Using continuous intravenous microdialysis sampling as an alternative to free fraction pharmacokinetics determination in plasma

JHA Folgering[1,2*], G Flik[1] and TIFH Cremers[1,3]

[1]Brains On-Line BV, Groningen, the Netherlands
[2]Encepharm Gmb, Göttingen, Germany
[3]University of Groningen, Research Institute of Pharmacy (GRIP), Groningen, The Netherlands

Introduction:

Plasma pharmacokinetic studies are based on total plasma concentrations in combination with ex-vivo free fraction determination by equilibrium dialysis. Here we present a method for direct continuous intravenous microdialysis sampling of free compound concentration in plasma. The method can easily be combined with plasma collection (for total plasma concentration), brain microdialysis and/or CSF sample collection. Remainder of collected samples may be used for pharmacodynamic studies as well (reducing the need of a separate animal study).

This makes it possible to compare multiple-compartment pharmacokinetics within a single species and across a range of species (rat-primate-human). The sampling method also strongly reduces the number of animals required compared to classical pharmacokinetics studies of compounds. In addition, we show a method for determining the in-vivo free concentration of a compound, which may be of more physiological relevance than the ex-vivo free fraction determined by total plasma sampling combined with rapid equilibrium dialysis.
Continuous plasma sampling with microdialysis can be performed without interaction with the animal except during compound administration, which reduces the handling stress during experiments.

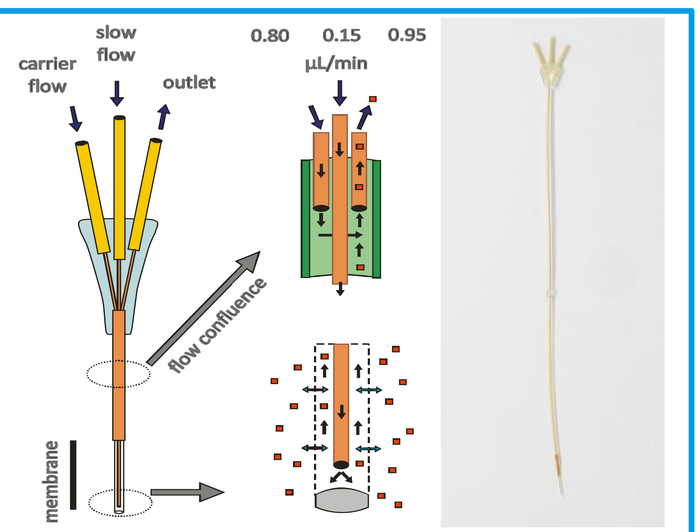

Figure 1 – Vascular MetaQuant™ microdialysis probe (Brainlink, the Netherlands)

Methods:

Modified intravenous slow flow microdialysis allows for compound exchange over a semipermiable membrane implanted in soft-tissue or in the lumen of a vein/artery. By application of the slow flow a near 100% recovery of compound over the membrane can be established. By combining the slow flow with a carrier flow it is still possible to collect sample volumes large enough for bio-analysis by LC-MS/MS.
After implantation of the Vascular MetaQuant™ (Brainlink, the Netherlands) it is possible to combine with a catheter in a second vein, a MetaQuant™ in the prefrontal cortex (for collection of brain microdialysate and/or, a probe in the cisterna magna for CSF collection. Further microdialysis probes can be implanted in other organs of interest.
After recovery from surgery microdialysis probes are perfused for sample collection. Blood samples are collected manually or automated by a DiLab Accusampler system.

Conclusion:

- Compound free-fraction pharmacokinetics in-vivo can be easily determined by application of Vascular MetaQuant™ microdialysis. The method can be combined with similar pharmacokinetic data from other compartments.
- The method results fast in-vivo plasma binding data.
- REFINEMENT: samples can be collected with limited interaction with the animals.
- REDUCTION: combining data from several organs/compartments, can significantly reduce the number of animals required for pharmacokinetic profiling.

Results:

