in large-molecule compounds has been well recognized because numerous protein drugs have been reported to have TMDD due to specific binding to their pharmacological targets. Although TMDD can also happen in small-molecule compounds, it has been largely overlooked. In this mini-review, we summarize the occurrence of TMDD that we discovered recently in a series of small-molecule soluble epoxide hydrolase (sEH) inhibitors. Our journey started with an accidental discovery of target-mediated kinetics of 1-(1-propanoylpiperidin-4-yl)-3-[4-(trifluoromethoxy)phenyl]urea (TPPU), a potent sEH inhibitor, in a pilot clinical study. To confirm what we observed in humans, we conducted a series of mechanism experiments in animals, including pharmacokinetic experiments using sEH knockout mice as well as in vivo displacement experiments with co-administration of another potent sEH inhibitor. Our mechanism studies confirmed that the TMDD of TPPU is due to its pharmacological target sEH. We further expanded our evaluation to various other sEH inhibitors and found that TMDD is a class effect of this group of small-molecule sEH inhibitors. In addition to summarizing the occurrence of TMDD in sEH inhibitors, in this mini-review we also highlighted the importance of recognizing TMDD of small-molecule compounds and its impact in clinical development as well as using pharmacometric modeling in facilitating quantitative understanding of TMDD.

Pharmacological target-mediated drug disposition (TMDD) represents a special source of nonlinear pharmacokinetics (PK), and it is caused by the binding of a compound to its high-affinity–low-capacity pharmacological target.[1] Along with the biotechnology revolution and the blossoming of large-molecule drug development, the concept of TMDD has gained broad attention because numerous protein drugs exhibit TMDD due to specific binding to their pharmacological targets. However, the phenomenon of TMDD is not unique to large-molecule compounds. Several lines of evidence indicate that TMDD can also happen in small-molecule compounds (eg, warfarin, imirestat, bosentan, linagliptin, selegiline).[2–4] Moreover, TMDD in small-molecule compounds appears to be a class effect. For example, ABT-384 and ASP3662, 2 11β-HSD1 inhibitors developed independently by Abbott and Astellas, respectively, demonstrated essentially same nonlinear PK behavior imparted by TMDD.[5,6] Similarly, a series of small-molecule endothelin receptor antagonists, including bosentan, clazosentan, and tezosentan, have also been reported to have TMDD.[7] In this mini-review, we summarize the occurrence of TMDD in a series of soluble epoxide hydrolase (sEH) inhibitors that we

recently found to show nonlinear PK mediated by their pharmacological target.[8,9] Two major factors contribute to increasingly common observation of the TMDD phenomenon. One factor is the advancement of increasingly sensitive analytical techniques such as liquid chromatography–tandem mass spectrometry. The other factor is the development of increasingly potent drugs such as the slow tight-binding transition state mimics described here.

