

Review Article

Theme: Integrating Microdialysis and Imaging Tools in Systems Pharmacology
Guest Editors: Robert E. Stratford, Nimita Dave, and Richard F. Bergstrom

Microdialysis as an Important Technique in Systems Pharmacology—a Historical and Methodological Review

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Abstract. Microdialysis has contributed with very important knowledge to the understanding of target-specific concentrations and their relationship to pharmacodynamic effects from a systems pharmacology perspective, aiding in the global understanding of drug effects. This review focuses on the historical development of microdialysis as a method to quantify the pharmacologically very important unbound tissue concentrations and of recent findings relating to modeling microdialysis data to extrapolate from rodents to humans, understanding distribution of drugs in different tissues and disease conditions. Quantitative microdialysis developed very rapidly during the early 1990s. Method development was in focus in the early years including development of quantitative microdialysis, to be able to estimate true extracellular concentrations. Microdialysis has significantly contributed to the understanding of active transport at the blood-brain barrier and in other organs. Examples are presented where microdialysis together with modeling has increased the knowledge on tissue distribution between species, in overweight patients and in tumors, and in metabolite contribution to drug effects. More integrated metabolomic studies are still sparse within the microdialysis field, although a great potential for tissue and disease-specific measurements is evident.

KEY WORDS: microdialysis; pharmacodynamics; pharmacokinetics; pharmacology; recovery methods; systems.

INTRODUCTION

Microdialysis has since its introduction been instrumental in improving the understanding of drug distribution into tissues in vivo and thereby contributed to target site measurement of concentrations to aid in relating concentrations and effects. The method has significantly improved drug pharmacokinetic measurements from relying on discrete plasma and whole tissue samples to more informed results on the pharmacologically more important unbound concentrations. It has specifically contributed to quantifying the role of drug transporters, where the brain is in particular focus. The development of microdialysis came in parallel to the development of highly sensitive analytical techniques, enabling quantification of low concentrations in small size samples.

Therefore, an important role of microdialysis in systems pharmacology is to measure and identify target site concentrations that may differ from plasma concentrations due to distributional consequences of transporters and other processes and in relating these concentrations to pharmacodynamic measurements (1–3). A potential and valuable role of microdialysis can be to use the technique as a local sampling device in metabolomics studies, thereby finding fingerprints of disease and the pharmacological interventions, and relate this to drug concentrations in the same tissue(s). However, such studies are still sparse and require state-of-the-art analytical equipment (4,5). Modeling, being an important part in systems pharmacology analysis, has been used rather extensively in microdialysis studies to refine the raw data and interpret the findings (1–3,5–14).

The unique feature of microdialysis is thus that it samples local unbound concentrations, thereby making it possible to directly relating tissue concentrations to pharmacological effects. A very valuable property is that repeated sampling can be performed in one animal with high time resolution, thereby saving animals and at the same time obtaining more information. It is made without the loss of

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fluid that is otherwise critical when sampling blood from rodents, which is also advantageous. The technique is rather advanced and time-consuming; however, direct information on drug distribution of unbound drug concentrations is not possible to obtain with any other technique. Issues related to adsorption of lipophilic drugs to tubings and probes are the main drawback of the method, hampering quantitative understanding and giving erroneous concentration-time profiles if not addressed.

Several review articles and book chapters are published on the use of microdialysis for quantitative studies of drugs. The references presented here are not complete but give a picture of the area (15–27).

HISTORICAL ASPECTS OF MICRODIALYSIS

The microdialysis field started in the early to mid 1980s with studies of neurotransmitters, an area having contributed to the major part of the microdialysis publications during all years. Human studies were performed already from early days and compose 24% of the microdialysis publications. Of all studies, 72% have focused on the brain, while the rest have studied peripheral phenomena.

In 10 years' time from 1985 to 1995, the number of articles on microdialysis exploded from 2 per year to over 600 per year, the reason being that the method made it possible to study neurotransmitter release in local brain regions *in vivo* in response to pharmacological interventions (Fig. 1). The number of microdialysis publications displayed a peak with 700–800 publications per year in the late 1990s–early 2000s and has since then balanced out to around 400–500 publications per year. One of the explanations to the decline is the increased use of other methods for neurochemical detection, including fluorescence methods and whole-brain imaging.

