Title

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

Journal

The Journal of Clinical Pharmacology, 61(4)

ISSN

0091-2700

Authors

An, Guohua
Lee, Kin Sing Stephen
Yang, Jun
et al.

Publication Date

2021-04-01

DOI

10.1002/jcph.1763

Peer reviewed

Published in final edited form as:

J Clin Pharmacol. 2021 April ; 61(4): 531–537. doi:10.1002/jcph.1763.

Target-Mediated Drug Disposition (TMDD) – a Class Effect of Soluble Epoxide Hydrolase (sEH) Inhibitors

Guohua An, MD, PhD[1,*], **Kin Sing Stephen Lee, PhD**[2,3], **Jun Yang, PhD**[4], **Bruce D. Hammock, PhD**[4]

[1]Division of Pharmaceutics and Translational Therapeutics, College of Pharmacy, University of Iowa, Iowa city, Iowa

[2]Department of Pharmacology and Toxicology, Michigan State University, East Lansing, Michigan

[3]Department of Chemistry, Michigan State University, East Lansing, Michigan

[4]Department of Entomology and Nematology and UCD Cancer Research Center, University of California at Davis, Davis, California

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 human, 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 utilizing pharmacometric modeling in facilitating quantitative understanding of TMDD.

Keywords

Target-mediate drug disposition; Soluble Epoxide Hydrolase Inhibitors; Nonlinear Pharmacokinetics; Drug Development

[*]**Correspondence**: Guohua An, MD, Ph.D., Division of Pharmaceutics and Translational Therapeutics, College of Pharmacy, University of Iowa, 115 S Grand Ave, Iowa City, IA, 52242,USA. Tel: (319) 467-4600; guohua-an@uiowa.edu.

Introduction

Pharmacological target-mediated drug disposition (TMDD) represents a special source of nonlinear pharmacokinetics, 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 (e.g. 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, two 11β-HSD1 inhibitors developed independently by Abbott and Astellas, respectively, demonstrated essentially same nonlinear pharmacokinetic behavior imparted by TMDD [5, 6]. Similarly, a series of small-molecule endothelin receptor antagonists (ERAs), 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 pharmacokinetics 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 (LC/MS/MS). The other factor is the development of increasingly potent drugs such as the slow tight binding transition state mimics described here.

Discovery in Human

TMDD of sEH inhibitor TPPU in human was discovered by accident

The sEH is a major enzyme involved in breaking down epoxyeicosatrienoic acids and other epoxyfatty acid chemical mediators (EpFA), leading to partial or complete loss of their initial biological activities, and sometimes generating product diols with inflammatory and other properties [10]. The EpFA 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]. Prior to the formal first-in-human study, a pilot study was conducted in one of the co-authors (BDH) to evaluate the clinical pharmacokinetics 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 (QD) from Day 1 to Day 9 to determine drug accumulation with repeated dosing. The calculated terminal half-life of TPPU was 93.9 h. Following what was thought to be an adequate washout period, another potent sEH inhibitor, Syn 29, then was administered at a single dose of 0.1 mg/kg on Day 25 (i.e. more than 4 half-lives of TPPU) and Syn 1 was administered at a single dose of 0.1 mg/kg on Day 31 (i.e. close to 6 half-lives of TPPU). As shown in Figure 1, TPPU has long terminal phase and the concentration was still measurable more than 2 weeks after the last dose. In addition, when Syn29 was given 16 days after the last dose of TPPU, a second peak of TPPU was observed. A similar pattern was also observed in Syn29 – a second peak of Syn29 was observed right after Syn1 was given. We hypothesize that the unexpected second peak of TPPU and Syn29 were caused by the co-administered 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, two types of experiments were conducted for TPPU, including pharmacokinetic experiments using sEH-knockout mice as well as *in vivo* displacement experiments with co-administration 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 wildtype mice and sEH global -knockout mice, and the TPPU pharmacokinetic profiles between these two groups were compared [8, 9]. As shown in Figure 2, different pharmacokinetic behaviors were clearly observed between these two groups - TPPU in wildtype mice has lower C_{max} (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 wildtype mice is larger than that in sEH-knockout mice and correspondingly the blood C_{max} of TPPU in wildtype 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 TCPU (a potent sEH inhibitor) at 168 hours on the 7th day in both wildtype mice and sEH-knockout mice. As shown in Figure 2c, in line with what we observed in human, a second peak of TPPU showed up right after the administration of TCPU in wildtype mice; this phenomenon was not 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 TCU, TIPU, APAU, TUPS, DFPU, and 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 prior to the displacer administration (i.e. 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 min and Ki of 4.33 nM) are weaker than the other two sEH inhibitors and correspondingly the impact of sEH binding to TPAU's disposition is mild. On the other hand, although APAU has strongest in vitro binding properties (Ki 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 (i.e. TCPU) has weaker binding property and was given at much lower dose (Table 1). Therefore, "pseudo" small 2nd 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 a 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 pharmacokinetics 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