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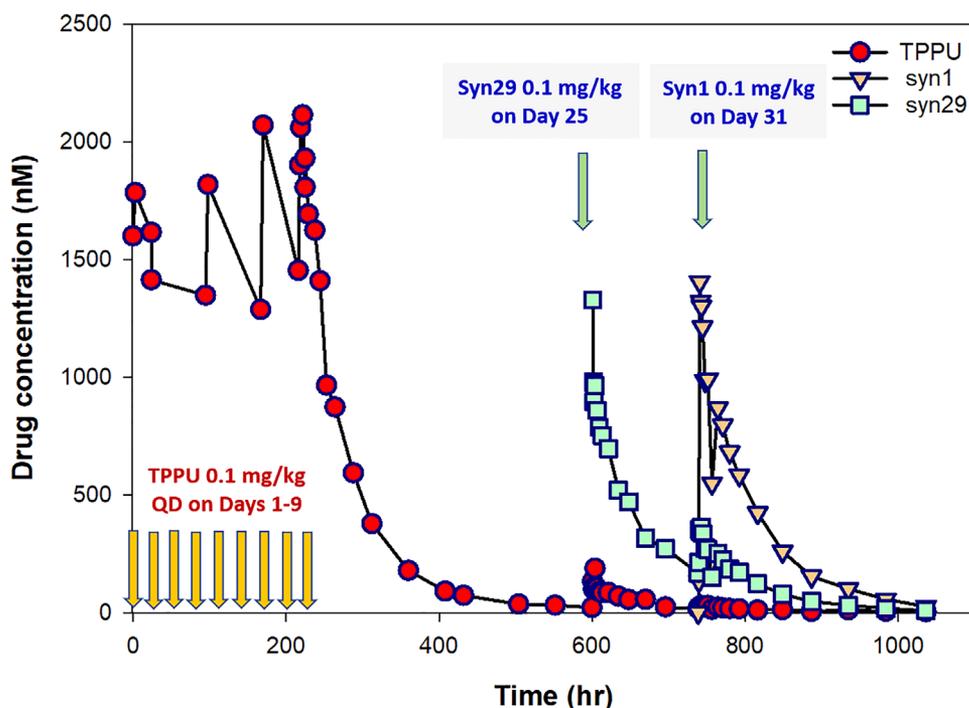
Figure 1. Clinical pharmacokinetic profiles of sEH inhibitors TPPU, Syn 29 and Syn 1 from a pilot study in which the target-mediated drug disposition was evaluated in 1 of the coauthors (B.D.H.). TPPU (0.1 mg/kg QD for 9 days) treatment followed by Syn 29 (0.1 mg/kg) at day 16 from the last dose of TPPU (ie, day 25), followed by Syn 1 (0.1 mg/kg) at day 22 from the last dose of TPPU (ie, day 31). QD, once daily; sEH, soluble epoxide hydrolase; TPPU, 1-(1-propanoylpiperidin-4-yl)-3-[4-(trifluoromethoxy)phenyl]urea.

Discovery in Humans

TMDD of sEH Inhibitor TPPU in Humans Was Discovered by Accident

The sEH is a major enzyme involved in breaking down epoxyeicosatrienoic acids and other epoxy fatty acid chemical mediators (EpFAs), leading to partial or complete loss of their initial biological activities, and sometimes generating product diols with inflammatory and other properties.[10] The EpFAs have a variety of biological activities including blood pressure regulation, control and prevention of heart disease, and prevention of pain. The sEH protein and mRNA are commonly induced in inflammation, so it is not surprising that inhibiting the sEH enzyme stabilizes the EpFA, leading to prevention and resolution of inflammation. Therefore, inhibition of sEH represents a promising strategy for the treatment of inflammation, pain, and cardiovascular diseases. We have successfully identified a series of small-molecule sEH inhibitors with good preclinical efficacy profiles and high potency as inhibitors of the sEH enzyme.[11] Before the formal first-in-human study, a pilot study was conducted in 1 of the co-authors (B.D.H.) to evaluate the clinical PK of several sEH inhibitors discovered in house, including a potent inhibitor, 1-(1-propanoylpiperidin-4-yl)-3-[4-(trifluoromethoxy)phenyl]urea (TPPU). TPPU was administered first, and then 2 weeks later a cassette

dose of 4 other sEH inhibitors were administered, and the blood concentrations of these compounds were measured at various time points. Surprisingly, following the cassette dosing of other sEH inhibitors, a second peak of TPPU was observed (data not shown). We confirmed that there was no time entry error and no bioanalytical assay error. To further support the initial observation, a second pilot study was conducted in the same investigator. In this study, TPPU was administered at 0.1 mg/kg once a day from day 1 to day 9 to determine drug accumulation with repeated dosing. The calculated terminal half-life of TPPU was 93.9 hours. Following what was thought to be an adequate washout period, another potent sEH inhibitor, Syn 29, then was administered as a single dose of 0.1 mg/kg on day 25 (ie, >4 half-lives of TPPU), and Syn 1 was administered as a single dose of 0.1 mg/kg on day 31 (ie, close to 6 half-lives of TPPU). As shown in Figure 1, TPPU has long terminal phase, and the concentration was still measurable >2 weeks after the last dose. In addition, when Syn 29 was given 16 days after the last dose of TPPU, a second peak of TPPU was observed. A similar pattern was also observed in Syn 29–a second peak of Syn 29 was observed right after Syn 1 was given. We hypothesize that the unexpected second peaks of TPPU and Syn 29 were caused by the coadministered sEH inhibitors

competing for their pharmacological target sEH. As a result, the drug molecules originally bound to sEH were displaced and subsequently distributed back from tissue to blood. To test our hypothesis that the binding with sEH plays important role in the disposition of sEH inhibitors, several mechanism experiments were conducted in experimental animals, and the key results are summarized in the next section.