Microdialysis studies on tissue distribution of drugs came a bit later and are still a smaller part of all microdialysis publications, although being extremely important for systems pharmacology. The two first articles measuring an administered drug came in 1988 on cocaine brain extracellular concentrations (28,29). Of the 132 articles from 1989 on PubMed, only two measured exogenous compounds (30,31). The area exploded from 1990 with several groups embracing the technique to study drugs. Groups who were pioneering the field include the one led by Tsuji in Japan (32,33). Other early groups were the ones headed by Sawchuk (34) and Lunte (35,36) in the USA, Ungerstedt (37) and our group (38) in Sweden, and also Breimer and de Lange (39) in the Netherlands, as well as Michotte (40) in Belgium.

Early methodological papers presented important principles of microdialysis, including solute exchange across the semi-permeable membrane, and highlighting the role of quantitative measurements as well as time aspects of the measurements (41–47). Theoretical aspects have been pursued further, all relying on the early papers (15,48–53).

THE TECHNIQUE OF MICRODIALYSIS

Microdialysis is performed with a probe comprised of tubings and a semi-permeable membrane placed in the tissue of interest (Fig. 2). A constant flow of perfusion fluid (perfusate) is pumped through the probe. Driven by passive diffusion, solutes diffuse into the probe and are sampled at

the end of the outlet tubing (dialysate), outside of the animal/human. In cases when the concentration is higher in the perfusate than in the extracellular fluid, the net movement of solute instead goes from the perfusate to the tissue, something that is used in the retrodialysis calibration method (see below).

Microdialysis Probes

Probes used today are generally i-shaped, so-called cannula style, as in Fig. 2. Linear probes are used, e.g., in dermal microdialysis. Loop probes are also available but less common. The latter can be used in larger animals and can have longer membranes. Shunt probes, for example bile duct cannulation, are also available. The materials in commercially available probes are cuprophane, polyarylethersulphone (PAES), polyethersulphone (PES), polyurethane, or cellulose. Inlet and outlet tubings are made of polyurethane, polyethylene (PE), teflon (FEP), polyetheretherketone (PEEK), or polyimide.

The cut-off of the membranes can be 6 kDa (cuprophane, PES), 15 kDa (PES), 20 kDa (PAES), 30 kDa (PAN), 35 kDa (PES, cellulosic), 55 kDa (PES), or 100 kDa (PES). There are also newer probes with cut-off of 1 or 3 MDa (PE). Outer probe diameters are 0.2–0.6 mm and the length varies from 1 to 10 mm. Clinically used probes are generally called “catheters.” Hsiao *et al.*, in a comparative study of different probe materials, concluded that the tissue properties are more important than the probe material when performing *in vivo* microdialysis of small molecules, as the main factor limiting extraction is tissue resistance to diffusion (45). Having said that, the probe and tubing materials are important regarding possible adsorption of the solute of interest (see 3.3).

Quantitative Microdialysis

Flow Rates and Probe Lengths

Exchange across the semi-permeable membrane will reach different degrees of equilibrium depending on how fast the buffer flows through the probe. Common flow rates are 0.3–2 $\mu\text{l}/\text{min}$. The lowest flow rate is used in clinical settings when measuring lactate, pyruvate, glucose and such, to ensure that the concentration inside the probe is as close as possible to that in the extracellular fluid. Flow rates of 0.5–2 $\mu\text{l}/\text{min}$ are used in preclinical studies, choosing the higher flow rates when more frequent sampling is needed, with the cost of obtaining a lower recovery across the probe membrane. This requires measuring the recovery *in vivo* for quantitative measurements. The more recently developed MetaQuant probe (PES membrane with a molecular weight cut-off of 18 kDa) has a specific construction with an ultraslow flow rate of $<0.2 \mu\text{l}/\text{min}$ at the membrane, increasing the recovery to close to 100% (54). This low flow is supported by a higher carrier flow at the exit from the semi-permeable part of the probe to remove the dialysate faster and thereby making it possible to sample more frequently and with higher volumes, aiding in the time resolution and chemical analysis of the samples.

