

Figure 1.: Schematic representation of a brain probe for preclinical use.

analytes. Smaller molecules in the tissue - including the **non-protein bound fraction of drug content** in the extracellular fluid - will passively diffuse across the surface of the membrane and thus enter the flow of the perfusate, which is sampled at regular intervals and analyzed either on-line or off-line. Over the last decade *in vivo* microdialysis (MD) has been increasingly applied to monitor drug distribution at peripheral tissue sites and the **penetration of various agents across the blood brain barrier** (Zhou and Gallo, The AAPS Journal 2005, Helmy et al., Current Medicinal Chemistry 2007). Applications of MD are being explored to address specific safety issues and MD data are likely to become an important part of new drug submissions to drug regulatory agencies (Chaurasia et al., The AAPS Journal 2007).

Microdialysis techniques have been developed to monitor changes in the chemistry of the extracellular space in living tissue. These techniques can be used for: a) **measuring drug and metabolite concentrations** in the interstitial space in brain and peripheral tissues including blood; b) continuous **monitoring of neurotransmitter release** to various stimuli; c) measuring concentrations of many analytes associated with tissue damage in CNS and in various organs. The microdialysis techniques require the introduction of an ultrathin, semi-permeable tube, a so-called probe in the tissue (Fig. 1) The probe is connected to a precision pump, which provides a steady flow of a tissue-compatible fluid through the probe at a very low flow rate (1–5 µl/min). Open circles on Fig. 1 depict the various endogenous compounds in the extracellular fluid or in blood; the closed circles depict exogenous compounds (**drugs to be tested** or calibrators for determination of the *in vivo* recovery) which can be delivered by the perfusion fluid. Squares represent extracellular macromolecules that may bind

BLOOD BRAIN BARRIER (BBB) PACKAGE

SOLVO's new microdialysis service is an integral *in vivo* part of our BBB package. The package consists of an assortment of crossvalidated *in vivo* and *in vitro* tools for studying the drug-transporter and/or drug-drug interactions in this important barrier. The BBB package focuses on two key efflux transporters, MDR1 and BCRP. **Membrane based ATPase and vesicular transport assays** represent a HTS assessment that provide transporter specific data to confirm *in vivo* detected interactions. **Living cell based BBB models**, like primary rat brain capillary endothelial cells (RBEC) are also validated with specific interactors and dedicated to explore efflux transporter related inter-species differences.

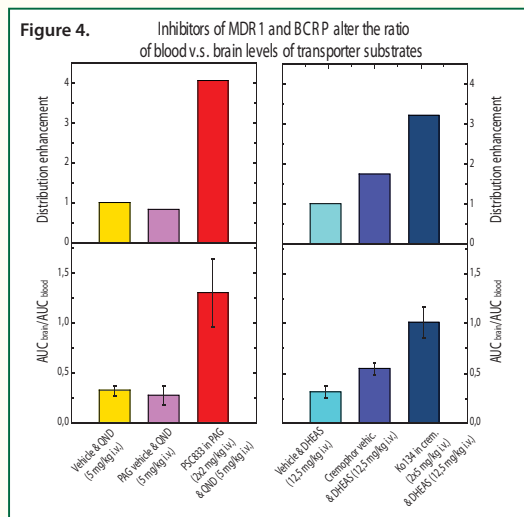
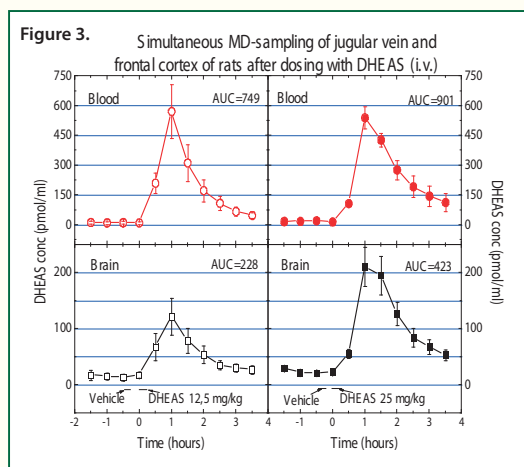
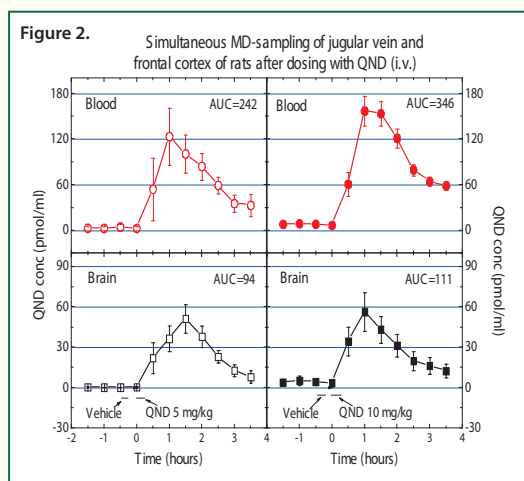
REFERENCE SUBSTRATES USED IN MDR1 AND BCRP DATA VALIDATION

The reporter substrate for BCRP is dehydroepiandrosterone sulfate (DHEAS). DHEA is a natural steroid prohormone, while DHEAS – its sulfated form – acts as a reservoir for the prodrug. It has been described as a BCRP substrate by several papers¹⁻³. BCRP transports both hydrophobic and hydrophilic compounds among which has a preference for sulfate conjugates. The **reference inhibitor** for BCRP-mediated BBB penetration of DHEAS *in vivo* is **Ko134, a BCRP-specific transporter inhibitor**⁴.