pharmacokinetics, their nonlinearity occurs at low doses [2, 3]. Because of this counter-intuitive 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* optimization of potency usually occurs in parallel with pharmacokinetics optimization. However, in man, clinical candidates usually are optimized *in vitro* before *in vivo* pharmacology and pharmacokinetics are evaluated. Thus, because relatively high doses are usually used in preclinical pharmacokinetic and toxicology studies, TMDD may not be apparent in preclinical stage and often first be encountered during clinical development, especially in first-in-human study where a wide dose range, including very low doses, are investigated.

Impact on dose regimen selection during clinical development

Because the nonlinearity in small-molecule TMDD occurs at low doses, one natural question is that why do we care 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 pharmacokinetics imparted by TMDD has no implication with safety endpoint, 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 pharmacokinetics and pharmacodynamics of ASP3662 were evaluated in the FIH 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 greater than 6 mg. Persistent and almost complete inhibition on hepatic 11β-HSD1 activity were observed even at daily dose of 0.7 mg of ASP3662 [5]. This result confirms that substantial nonlinear pharmacokinetics occurring at low doses reflect 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 its 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 study, the dose is defined as no greater than 100 ug or 1/100th of the No Observed Adverse Effect Level (NOAEL), whichever is the lower [14]. The prerequisite for the full implementation of this approach is that the pharmacokinetics of the compound is linear over the range of dose of interest so that the pharmacokinetics 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 pharmacokinetic studies performed over a range of doses in experimental animals and compared to the pharmacokinetics in the human subjects. While it works for compounds with linear pharmacokinetics or compounds whose nonlinearity occurs at high doses (e.g.

drugs with capacity-limited metabolism), 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 cross-over 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 pharmacokinetics following the first dose and that from the following dose(s). This feature may lead to order/sequence effect and potentially could significantly influence the results in those cross-over clinical studies, such as bioequivalence and bioavailability studies. To ensure the quality of those clinical studies, utilizing TMDD principals to select appropriate dose(s) as well as sufficient wash out phase will be critical.

Pharmacometric Modeling in Facilitating Quantitative Understanding of TMDD

For small-molecule compounds exhibiting TMDD, due to their nonlinear and complex pharmacokinetics, 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 (LLOQ) [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 two 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 residence time (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 Kd[8]. Our model results indirectly support this recommendation considering that the koff values determined *in vitro* are consistent with those estimated from the mathematical modeling using the *in vivo* data. In addition to pharmacokinetics 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 ≥ 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 a small-molecule sEH inhibitor TPPU in human accidently due to careful attention by the mass spectrometry scientist (JY), 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, as well as data interpretation.

ACKNOWLEDGEMENT

This work is partially supported by National Institute of Environmental Health Sciences (NIEHS) Superfund Research Program P42 ES004699, NIEHS RIVER Award R35 ES030443, and NIEHS R00 ES024806.