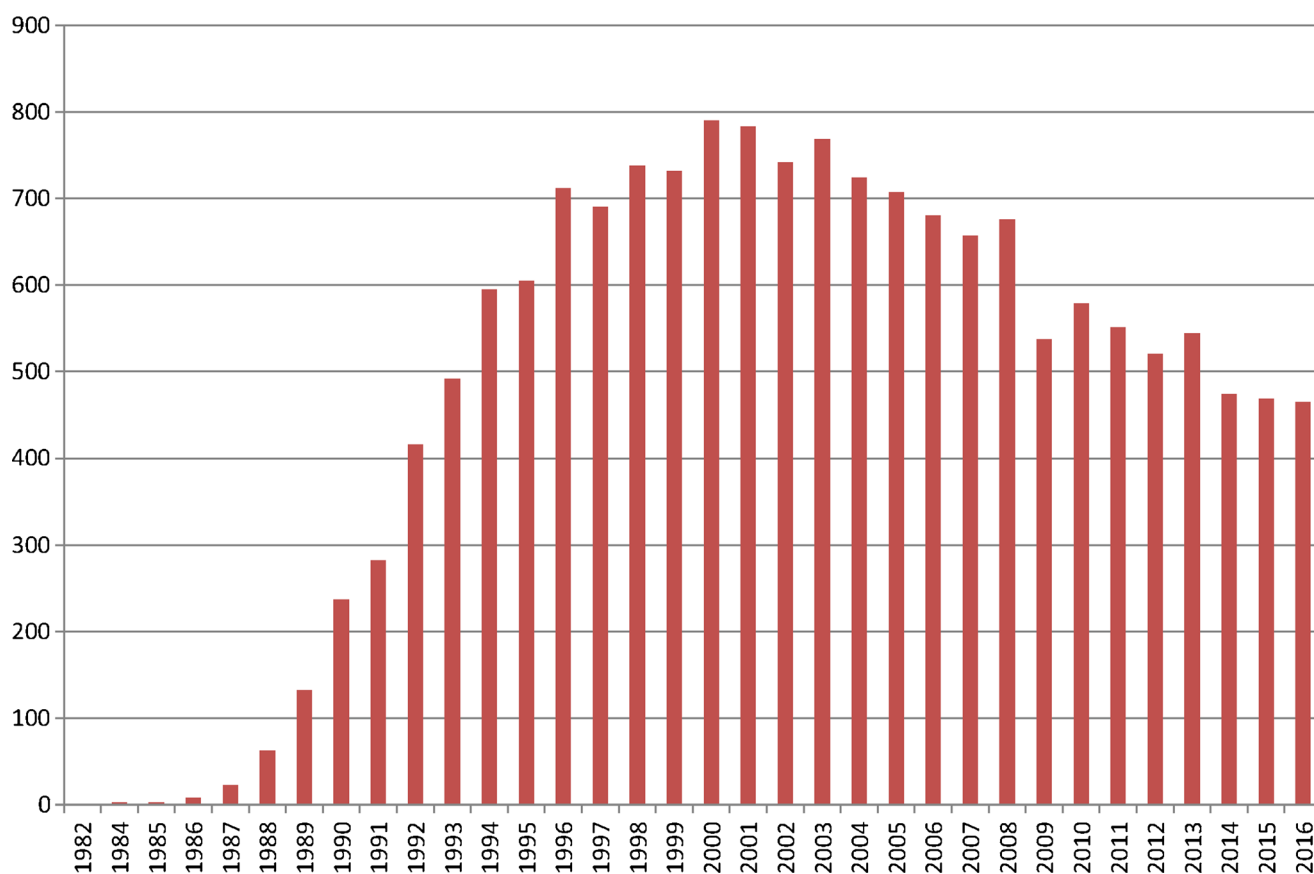


Fig. 1. Number of publications per year on “microdialysis,” of which 71% are brain microdialysis studies and 24% are microdialysis studies in humans, according to information on PubMed

The exchange across the microdialysis probe is, apart from the flow rate, determined by the length of the semi-permeable membrane of the probe (increasing length gives increasing degree of exchange), as well as of the probe material and the cut-off of the membrane. Depending on the tissue studied, it is difficult to influence the probe length. When measuring CSF concentrations in cisterna magna in rats, only a 1-mm probe length is possible (55), while a 3-mm probe length can be used in striatum, and a 10-mm probe length in vena jugularis (56).

