In Situ, In Vitro, and In Silico Permeability Values as Inputs for Blood-Brain Barrier Penetration Prediction: Impact on Brain Exposure for Passively Diffusing Compounds, with Ethanol as a Case Study

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BACKGROUND

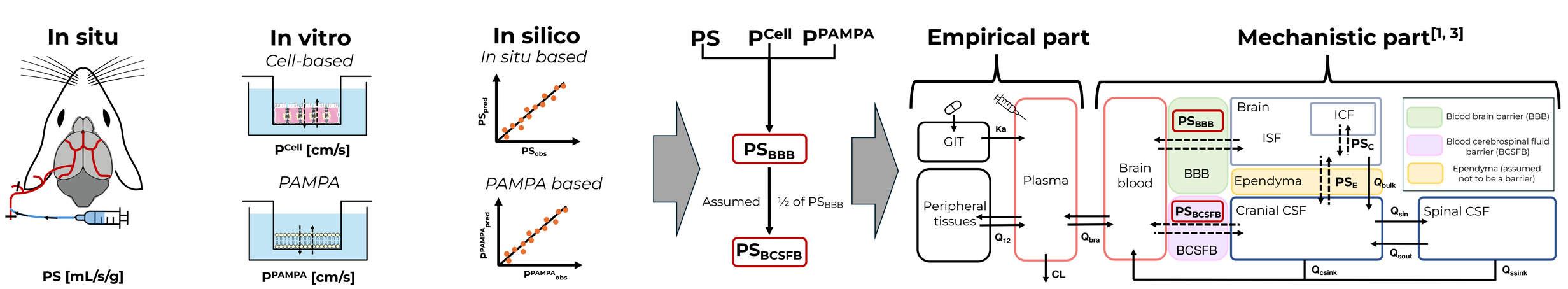
- Brain drug levels often differ from plasma due to the blood-brain barrier (BBB)
- Physiologically based pharmacokinetic models (PBPK) are used to predict brain exposure of central nervous system (CNS) active drugs[1, 2]
- **Permeability × surface area of BBB (PS_{BBB})** is one of the **key input parameters** in **PBPK CNS models**, but there is no consensus in the literature on its selection[3,4]

OBJECTIVES

- Investigate **variability of PS_{BBB}** values from in situ, in vitro and in silico sources
- Demonstrate **PS_{BBB} impact** on **human brain exposure** using ethanol as a case study

METHODS

- Permeability **data collection**[5, 6]:
  - In situ
  - In vitro (cell-based, PAMPA)
  - In silico[7, 8]
    - $PS = 10^{-2.06+0.448*logP-0.366*MW/100}$
    - $P^{PAMPA} = 10^{0.939*logP-6.210}$
- **Translation** permeability to PS_{BBB}
  - In situ: $PS_{BBB} = PS \times BrainWT$ [9]
  - In vitro: $PS_{BBB} = P \times SA_{BBB}$ [10]
- **Focus on** compounds crossing BBB via **passive diffusion** ($0.3 < K_{p,uu,brain} < 3$)
- **Brain exposure prediction** for ethanol using different PS_{BBB} values
- **Validation** of ethanol brain exposure prediction using human data[11-14] and **2-Fold Error (FE)** as criterion



RESULTS

Permeability variability

- Permeability data were collected and translated to PS_{BBB} values for **10 CNS-active compounds**
- **PS_{BBB}** values differed from 3-fold for lidocaine to 1385-fold for ethanol with an **average difference of 230-fold**
- For half the compounds, **in situ perfusion** gave the **highest PS_{BBB}** values, while **PAMPA** gave the **lowest**. **In vitro cell-based** showed **intermediate PS_{BBB}** values with the least variability.
- **Maximum PS_{BBB}** values among the 10 selected compounds were **2357 L/h (PAMPA)**, **522 L/h (in situ)** and **40 L/h (cell-based)**