References

1. Levy G Pharmacologic target-mediated drug disposition. Clinical Pharmacology & Therapeutics. 1994;56(3): 248–252. [PubMed: 7924119]
2. An G Concept of Pharmacologic Target-Mediated Drug Disposition in Large-Molecule and Small-Molecule Compounds. J Clin Pharmacol. 2020;60(2): 149–163. [PubMed: 31793004]
3. An G Small-Molecule Compounds Exhibiting Target-Mediated Drug Disposition (TMDD): A Minireview. The Journal of Clinical Pharmacology. 2017;57(2): 137–150. [PubMed: 27489162]
4. Retlich S, Withopf B, Greischel A, Staab A, Jaehde U, Fuchs H. Binding to dipeptidyl peptidase-4 determines the disposition of linagliptin (BI 1356)–investigations in DPP-4 deficient and wildtype rats. Biopharmaceutics & drug disposition. 2009;30(8): 422–436. [PubMed: 19771584]
5. Bellaire S, Walzer M, Wang T, Krauwinkel W, Yuan N, Marek GJ. Safety, Pharmacokinetics, and Pharmacodynamics of ASP3662, a Novel 11beta-Hydroxysteroid Dehydrogenase Type 1 Inhibitor, in Healthy Young and Elderly Subjects. Clin Transl Sci. 2019;12(3): 291–301. [PubMed: 30740895]
6. An G, Liu W, Dutta S. Small-molecule compounds exhibiting target-mediated drug disposition - A case example of ABT-384. J Clin Pharmacol. 2015;55(10): 1079–1085. [PubMed: 25931139]
7. Volz AK, Dingemanse J, Krause A, Lehr T. Target-Mediated Population Pharmacokinetic Modeling of Endothelin Receptor Antagonists. Pharm Res. 2019;37(1): 2. [PubMed: 31823033]
8. Lee KSS, Yang J, Niu J, et al. Drug-Target Residence Time Affects in Vivo Target Occupancy through Multiple Pathways. ACS Cent Sci. 2019;5(9): 1614–1624. [PubMed: 31572788]
9. Wu N, Hammock BD, Lee KSS, An G. Simultaneous Target-Mediated Drug Disposition Model for Two Small-Molecule Compounds Competing for Their Pharmacological Target: Soluble Epoxide Hydrolase. J Pharmacol Exp Ther. 2020;374(1): 223–232. [PubMed: 32238455]
10. Kodani SD, Hammock BD. The 2014 Bernard B. Brodie award lecture-epoxide hydrolases: drug metabolism to therapeutics for chronic pain. Drug Metab Dispos. 2015;43(5): 788–802. [PubMed: 25762541]
11. Lee KSS, Liu J-Y, Wagner KM, et al. Optimized inhibitors of soluble epoxide hydrolase improve in vitro target residence time and in vivo efficacy. Journal of medicinal chemistry. 2014;57(16): 7016–7030. [PubMed: 25079952]
12. Anandan SK, Webb HK, Chen D, et al. 1-(1-acetyl-piperidin-4-yl)-3-adamantan-1-yl-urea (AR9281) as a potent, selective, and orally available soluble epoxide hydrolase inhibitor with efficacy in rodent models of hypertension and dysglycemia. Bioorg Med Chem Lett. 2011;21(3): 983–988. [PubMed: 21211973]

13. Chen D, Whitcomb R, MacIntyre E, et al. Pharmacokinetics and pharmacodynamics of AR9281, an inhibitor of soluble epoxide hydrolase, in single- and multiple-dose studies in healthy human subjects. J Clin Pharmacol. 2012;52(3): 319–328. [PubMed: 21422238]
14. Burt T, Yoshida K, Lappin G, et al. Microdosing and Other Phase 0 Clinical Trials: Facilitating Translation in Drug Development. Clin Transl Sci. 2016;9(2): 74–88. [PubMed: 26918865]
15. Mager DE, Jusko WJ. General pharmacokinetic model for drugs exhibiting target-mediated drug disposition. J Pharmacokinet Pharmacodyn. 2001;28(6): 507–532. [PubMed: 11999290]
16. An G, Liu W, Katz DA, Marek GJ, Awni W, Dutta S. Population pharmacokinetics of the 11beta-hydroxysteroid dehydrogenase type 1 inhibitor ABT-384 in healthy volunteers following single and multiple dose regimens. Biopharm Drug Dispos. 2014;35(7): 417–429. [PubMed: 25041811]
17. Retlich S, Duval V, Graefe-Mody U, et al. Population Pharmacokinetics and Pharmacodynamics of Linagliptin in Patients with Type 2 Diabetes Mellitus. Clin Pharmacokinet. 2015;54(7): 737–750. [PubMed: 25637172]