In Vitro Preparations and Adsorption to Tubings and Probe

The major disadvantage with microdialysis when studying drugs is that the material in the probe and tubings may adsorb the drug. This is not the case when studying neurotransmitters as they are generally much more hydrophilic. Adsorption will influence the concentration in the dialysate so that the dialysate will not mirror the extracellular fluid concentration with time. There will be a time delay, and observed concentration-time profiles will be more a result of delayed “tubing pharmacokinetics” than real tissue behavior.

In vitro preparations are therefore recommended before starting *in vivo* microdialysis studies to save time and to ascertain good quality of the results. This is performed by measuring (1) outlet tubing concentrations with time and (2) loss and gain across the probe in relation to a buffer solution of the drug of interest. The optimal performance is a direct change in concentrations when the buffer solution is changed from blank to one containing the drug or

the opposite. Alternatives to solving an adsorption problem are to test different tubing materials that may act differently and/or to add 0.25 or 0.5% albumin to the perfusate. Woo and Lunte concluded that the microdialysis behavior of some compounds improved after adding albumin, but not all, and that lipophilicity per se was not the issue, making the problem difficult to solve (57). If the measures of adding albumin or changing tubing material do not solve the problem, it is recommended not to perform microdialysis. More detailed recommendation on how to address the problem with adsorption can be found in the AAPS-FDA workshop white paper (16), as well as in (18,24,57,58). However, in the paper by Nirogi *et al.*, a 60-min pre-equilibration was used, which may hide possible adsorption (58).

If protein is needed in the perfusate in order to prevent sticking to tubing and probe material, it may influence the loss and gain relationship and thereby give erroneous recovery estimations. This has been found for highly protein-bound drugs, while drugs with lower degree of protein binding do not seem to be influenced (unpublished observations). The same phenomenon has been found by others (59,60). It can thus be concluded that albumin present in the dialysate will likely influence recovery of highly protein-bound drugs but not be of importance for drugs that bind to a lower extent to proteins.

Influence of Tissue Factors on the Recovery

Early papers described important aspects of how quantitative microdialysis *in vivo* works in relation to

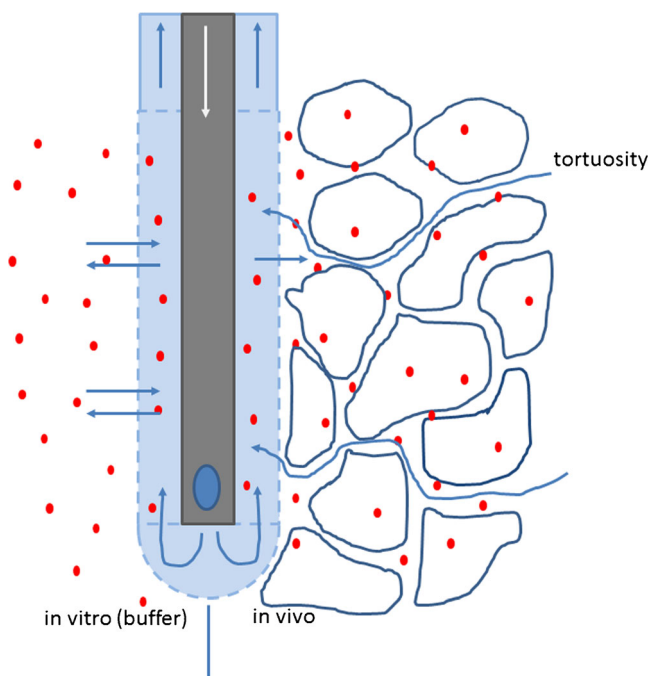


Fig. 2. Movement of molecules across the semi-permeable microdialysis probe membrane *in vitro* and *in vivo*. Flow of perfusate is from the inner metal shaft out through the hole (blue oval) and then along the membrane with collection of dialysate outside of the body. In a buffer solution, the molecules can move freely, while in the tissue, there is tortuosity, as the molecules need a longer path to reach the probe due to the presence of cells. The volume fraction is also smaller

concentrations in the probe and the surrounding tissue, and that *in vitro* recovery is not sufficient for *in vivo* estimations of extracellular concentrations (42,44–47,61). This is due to the difference in movement of molecules in the tissue vs in a buffer solution (Fig. 2), where the rate of movement will determine the recovery, e.g., through active transport at the blood-brain barrier or at another site. This indicates that influencing active transport will also influence the recovery.