CASE 1

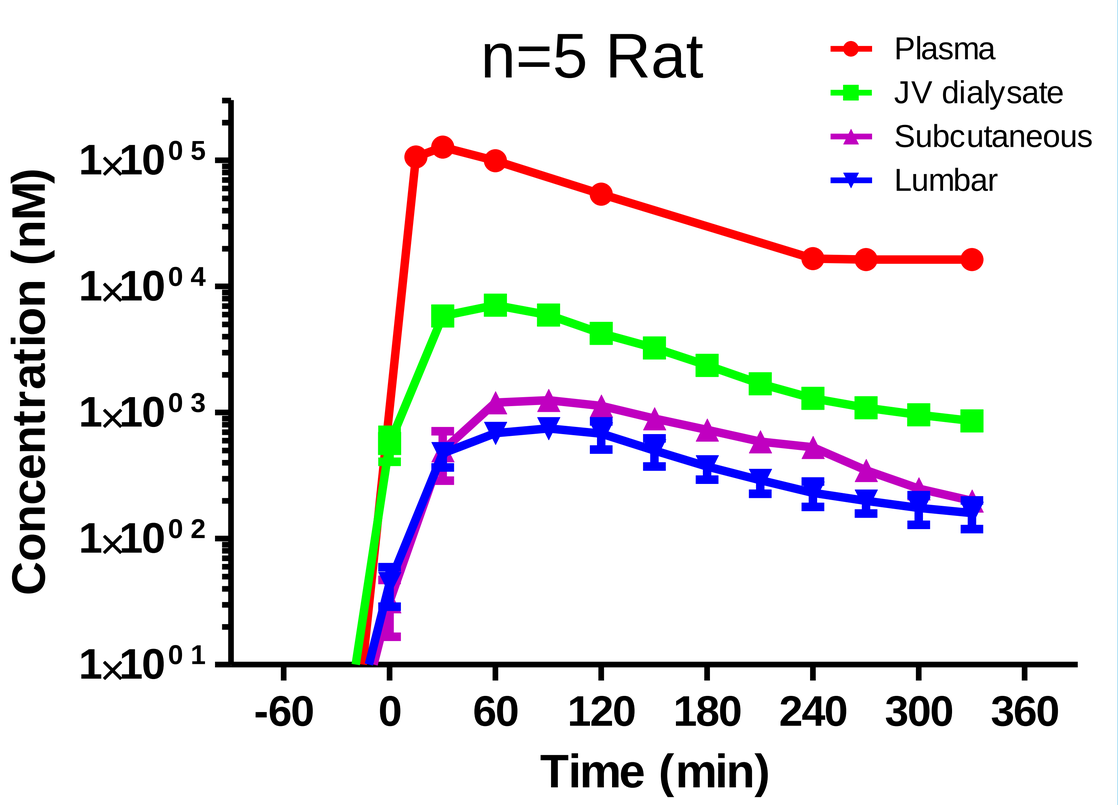

Figure 2 – Multi-compartment sampling in anesthetized rat. All animals received 30 mg/kg of compound P i.p. at t= 0 In-vitro plasma free fraction was reported to be 3-5%. In-vivo plasma free fraction was found to be 5.4%.