Mechanism Studies in Animals

Mechanism Studies Confirmed That the TMDD of TPPU Is Due to Its Pharmacological Target sEH

To test our hypothesis, 2 types of experiments were conducted for TPPU, including PK experiments using sEH knockout mice as well as in vivo displacement experiments with coadministration of another potent sEH inhibitor 1-(4-trifluoro-methoxy-phenyl)-3-(1-cyclopropanecarbonyl-piperidin-4-yl)-urea (TCPU).[8] In the first experiment, a single low dose of 0.3 mg/kg TPPU was administered alone in both wild-type mice and sEH global knockout mice, and the TPPU PK profiles between these 2 groups were compared.[8,9] As shown in Figure 2, different PK behaviors were clearly observed between these 2 groups–TPPU in wild-type mice has lower maximum concentration (Figure 2B) and much longer terminal phase than that in sEH knockout mice (Figure 2A). These phenomena can be explained by the high-affinity target binding of the drug.

sEH is mainly expressed in tissues. Following a low dose, the tissue sEH enzyme rapidly acquires a considerable fraction of the administered dose so that only a portion of TPPU molecules were available for systemic circulation. As a result, the apparent volume of distribution of TPPU in wild-type mice is larger than that in sEH knockout mice, and correspondingly the blood maximum concentration of TPPU in wild-type mice is lower than that in sEH knockout mice. This high-affinity and tight target binding not only affects TPPU's distribution phase but also its elimination phase. Because of the firm and long-lasting target binding, slow off rate, and likely reassociation with other sEH molecules, the TPPU-sEH complex in tissues dissociated back to free TPPU and free target slowly. This slow dissociation process became the rate-limiting step for drug elimination, leading to the long terminal phase and long half-life. In the second experiment, a single 0.3 mg/kg dose of TPPU was given at time 0, followed by 3 mg/kg of TCPU (a potent sEH inhibitor) at 168 hours on the seventh day in both wild-type mice and sEH knockout mice. As shown in Figure 2C, in line with what we observed in humans, a second peak of TPPU showed up right after the administration of TCPU in wild-type mice; this phenomenon was not
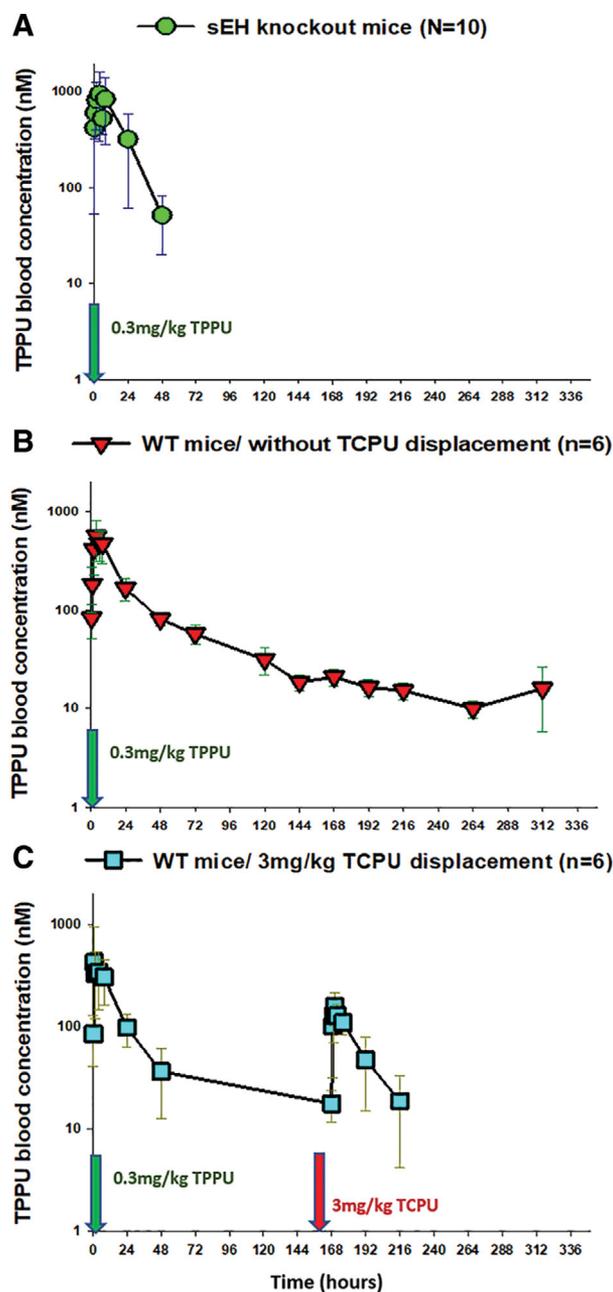
Figure 2. Time courses of mean observed TPPU blood concentrations following 0.3 mg/kg TPPU at time 0 in (A) sEH knockout mice with or without TCPU displacement (N = 10; 6 mice without TCPU displacement and 4 mice with 3 mg/kg TCPU displacement at 168 hours); (B) wild-type mice without TCPU displacement (N = 6); and (C) wild-type mice with 3 mg/kg TCPU displacement at 168 hours (N = 6). The lower limit of quantification of TPPU was 0.5 nM. In A, data in sEH knockout mice were combined since TPPU demonstrated same pharmacokinetic behavior no matter it was coadministered with TCPU or not (ie, no displacement, no second peak). (Adapted from Wu et al.[9]) sEH, soluble epoxide hydrolase; TCPU, 1-(4-trifluoro-methoxy-phenyl)-3-(1-cyclopropanecarbonyl-piperidin-4-yl)-urea; TPPU, 1-(1-propanoylpiperidin-4-yl)-3-[4-(trifluoromethoxy)phenyl]urea; WT, wild-type.