An important paper was written by Smith and Justice on how different processes *in vivo*, like inhibition of synthesis, release, metabolism, and uptake, influenced the recovery of dopamine (62). The only process that influenced the recovery was the elimination of dopamine from the site of measurement, where inhibition of reuptake decreased the recovery. Another study showing the same phenomena found that serotonin and norepinephrine recovery was only influenced by changing the clearance rate from the measurement site (63). These principles are also valid for exogenous compounds like drugs, shown by de Lange *et al.* to be of importance for the interpretations of the results (64). Thus, if interventions are performed in the *in vivo* studies, e.g., blocking an active transporter, this has been shown to drastically influence the size of the recovery (64–67). In order to obtain correct results, recovery thus has to be measured throughout such a study. Also, if comparing results from different strains of rats, e.g., P-glycoprotein knock-out mice compared with wild-type mice, there will be differences in recovery between the two groups, making it necessary to measure recovery (64,68). The recovery will also vary depending on in which tissue the probe is placed due to different tissue properties (69).

Another important study described inhibition of efflux transport at the blood-brain barrier and its influence on the recovery, comparing experimental results and mathematical models of transporter kinetics (70). The authors conclude that “only in certain circumstances will efflux inhibition at the blood-brain barrier and blood-cerebrospinal fluid barrier influence the microdialysis probe recovery, and this may depend upon the substrate and inhibitor examined and their routes of administration, the localization and mechanism of the membrane transporter, as well as the microenvironment surrounding the probe” (70). Thus, it is crucial to always measure recovery, preferably during the whole study, to ensure correct estimation of true extracellular fluid concentrations of the solute of interest. Thus, *in vitro* recovery measurements do not map the recovery *in vivo*.

RECOVERY METHODS

Quantitative estimations of extracellular fluid concentrations require recovery estimations. Recovery can be equated with the extraction efficiency across the probe membrane according to Eq. 1:

Extraction efficiency

$$= \frac{\text{Concentration in dialysate}}{\text{Concentration in the surrounding tissue or buffer}} \quad (1)$$

Several methods have been developed to estimate recovery. They can be divided into three different categories:

Flow Methods

Flow methods are extrapolation-to-zero flow (71) and the modified ultraslow microdialysis (54). The flow rate methods all assume that at a really slow flow rate, the contact time between extracellular fluid and perfusate is enough to fully equilibrate. These methods thus do not need any further recovery calculations.

No-Net-Flux Methods

The no-net-flux methods are the no-net-flux method (71) and the dynamic no-net-flux method (68,71,72). While the no-net-flux method requires constant concentrations of the solute of interest in the extracellular fluid, the dynamic no-net-flux method can estimate recovery when concentrations are changing. Several concentrations are needed in the incoming perfusate to find the point of no-net-flux across the probe membrane. A between-group design rather than a within-group design was described by Olson and Justice for transient conditions (72).

As there are always neurotransmitters present *in vivo*, the methods of no-net-flux are suitable. They were therefore early investigated (65,73). The dynamic no-net-flux method was used by Parsons *et al.* to study dopamine release after cocaine administration, showing that the recovery changed in relation to when the dopamine levels were measured after cocaine administration (73). This was an important finding illustrating the need for *in vivo* measurements over time when the tissue conditions for movement of solutes change.

Loss = Gain Methods

The loss = gain methods are retrodialysis-by-drug (74) and retrodialysis-by-calibrator (61,75,76) or a combination of the two. Retrodialysis in this context means that the calibrator or drug is added to the perfusate while there is no calibrator or drug present in the tissue. These methods assume that the fractions lost and gained across the probe membrane are the same independent of whether the calibrator or drug is added to the perfusate or in the external buffer in vitro or tissue in vivo.

Retrodialysis recovery is estimated as

$$\text{Retrodialysis recovery} = \frac{\text{Conc}_{\text{perfusate}} - \text{Conc}_{\text{dialysate}}}{\text{Conc}_{\text{perfusate}}} \quad (2)$$

where Conc is the concentration in the perfusate (into the probe) or dialysate (out from the probe).