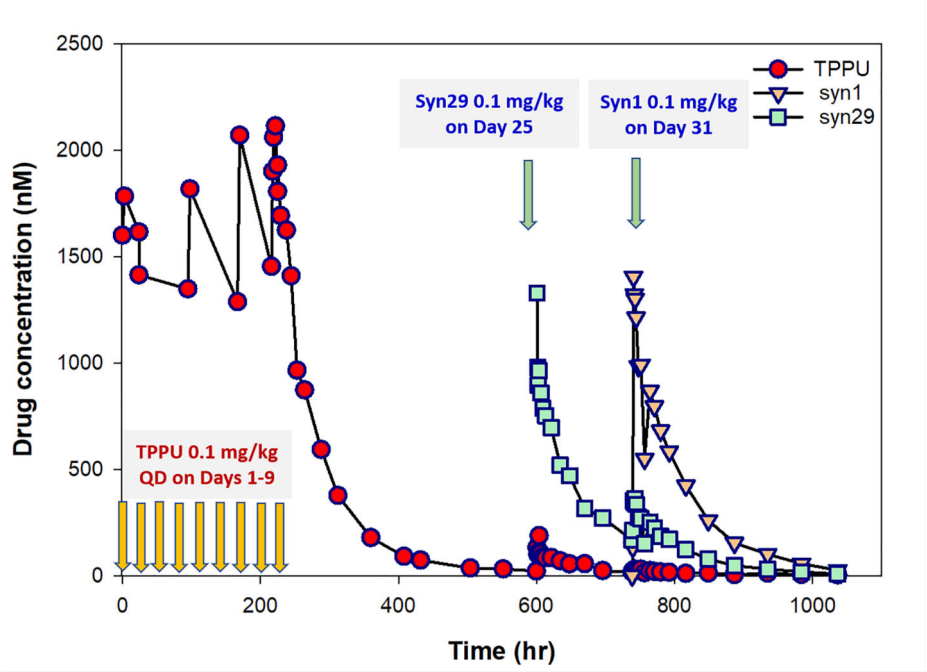

Figure 1.

Clinical PK profiles of sEH inhibitors TPPU, Syn 29 and Syn 1 from a pilot study in which the target-mediated drug disposition was evaluated in one of the co-authors (BDH). 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 (i.e. Day 25), followed by Syn 1 (0.1 mg/kg) at day 22 from the last dose of TPPU (i.e. Day 31).