Table 1. In Vitro and In Vivo Parameters of sEH Inhibitors Evaluated in the Displacement Study Conducted in Mice[a]

sEH Inhibitor	K_i (nM)	In Vitro Drug Target Residence Time (t_R)[b] (min)	Dose (mg/kg)	AUC_{inf} (nmol·h/L)	$AUC_{2nd-Peak}$ (nmol·h/L)	Ratio of $AUC_{2nd-Peak}$ Over AUC_{inf}
TPPU	2.50	28.6	0.3	14 060	3045	0.22
TUPS	2.09	14.4	0.3	11 110	2082	0.19
TPAU	4.33	8.7	1	18 360	484.5	0.026
APAU	1.88	45.2	20	29 700	708.6	0.024

APAU, 1-(1-acetypiperidin-4-yl)-3-adamantanylurea; AUC, area under the concentration-time curve; K_i, inhibitory constant; t_R, residence time; TPAU, 1-trifluoromethoxyphenyl-3-(1-acetylpiperidin-4-yl) urea; TPPU, 1-(1-propanoylpiperidin-4-yl)-3-[4-(trifluoromethoxy)phenyl]urea; TUPS, 1-(1-methanesulfonyl-piperidin-4-yl)-3-(4-trifluoromethoxy-phenyl)-urea.
Adapted from Lee et al.[8]
[a] TCPU (at the dose of 3 mg/kg) was used as the displacer in all experiments. The K_i and t_R of TCPU were 0.92 nM and 23.8 minutes, respectively.
[b] t_R is the reciprocal of the dissociation rate constant k_{off} (ie, $t_R = 1/k_{off}$).

observed in sEH knockout mice (Figure 2A), further supporting that the TMDD of TPPU is due to its pharmacological target sEH.