If the drug itself is used as the calibrator, it has to be studied either before drug administration or when drug concentrations have decreased to a minimum again to avoid contamination. Using other compounds than the drug itself may give erroneous results, especially when active transport properties in the tissue studied differ between the two, something difficult to know prior to the study start. The most elegant method is when a deuterated drug is used as the calibrator, allowing measurements throughout the study and coming as close as possible to the behavior in vivo of the drug studied (75,77).

The retrodialysis methods are the easiest to use and can be used before or throughout the experiment, gaining information about possible changes in the probe behavior with time. A caveat is that at low recoveries (<20%, which is quite often the case), the method calculates a difference between two larger numbers (Eq. 2), which may give calculation errors due to the variability in the chemical analysis of the samples and thereby uncertainties regarding the size of the recovery (76).

Bungay and coauthors studied the theoretical basis for how to calibrate microdialysis probes in vivo regarding pharmacokinetic investigations, where the concentration varies with time (48) and has since also developed further mathematical models for microdialysis (50). Other theoretical papers were written by Chen *et al.* in relation to active clearance processes and using one or dual probes (52,53). Articles comparing different recovery methods can be found in (67,78,79).

STUDY DESIGN IN VIVO

Microdialysis Probe Placement and Tissue Reactions

It is debated when the probe should be inserted in relation to the start of the experiment, as there will be temporary damages in the tissue that is to be measured. Tissue injury when placing the probe in vivo was already mentioned in 1992 by Dykstra *et al.* (47). This has led to experimental strategies allowing for stabilization of the tissue after probe placement. Based on experience from our own lab and from others, it is not enough to exchange the dummy

probe with the measuring probe just before the start of the experiment. This is especially important for the brain where drug concentrations in brain and blood can be very different. A leakage of the BBB due to the probe placement will profoundly influence the obtained concentrations. We found that 3 h was not enough between probe placement and experiment when studying blood-brain barrier transport of the low permeable compound morphine-3-glucuronide and extended the time to 24 h (80). There was no further change in recovery between 24 and 48 h. The probe should therefore be placed in the brain tissue at least 1 day before the experiment in order to minimize the damage made by the insertion. If placed in other tissues like a muscle, it is more difficult to place the probe long before the experiment, as the animal or human then would need to be very still until the start of the study in order not to damage the probe membrane. However waiting for at least 1 h is advisable in these instances. Subcutaneous probes can be placed at the site a bit longer in advance. Tissue damage when inserting the probe and the possibility of using dexamethasone to decrease the damage was studied by Nesbitt *et al.*, with positive findings on diminishing the tissue reactions (81).

Having the probe placed in the tissue for too long can also be a problem, with the formation of scar tissue that may alter the drug behavior in the tissue (82–84). Histological studies were performed to investigate the extent of tissue damage, resulting in findings of gliosis 200–300 μm from the track 3–7 days after implantation of microdialysis probes in the brain, while the integrity of the blood-brain barrier was intact up to 1 week (85). Stenken and colleagues commented on the cytokine response and recommended a cautious approach when interpreting results from measuring these molecules, as chemokines and cytokines are produced by the inflammatory response cause by microdialysis probe placement (86).

The results are likely not much influenced by the procedure if these precautions are followed when studying exogenously administered small drugs. As for all methods, it is important to evaluate the results in relation to methodological limitations.

Trade-Offs When Designing a Microdialysis Study

There is always a trade-off when designing an *in vivo* microdialysis study between methodological issues related to the microdialysis technique and the analytical capacity regarding detection limits vs the aim of the study from an *in vivo* perspective (24). If a rapid pharmacokinetic profile is to be measured, sampling needs to be done frequently with the cost of lower volume samples and/or higher flow rates and thereby lower recovery. One example is the small peptide DAMGO ([H-Tyr-D-Ala-Gly-MePhe-Gly-ol]), with a half-life of 9 min in plasma, which posed quite some challenges when being studied with microdialysis, although possible (87). To circumvent rapidly changing concentrations in the measured tissue, it is advised to administer a constant infusion of the drug of interest, as was also done in the DAMGO study. This will result in more stable measurements over time, e.g., when studying BBB transport by comparing brain and blood unbound concentrations. To more rapidly obtain steady state, a bolus dose or higher infusion rate can be administered in

the beginning, shortening the time needed for the experiment. It is also beneficial when the half-life is long, so that stable concentrations are achieved within the time-frame of one study day.