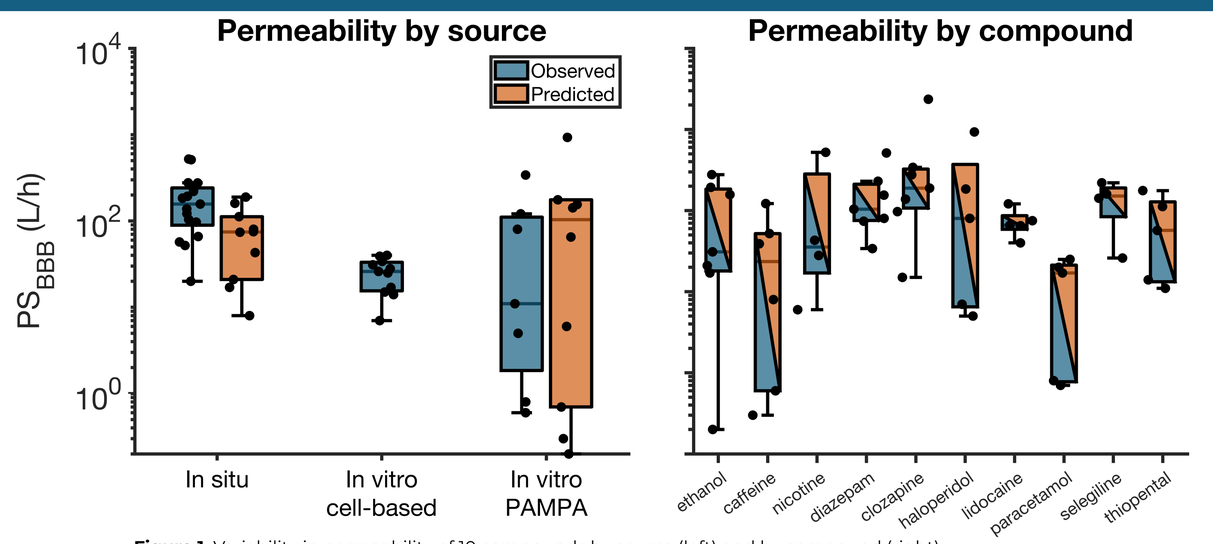


Figure 1. Variability in permeability of 10 compounds by source (left) and by compound (right).

PS_{BBB} impact on brain exposure for Ethanol

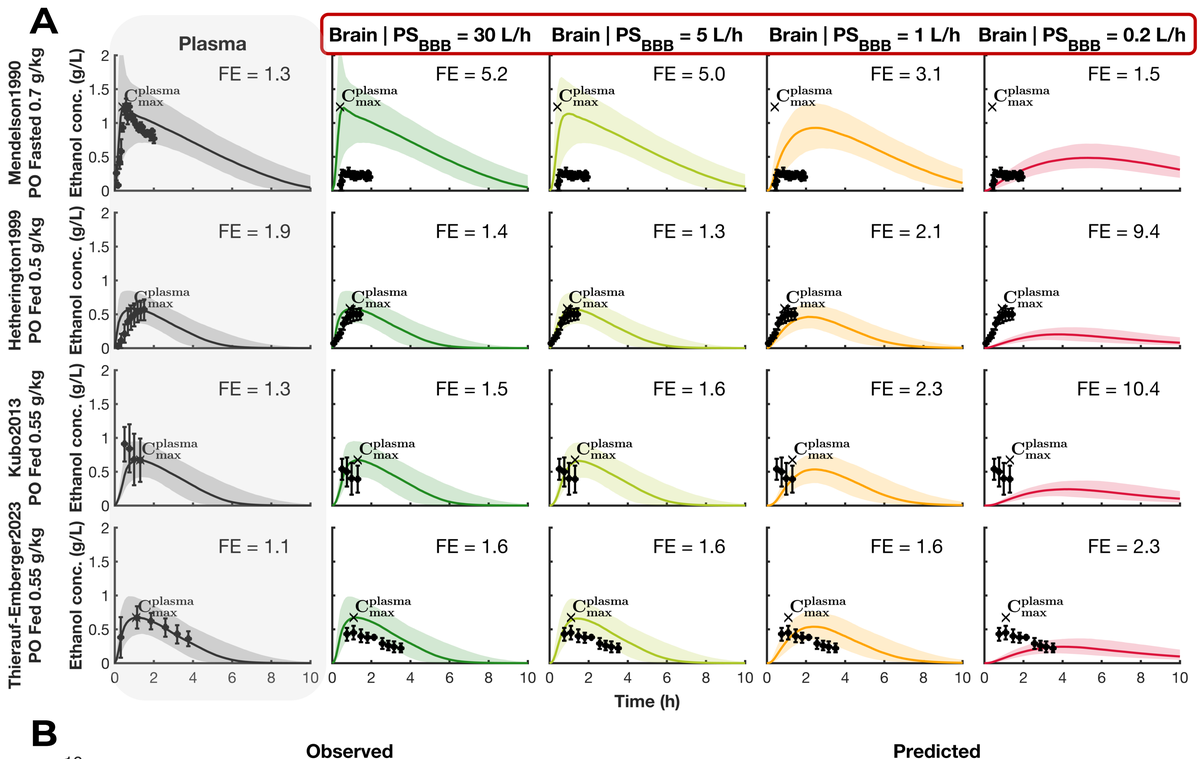


Figure 2. Concentration-time profiles (**A**) and Brain:Plasma ratios (**B**) following oral administration (PO) of ethanol for different PS_{BBB} values. Experimental data were obtained from 4 human studies using magnetic resonance spectroscopy and are presented as mean ± SD. Simulation results are shown as median and 90% prediction interval (PI) based on a virtual population of 1000 subjects. Each color corresponds to a specific PS_{BBB} value. FE is the absolute average error of the prediction based on the averaged data.