CASE 2

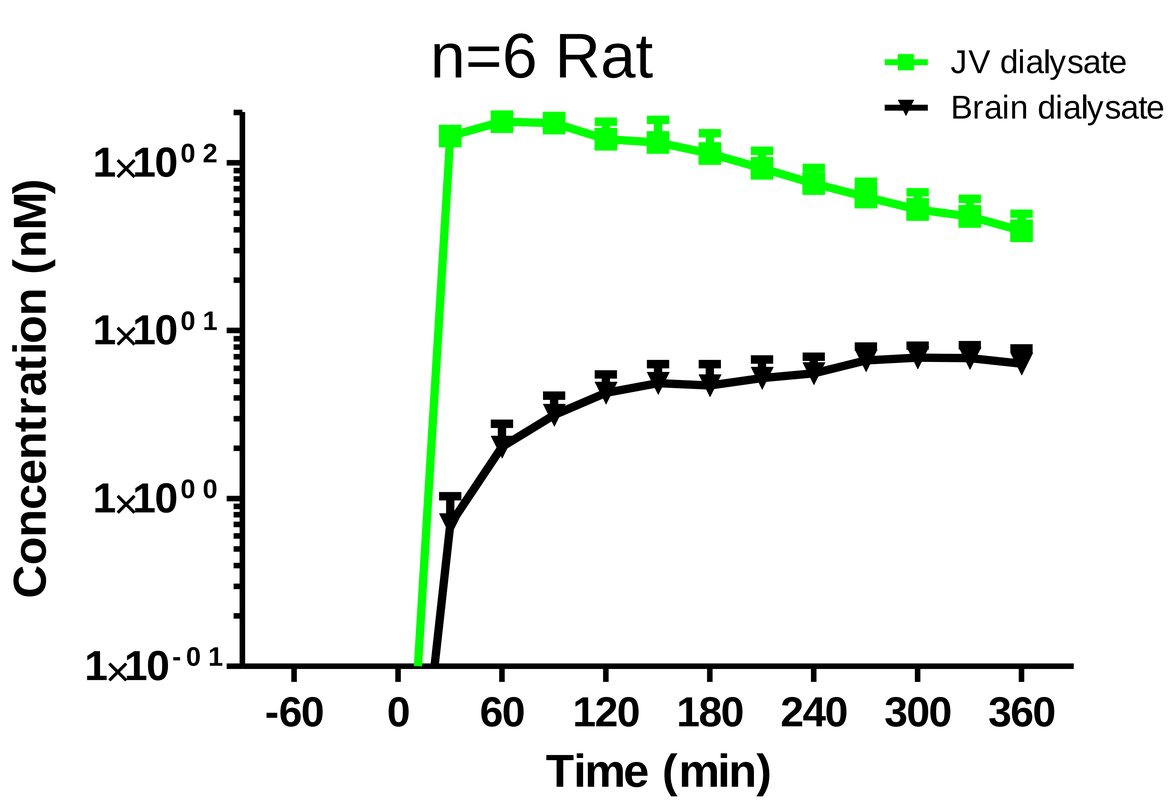

Figure 3 – Multi-compartment sampling in freely moving rat. All animals received 30 mg/kg of compound M 250 mg/kg p.o. at t = 0. In-vitro plasma free fraction was reported to be 1% (equilibrium dialysis and spin-fliter). In-vivo plasma free fraction was found to be 0.035% (based on terminal total plasma). Brain free fraction was 0.002% (based on terminal tissue). Plasma : brain ratio was about 6:1

CASE 3

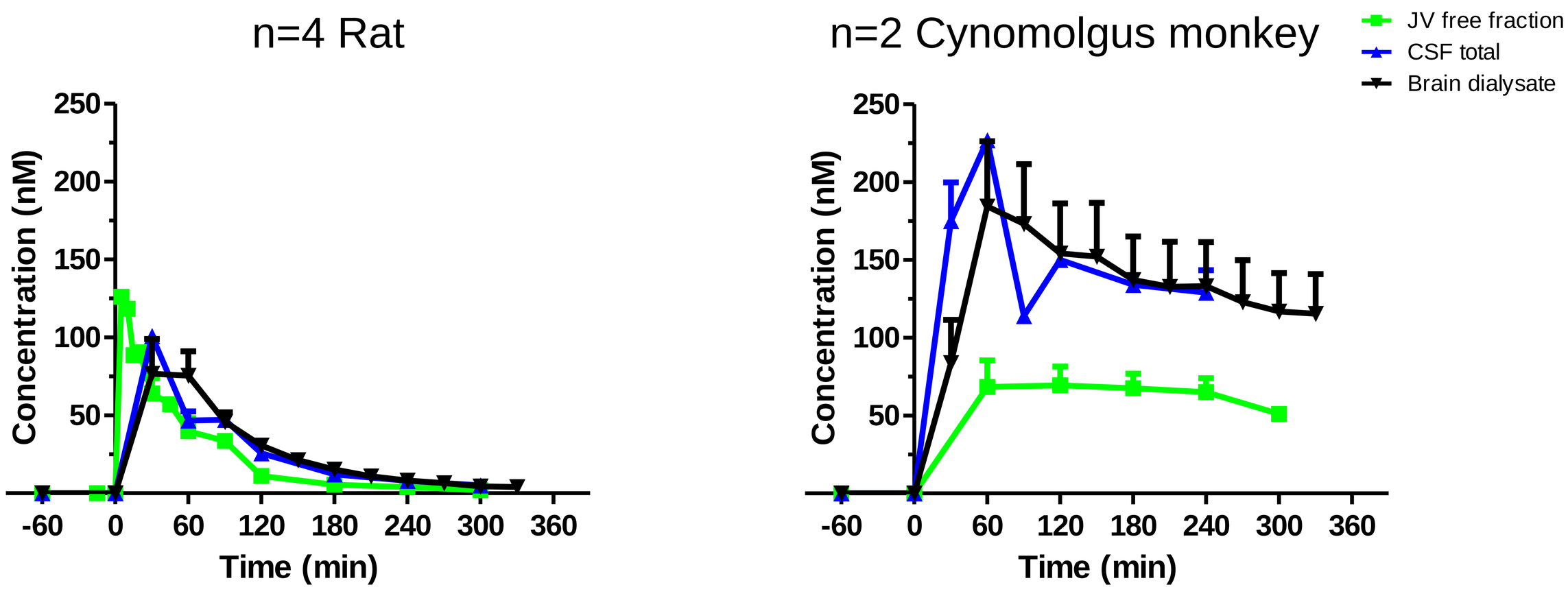

Figure 4 – Multi-compartment sampling in different species. All animals received 0.1 mg/kg amphetamine i.v. Even with low animal numbers PK of compound free fraction shows relatively low variation and very clear inter-species difference in PK. Remainder of brain dialysate was used for determination of monoamine levels (data not shown).