With high analytical sensitivity, the sampling interval can be shorter. A 2-min interval was applied when studying heroin and its metabolites 6-acetylmorphine and morphine in rats, giving a very high time resolution of the pharmacokinetics of brain uptake of the compounds in relation to pharmacological effect (88,89).

DEVELOPMENT OF MICRODIALYSIS AND ALTERNATIVES

The development of microdialysis has during later years focused on increasing the cut-off of the probe membranes, in order to increase the utility of the technique for studying larger molecules (90). With increasing cut-off up to 1 or even 3 MDa, there is an increased sensitivity of the method to pressure differences between inlet and outlet tubings and one has to check that flow rate into and out from the site of measurement are the same. Recovery estimations are more difficult under these circumstances. The group of Stenken has made important contributions to the area. Among other

things, the group found that the membrane provides more of the resistance to transport for larger macromolecules, than the tissue movement, thus different from small molecules (91,92). A theoretical and *in vitro* investigation of transmembrane convection with diffusion was made by Bungay *et al.* with the 100-kDa molecular weight cut-off membranes, as a modification of sampling large molecules (51).

Another area of development is the use of microdialysis for cutaneous bioavailability of drugs that has not come too far in spite of promising results (93–96). In comparison to blister techniques, it can be said to be very sophisticated, although still being limited by adsorption phenomena.

The open-flow microperfusion is a push-pull method that may be an alternative to microdialysis for sampling larger molecules (97,98). It is however even more specialized than microdialysis. Another push-pull technique was studied regarding possible tissue damage, with the conclusion that push-pull perfusion at low flow rates works well for sampling from brain, resulting in high temporal and spatial resolution, and that the probe insertion is what causes the tissue damage (99).

The use of microdialysis for drug studies in humans is of natural reasons mainly limited to peripheral studies where the probes are placed subcutaneously, cutaneously, or in muscles. Drug studies in brain have been performed in patients after