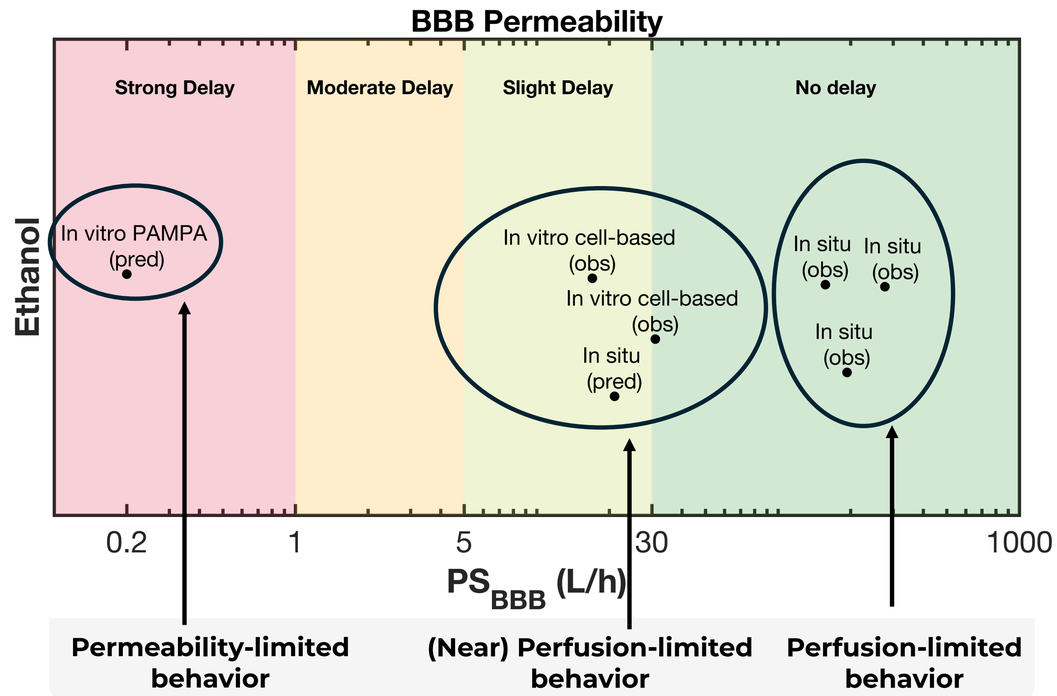


Figure 3. Comparison of the impact of PS_{BBB} values derived from different sources on ethanol brain penetration kinetics.

- Despite high PS_{BBB} variability, 3 out of 4 brain profiles were **predicted within 2-FE**
- **Permeability impact** on ethanol brain exposure was evident only **at PS_{BBB} < 5 L/h**, while **experimental values** ranged **from 17 to 277 L/h**
- Only one **PS_{BBB}** value, **predicted** by **in silico PAMPA** based model, resulted in a **strong brain-plasma delay** and large **prediction error (up to 10-fold)**

CONCLUSIONS

- **PS values** for BBB passive compounds can vary by up to **4 orders of magnitude**
- For **ethanol,** brain exposure **predictions were insensitive** to **large PS_{BBB} differences** due to **perfusion-limited distribution**
- **In silico PAMPA based** method yielded an **unrealistic PS_{BBB}** prediction for **ethanol**, resulting in **permeability-limited distribution**

REFERENCES and CONTACTS

[1] Gaohua L et al. Drug Metab Pharmacokinet. 2016;31(3):224–33. [2] Saleh M et al. J Pharmacokinet Pharmacodyn. 2021;48:725–741 [3] Bowman C et al. Biopharm Drug Dispos. 2023;44(1):60–70. [4] van Valkengoed et al. J Pharmacokinet Pharmacodyn. 2025;52(16). [5] Avdeef A. John Wiley & Sons. 2012:575-680. [6] Smith Q R et al. Fluid Barriers CNS. 2024;21(1):100. [7] Luco J et al. Current Computer-Aided Drug Design 2006;2(1):31–55. [8] Grumetto L et al. Mol Pharm. 2016;13(8):2808-2816. [9] Int. Comm. on Radiolog. Prot. (ICRP). ICRP Publication 2002;89. [10] Abbott N J et al. Neurobiol Dis. 2010;37(1):13-25. [11] Mendelson et al. Alcohol. 1990;7(5):443-447. [12] Hetherington H P et al. Magn Reson Med. 1999;42(6):1019-1026. [13] Kubo H et al. Magn Reson Med Sci. 2013;12(3):235-240. [14] Thierauf-Emberger A et al. Int J Mol Sci. 2023;24(17):13499.

Acknowledgment

is given to Ralf Engbert and Daniel Schad for valuable discussions on ethanol distribution in the brain.

PAGE meeting

4-6.06.2025, Thessaloniki, Greece

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