TMDD Appears to Be a Class Effect of Slow Tight-Binding sEH Inhibitors

The interesting result on TPPU motivated us to expand our evaluation to other sEH inhibitors to see if TMDD is a class effect of the sEH inhibitors with urea pharmacophores. In vivo displacement experiments were conducted for a series of sEH inhibitors, including 1-(1-(cyclopropanecarbonyl)piperidin-4-yl)-3-(4-[trifluoromethoxy]phenyl)urea, 1-(1-isobutyrylpiperidin-4-yl)-3-(4-[trifluoromethoxy]phenyl)urea, 1-(1-acetypiperidin-4-yl)-3-adamantanylurea (APAU), 1-(1-methanesulfonyl-piperidin-4-yl)-3-(4-trifluoromethoxy-phenyl)-urea (TUPS), (S)-1-(2,6-difluorophenyl)-3-(1-[2-methylbutanoyl]piperidin-4-yl)urea, and 1-trifluoromethoxyphenyl-3-(1-acetylpiperidin-4-yl) urea (TPAU).[8] As shown in Figure 3, target-mediated kinetics was observed in most of the sEH inhibitors evaluated, indicating that TMDD is a class effect of these slow tight-binding sEH inhibitors. Our data also indicate that the magnitude of TMDD among sEH inhibitors is dependent on their binding affinities as well as dissociation rate constants. The magnitude of TMDD can be evaluated by calculating the ratio of the second peak area over the total peak area, as this ratio reflects the percentage of drug amount that still bound to sEH at the time before the displacer administration (ie, at 168 hours after the dose in our study). As shown in Figure 3 and Table 1, the ratio of TPAU is smaller than TPPU and TUPS, which is anticipated as in vitro binding properties of TPAU (drug-target residence time [t_R] of 8.7 minutes and inhibitory constant [K_i] of 4.33 nM) are weaker than the other 2 sEH inhibitors and correspondingly the impact of sEH binding to TPAU's disposition is mild. Although APAU has strongest in vitro binding properties (K_i is the smallest and t_R is the longest among all tested sEH inhibitors),

the displacement peak is unusually small. Compared with APAU, the displacer (ie, TCPU) has a weaker binding property and was given at much lower dose (Table 1). Therefore, a "pseudo" small second peak was observed because TCPU did not successfully displace the APAU bound in tissues. We anticipate that the actual amount of APAU trapped in tissues could be much larger than it looks in murine systems. A caution is that APAU (UC1153 or AR9281) was developed through human phase 2a trials. As shown in Table 1, APAU is surprisingly potent on the murine sEH enzyme, although even in rodents it has the liability of a short half-life.[12] However, whether IC_{50}, K_i, or drug target residence time is used as an indicator of potency on the target, it is a far weaker inhibitor than TPAU, TUPS, TPPU, or more modern sEH inhibitors for human sEH. Although APAU proved safe in phase 1 human trials,[13] it failed to show a commercial level of efficacy in the human phase 2a trial cautioning against uncritical extrapolation from animal models to man and that potency and TMDD studies should be evaluated in the target organism.

