

In vitro Brain Exposure Prediction

DMPK R&D



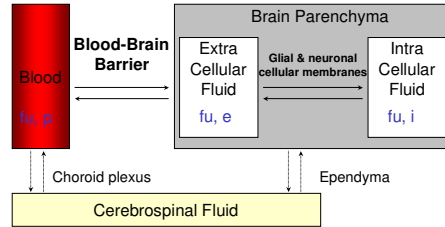
Primary rat *in vitro* tools to predict drug brain concentrations

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Drug kinetics into the brain : Need for Modelization

The unbound fraction of a molecule in plasma (f_u, p) can migrate from the blood to the brain mainly through the Blood Brain Barrier (BBB).

Once into the Brain, the active forms of the molecule are the unbound fraction in the Extra Cellular Fluid (f_u, e), and in the Intra Cellular Fluid (f_u, i) if the molecule can cross the brain cell membranes.



Hypothesis for the modelization : in our PBPK model, we consider that

- ✓ The targeted receptors are located at the surface of the brain cells
- ✓ The fluxes from ECF to CSF and from blood to CSF are negligible
- ✓ Brain distribution is homogeneous and instantaneous
- ✓ Metabolism by the BBB cells and in the brain is negligible

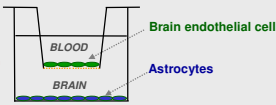
If needed these parameters could be included in the model.

Brain Exposure Prediction to a molecule requires the integration of two fundamental parameters

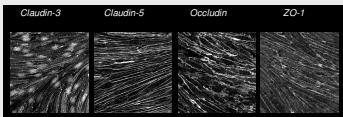
1 Kinetic Clearance of passage through the BBB

In vivo characteristics preserved using our *in vitro* standardized model

- Primary cultures
- Rat syngenic coculture (endothelial cells [RBECs] + glial cells)
- Freshly isolated cells



Tight junction expression, localization and functionality

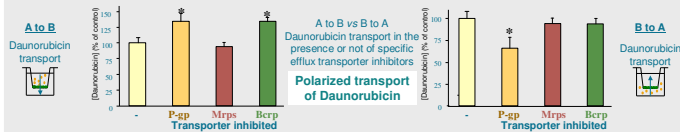


Paracellular permeability ($P_e : 10^{-3}$ cm/min)

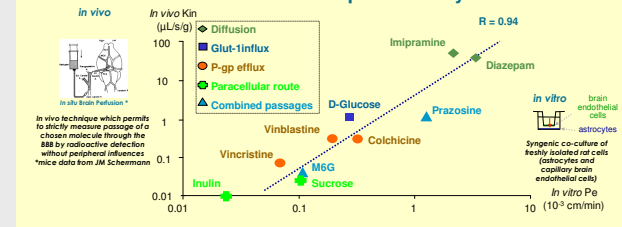
Sucrose	Inulin	Fluorescein	FD4	FD40	FD70
0.10	0.05	0.12	0.05	0.015	0.01

Low permeability values similar to *in vivo*

Transporter expression, polarization and functionality



In vivo / *In vitro* permeability correlation

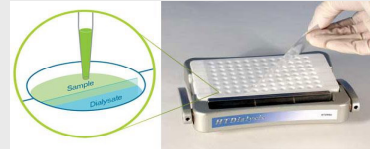


Standardized protocol - Minimization of cell culture variability

- Standardized strain: Cri:OFA(SD) rat, male, 2 weeks old
- Positive selection of brain endothelial cells with BBB phenotype
- Seeding on inserts at D4 with a defined cellular density
- Permeability study realized at D10

2 Thermodynamic Brain/Plasma Partition Coefficient

Equilibrium dialysis with plasma and brain homogenates (both from rat)

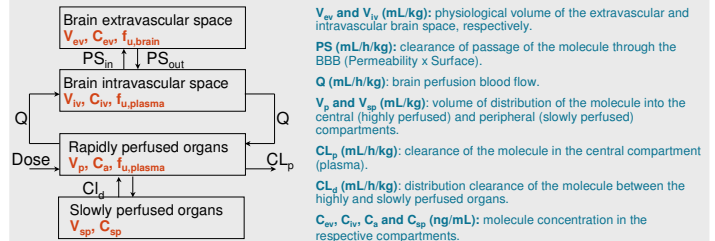


- High-throughput
- Rat brain homogenates, from the same animals
- 96 wells format, assays in triplicates

Plasma and brain homogenate are dialyzed against PBS buffer pH 7.4, during 5 h at 37 °C and under shaking, molecule assay performed by LC-MS/MS

$$K_p = \frac{f_u \text{ plasma}}{f_u \text{ brain}}$$

Brain Exposure Prediction Calculation Methodology



Mass balance equations of the PBPK model

Extravascular route, first order absorption, no slowly perfused organs

$$\frac{dDose}{dt} = -ka \cdot Dose$$

$$V_p \cdot \frac{dCa}{dt} = ka \cdot Dose - CL_p \cdot Ca - Q(Ca - C_{iv})$$

$$V_{iv} \cdot \frac{dC_{iv}}{dt} = Q(Ca - C_{iv}) - PS \cdot f_{up} \left(C_{iv} - \frac{C_{ev}}{K_p} \right)$$

$$V_{ev} \cdot \frac{dC_{ev}}{dt} = PS \cdot f_{up} \left(C_{iv} - \frac{C_{ev}}{K_p} \right) \quad \text{with} \quad K_p = \frac{f_u \text{ plasma}}{f_u \text{ brain}}$$

Initial conditions:
Dose = Dose
Ca = C_{iv} = C_{ev} = 0

The Must in time-course Brain exposure prediction

- Physiological *in vitro* model that maintains *in vivo* BBB specific properties
- *In vitro* Brain permeability data, correlated to the most relevant *in vivo* BBB model (*In Situ Brain Perfusion*) independent of the passage mechanisms
- High reproducibility

Full in house single integrated package
Standardized protocols and animal supply

- *In vitro* f_u of the test molecules in both plasma and brain under a high throughput format
- Simulation of brain and Blood/Plasma concentration time-courses using the most complete and versatile Brain PBPK model available to date, integrating the fundamental brain and plasma f_u parameter

Support to medicinal chemists via several key parameters:
 K_{el} , K_{out} , K_p , half-lives to plateau in the brain, f_{up} .

Full *in vitro* system with relevant primary cells & tissues
We pride ourselves on fast experimental results delivery

Publications

Perrière N. et al. *J Neurochem.* 2005; Perrière N. et al. *Brain Res.* 2007; Kalvass J. & Maurer T. *Biopharm Drug Dispos.* 2002; Liu X. et al. *JPET* 2005

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