MDR1 is the most commonly studied efflux transporter in the BBB. The **reporter substrate** in SOLVO's microdialysis package, Quinidine (QND), has first become known for its interaction with digoxin absorption in the intestine⁵, but it was also revealed that QND is indeed a transported substrate of MDR1⁶. QND is an antiarrhythmic drug producing many adverse effects when coadministered with other Pgp interactors. The **reference inhibitor PSC 833 (Valspodar)** is a second generation multidrug resistance-reversing agent⁷. It was reported to increase the brain penetration of colchicine in a study using brain and blood microdialysis⁸.

1. Suzuki et al JBC 2003;278:22644	2. Glavinas et al DMD 2007;35:1533
3. Grube et al DMD 2007;35:30	4. Allen et al Molecular Cancer Therapeutics 2002, 1:417
5. Kondo et al Pharm Res 2004;21:1895	6. Muller et al FEBS Letters 1994;343:168
7. Pourtier-Manzanedo et al Anti-Cancer Drugs 1992;3:419	8. Desrayaud et al Life Sciences 1997, 61:153

Types of microdialysis studies performed at Solvo



I. Determination of brain penetration of test molecules in rats: We have the capability to perform **double microdialysis experiments** using anesthetized rats to simultaneously assess unbound levels of test molecules in blood and in brain. We implant a femoral vein catheter for dosing; peripheral and brain microdialysis probes for sampling blood and brain. After the implantation of the femoral vein catheter and a jugular vein probe, the rat is placed in a stereotaxic frame for implanting a brain microdialysis probe in the specified brain region and for running the microdialysis (MD) experiment. Vascular and brain MD sampling are performed 90 min before and 210 min after dosing the animal intravenously (i.v.). This type of double MD experiment is shown in Fig. 2 and Fig 3 using two doses of QND and DHEAS. In these experiments CMA/20 probes were used for vascular, and CMA/12 probes for brain sampling with a perfusion rate of 1.0 μ l/min. Prior to *in vivo* MD experiments on new compounds, *in vitro* experiments are performed to check the adsorption of the compound to the tubing and to determine the recovery of the probes for the test compound. Upon request *in vivo* recovery of probes can be also determined.

II. Determination of test molecule interactions with transporters:

II/1: This type of *in vivo* MD studies are performed if *in vitro* transporter interaction or other studies suggest (see section-2) that a test molecule might be an **inhibitor** of a specific efflux transporter. At least 3 groups will be used (n=5 per group): **Group-1:** animals receive a vehicle injection at -20 min and a reference substrate injection at 0 time; **Group-2:** animals receive an injection of a test molecule at -20 min and a reference substrate at 0 time; **Group-3:** a reference inhibitor of the transporter at -20 min and a reference substrate injection at 0 time. Two doses of test molecule/reference inhibitor/reference substrate will be used upon request. Results of experiments of this type using PSC-833 and Ko-134 as inhibitors are shown on Fig. 4. **II/2:** This type of *in vivo* MD studies are performed if *in vitro* transporter interaction or other studies suggest (see section-2) that a test molecule might be a substrate of a specific efflux transporter. At least 4 groups will be used (n=5 per group): **Group-1:** vehicle injection at -20 min and a test molecule injection at 0 time; **Group-2:** an injection with a reference inhibitor of the transporter at -20 min and a test molecule at 0 time; **Group-3:** vehicle injection at -20 min and a reference substrate injection at 0 time; **Group-4:** injections with a reference inhibitor of the transporter at -20 min and with a reference substrate at 0 time. Two doses of test molecule/reference inhibitor will be used upon request.

III. Determination of brain penetration of test molecules and simultaneous monitoring of neurotransmitter release in specified brain regions: Upon request, **brain probes** will be placed in anesthetized rats into brain regions that are rich in specific neurotransmitters. Aliquots of brain dialysate samples and blood dialysate samples will be analyzed for test molecules; further aliquots from the same brain samples will be analyzed for specific neurotransmitters to explore possible drug interactions and/or adverse reactions. Water soluble drugs/test molecules can be applied by retrodialysis if it is requested.

In vivo MD studies currently available: Double MD studies described above in anesthetized rats with i.v. and/or i.p. drug administration; Double/triple MD studies described above in rats with i.p. or p.o. administration using a movement-responsive animal system; Brain MD studies in rats with i.p. or p.o. treatment using CMA/120 system for freely moving animals.

Additional MD studies available from Q4 in 2009: Double MD studies in anesthetized mice with i.p. drug administration; Brain MD studies in mice with i.p. drug administration using a mouse MD system from Linton Instrumentation.



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