Importance of Recognizing TMDD of Small-Molecule Compounds

Regarding nonlinearity in PK of small-molecule compounds, the most common reason is due to the Michaelis-Menten kinetics that are caused by saturation of drug-metabolizing enzymes. For those drugs with capacity limited metabolism, the typical behavior is that the nonlinear pharmacokinetics occur at high doses. However, for small-molecule compounds undergoing pharmacological target-mediated nonlinear PK, their nonlinearity occurs at low doses.[2,3] Because of this counterintuitive behavior, the concept of TMDD in small-molecule compounds has not been widely recognized/appreciated. Interestingly, the phenomenon tends to become more important with the most potent and often most desirable analogs. In rodents, in vitro
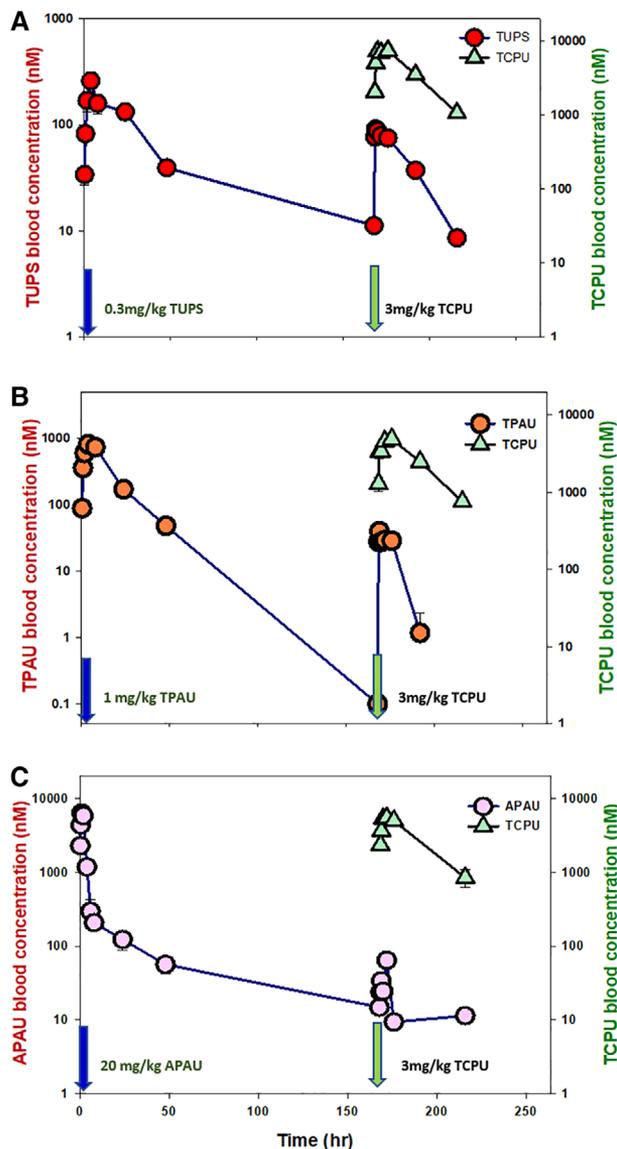
Figure 3. (A) Time courses of the mean observed TUPS and TCPU blood concentrations in wild-type mice following 0.3 mg/kg TUPS at time 0 and 3 mg/kg TCPU at time 168 hours. (B) Time courses of the mean observed TPAU and TCPU blood concentrations in wild-type mice following 1 mg/kg TPAU at time 0 and 3 mg/kg TCPU at time 168 hours. (C) Time courses of the mean observed APAU and TCPU blood concentrations in wild-type mice following 20 mg/kg APAU at time 0 and 3 mg/kg TCPU at time 168 hours. (Adapted from Lee et al.[8]) APAU, 1-(1-acetypiperidin-4-yl)-3-adamantanylurea; TPAU, 1-trifluoromethoxyphenyl-3-(1-acetylpiperidin-4-yl) urea; TCPU, 1-(4-trifluoro-methoxy-phenyl)-3-(1-cyclopropanecarbonyl-piperidin-4-yl)-urea; TUPS, 1-(1-methanesulfonyl-piperidin-4-yl)-3-(4-trifluoromethoxy-phenyl)-urea.

optimization of potency usually occurs in parallel with PK optimization. However, in man, clinical candidates usually are optimized in vitro before in vivo pharmacology and PK are evaluated. Thus, because relatively high doses are usually used in preclinical PK and toxicology studies, TMDD may not be apparent in preclinical stage

and often first be encountered during clinical development, especially in a first-in-human study where a wide dose range, including very low doses, is investigated.

Impact on Dose Regimen Selection During Clinical Development

Because the nonlinearity in small-molecule TMDD occurs at low doses, 1 natural question is why do we care about this nonlinear behavior considering that it is unlikely to raise safety issues that are commonly seen in those drugs with capacity-limited metabolism. We should care because it matters–while nonlinear PK imparted by TMDD has no implication with the safety end point, it ties closely with pharmacodynamics and can provide valuable insight on target engagement. For TMDD in small-molecule compounds, the nonlinear kinetics occurring at low doses is a strong sign of significant target engagement. A good example is ASP3662, a potent 11β-HSD1 inhibitor. The PK and pharmacodynamics of ASP3662 were evaluated in the first-in-human study in which both single ascending doses (1-60 mg) and multiple ascending doses (0.2–50 mg) were investigated.[5] ASP3662 exhibited substantial nonlinear PK at low doses and demonstrated essentially linear PK at doses >6 mg. Persistent and almost complete inhibition of hepatic 11β-HSD1 activity was observed even at a daily dose of 0.7 mg of ASP3662.[5] This result confirms that substantial nonlinear PK occurring at low doses reflects the extent of target occupancy, which means that we can get a good sense of what would be the potential efficacious dose based on the doses at which the nonlinearity occurs and the "turning point" dose at which the nonlinearity tends to disappear.