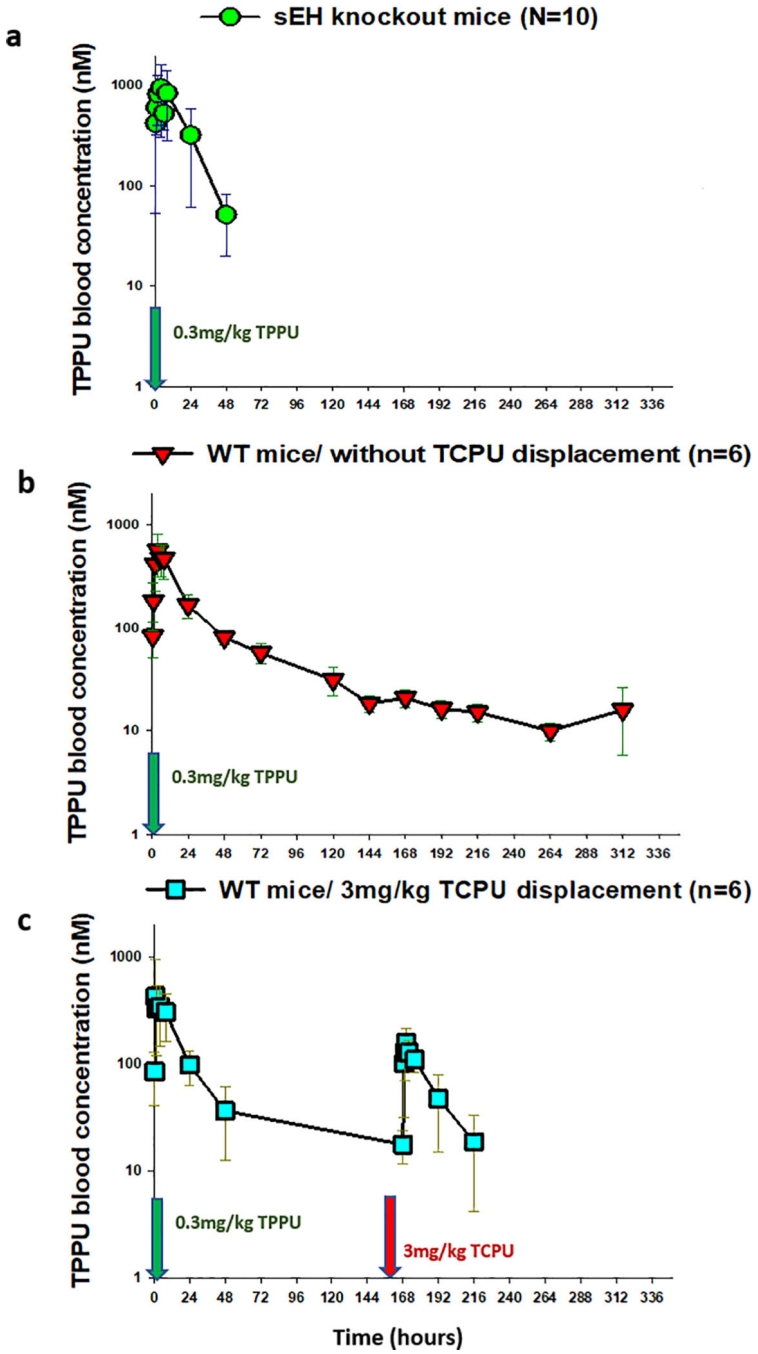

Figure 2.

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 Figure 2a, data in sEH knockout mice were combined since TPPU demonstrated same

pharmacokinetic behavior no matter it was co-administered with TCPU or not (i.e. no displacement, no second peak). *(Adapted from Wu N et al. JPET 2020)* [9]