Impact on Microdosing Studies

Microdosing studies are phase 0 clinical trials, and they have received considerable attention over the past decade due to their application in drug candidate selection before full phase 1 development. Initially, the exquisite sensitivity of accelerator mass spectrometry was needed in most cases to reach microdosing levels. As illustrated here, with improvements in mass spectrometry, such microdosing studies are becoming more feasible with conventional equipment. For microdosing studies, the dose is defined as no greater than 100 μg or 1/100th of the no observed adverse effect level, whichever is lower.[14] The prerequisite for the full implementation of this approach is that the PK of the compound is linear over the range of dose of interest so that the PK obtained following microdosing can be reliably extrapolated to predict drug exposure at clinical doses. The microdosing results are particularly useful when employed in the context of PK studies performed over a range of doses in experimental animals and

compared to the PK in the human subjects. While it works for compounds with linear PK or compounds whose nonlinearity occurs at high doses (eg, drugs with capacity-limited metabolism), a microdosing study should be conducted with extra caution for those compounds exhibiting TMDD. This is because TMDD occurs at low doses, and the lower the dose, the more pronounced nonlinearity. As a result, TMDD can confound microdosing studies, leading to significant underprediction on drug exposure at therapeutic doses.

Impact on Studies With Crossover Study Design

For a small-molecule compound exhibiting TMDD, they usually have a very long terminal phase caused by slow release of the drug from the tight target binding. As a result, there could be a substantial difference between the PK following the first dose and that of the following dose(s). This feature may lead to order/sequence effect and potentially could significantly influence the results in those crossover clinical studies, such as bioequivalence and bioavailability studies. To ensure the quality of those clinical studies, using TMDD principles to select appropriate dose(s) as well as a sufficient washout phase will be critical.

Pharmacometric Modeling in Facilitating Quantitative Understanding of TMDD

For small-molecule compounds exhibiting TMDD, due to their nonlinear and complex PK, the relationship among dose, drug exposure, and response is no longer intuitive, and consequently the dose regimen design can be challenging. Indeed, there was evidence of significant 11β-HSD1 inhibition following a single dose of ASP3662 1 mg even though the plasma levels were below the lower limit of quantification.[5] To optimize the dose regimen, there has been a growing interest in developing pharmacometric models to quantitatively characterize TMDD in small-molecule compounds. TMDD models have been developed for many small-molecule compounds, such as imirestat,[15] bosentan,[7] ABT-384,[16] and linagliptin.[17] However, most of the TMDD models reported so far were established in a single compound scenario. Based on the results from our in vivo displacement experiments, recently we developed a novel TMDD model for TPPU and TCPU competing for sEH,[9] which represents the first TMDD interaction model for 2 small-molecule compounds competing for the same pharmacological target. Our model predicted the total amount of in vivo sEH enzyme as well as dissociation rate constants (K_{off}) of TPPU and TCPU were all close to the values obtained from in vitro experiments.[9] Recently, a number of studies have suggested that drug-target t_R, which is calculated as $1/K_{off}$, is a better in vitro parameter to predict in vivo efficacy than those standard in vitro potency parameters, such as K_d.[8] Our model results indirectly support this recommendation considering that the K_{off} values determined in vitro are consistent with those estimated from the mathematical modeling using the in vivo data. In addition to PK characterization, we also used our TMDD interaction model to predict sEH target occupancy, and our results indicated that 90% of the sEH will be occupied shortly after a low dose of 0.3 mg/kg TPPU administration, with $\geq$40% of sEH remaining bound with TPPU for at least 7 days.[9] If sEH target occupancy ties closely with the pharmacodynamics effect, then long-lasting efficacy is expected following a single dose of TPPU. Further efficacy experiments are warranted to confirm our prediction.

Conclusion

Compared with large-molecule compounds undergoing TMDD, which has been well recognized due to its high prevalence, TMDD in small-molecule compounds is more counterintuitive and has been an overlooked area. We discovered the TMDD of the small-molecule sEH inhibitor TPPU in humans accidentally due to careful attention by the mass spectrometry scientist (J.Y.), and then confirmed that the TMDD of TPPU is due to its pharmacological target sEH through conducting a series of mechanism experiments in wild-type and sEH knockout mice. Our studies summarized in this mini-review provide solid evidence on the occurrence of TMDD in a series of small-molecule compounds acting potently and specifically on sEH. For small-molecule compounds exhibiting TMDD, recognizing TMDD is important, as it plays important role in dose regimen optimization, clinical trial design, and data interpretation.

Conflicts of Interest

The authors declare no conflicts of interest.

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