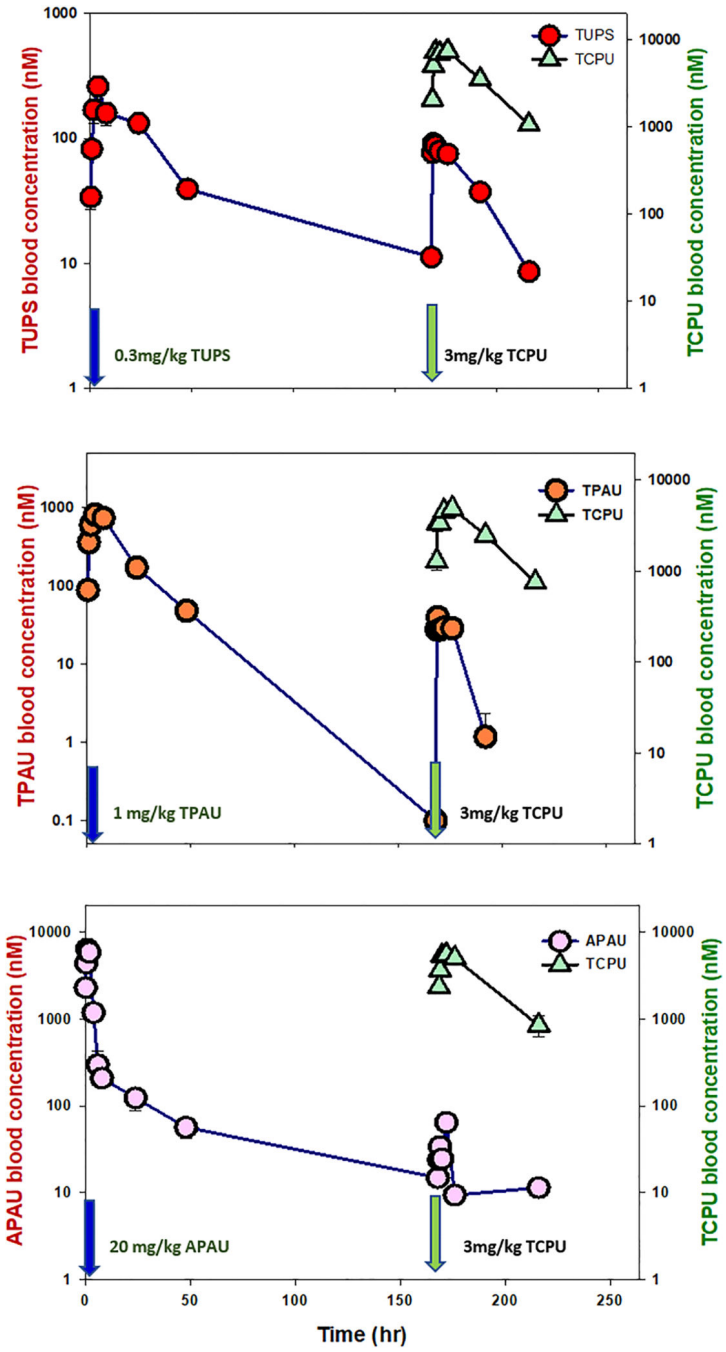

Figure 3.

(Top panel) 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; (Middle panel) 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; ; (Bottom panel) 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 KSS et al. ACS central science. 2019)*[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-cyclopropanecarbonylpiperidin-4-yl)-urea; TUPS, 1-(1-methanesulfonyl-piperidin-4-yl)-3-(4-trifluoromethoxy-phenyl)-urea.

Table 1.

In vitro and *in vivo* parameters of sEH inhibitors evaluated in the displacement study conducted in mice[#].

sEH inhibitor[^]	Ki (nM)	*In vitro* drug target residence time (t_R)[*] (min)	Dose (mg/kg)	AUC_{inf} (nM[*]h)	$AUC_{2nd\text{-}Peak}$ (nM[*]h)	Ratio of $AUC_{2nd\text{-}Peak}$ over AUC_{inf}
TPPU	2.50	28.6	0.3	14060	3045	0.22
TUPS	2.09	14.4	0.3	11110	2082	0.19
TPAU	4.33	8.7	1	18360	484.5	0.026
APAU	1.88	45.2	20	29700	708.6	0.024

[#]TCPU (at the dose of 3 mg/kg) was used as the displacer in all experiments. The ki and t_R of TCPU were 0.92 nM and 23.8 min, respectively.

[^]TPPU, 1-(1-propanoylpiperidin-4-yl)-3-[4-(trifluoromethoxy)phenyl]urea; TPAU, 1-trifluoromethoxyphenyl-3-(1-acetylpiperidin-4-yl) urea; TUPS, 1-(1-methanesulfonyl-piperidin-4-yl)-3-(4-trifluoromethoxy-phenyl)-urea; APAU, 1-(1-acetypiperidin-4-yl)-3-adamantanylurea

[*]t_R is the reciprocal of the dissociation rate constant k_{off} [i.e. $t_R = 1/k_{off}$]

(This table was adapted from Lee KSS et al. ACS central science. 2019)