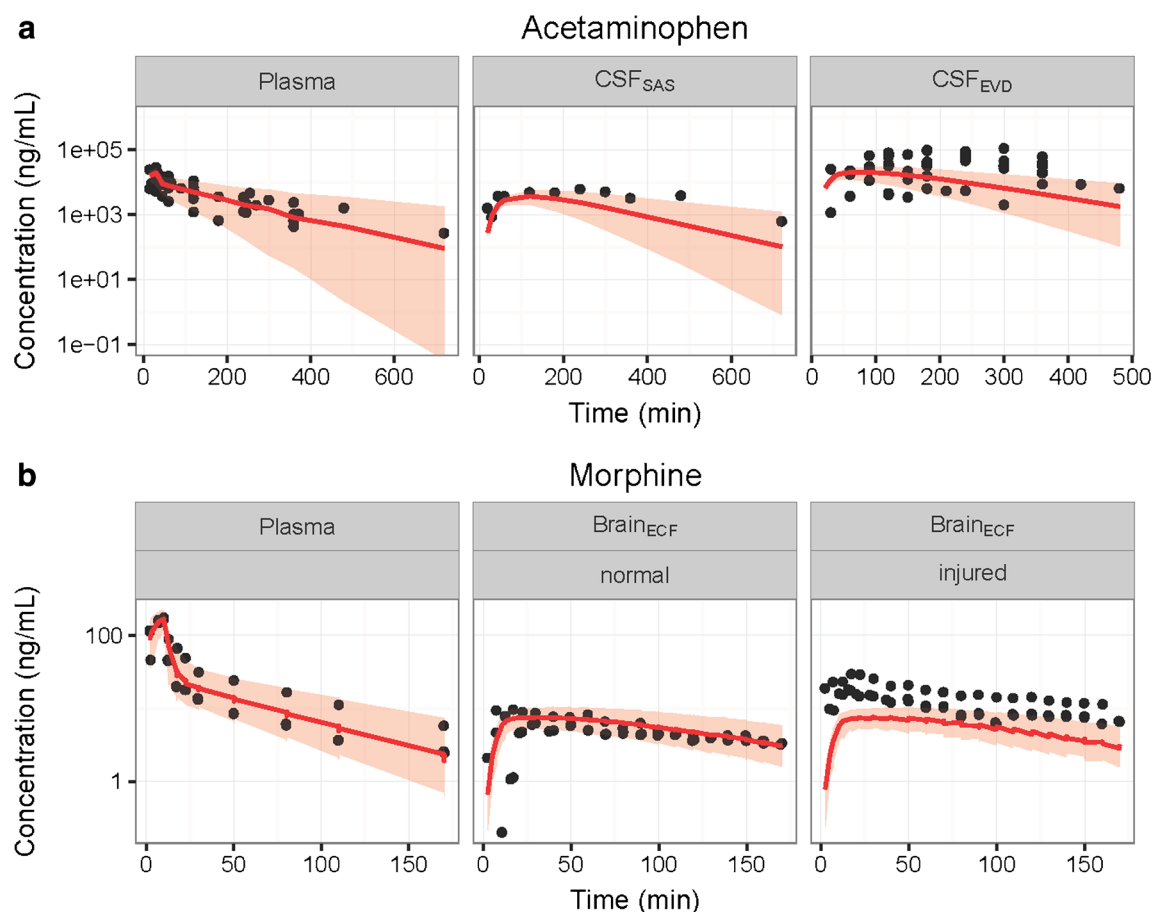


Fig. 3. Example of translation of microdialysis data from rat to humans based on a physiologically based pharmacokinetic model. Concentration-time profiles of human brain interstitial fluid and CSF concentrations (*filled circles*) and prediction from the translational model (*red lines*). The *shaded area* corresponds to a 95% prediction interval. **a** Acetaminophen data was obtained from plasma, subarachnoidal CSF (CSF_{SAS}), and CSF from external-ventricular drainage (CSF_{EVD}). **b** The morphine data was obtained from plasma, brain interstitial fluid through microdialysis in normal and injured patients (brain_{ECF}). From ref. (104) with permission from the publisher

brain injuries, where microdialysis catheters are placed for surveillance of the status of the brain tissue (27,100–102).

An alternative to microdialysis for brain studies in humans is positron emission tomography (PET). However, PET measures total concentrations including radioactive metabolites. A study in our laboratory has recently compared the two techniques regarding brain uptake of oxycodone in rats, as a step in the translation of preclinical findings of unbound concentrations into the clinic (103). We found that the two methods are comparable, after having compensated for non-specific binding in the brain of the PET data, but that radioactive metabolites may disturb the interpretations at later time points. MALDI-IMS has a higher spatial resolution but has other related problems when the aim is to quantify tissue concentrations.

SYSTEMS PHARMACOLOGY-RELATED EXAMPLES UTILIZING MICRODIALYSIS

Yamamoto *et al.* recently published the most extensive systems-pharmacology-related interpretation of brain drug delivery between rodents and humans based on microdialysis studies (104). It is a generic multi-compartmental CNS distribution model that allowed prediction of human brain target site concentrations of nine different drugs. Very good correlations were found between the physiologically based pharmacokinetic model from rat data, predicting human data from the literature (Fig. 3).

Morphine was studied in rats, in pigs with induced meningitis, and in humans with traumatic brain injury (1,100,105). Modeling of the blood-brain barrier transport was made on the rodent data but not of the pig or human data. Very similar blood-brain barrier transport properties were found between rodents, pigs, and humans, with a partitioning of the unbound concentration across the BBB ($K_{p,uu,brain}$) of 0.3–0.5, indicating moderate efflux at the blood-brain barrier. In sheep, however, the $K_{p,uu,brain}$ was above unity, showing a different transporter behavior from the other species and a change in transport with age (106). Li *et al.* predicted brain clozapine and nortriptyline concentrations in humans with a pharmacokinetic extrapolation from rat brain and plasma pharmacokinetics (107).

Target engagement of ertapenem was studied in plasma, subcutaneous tissue, and peritoneal fluid, in morbidly obese patients (108). By performing Monte Carlo simulations and population pharmacokinetic modeling of the microdialysis data, it was found that satisfactory concentrations were reached in all sites, albeit with only 25–50% of the unbound plasma concentration in subcutaneous tissue. Pigatto *et al.* studied etoposide distribution in a tumor model in rats with the help of microdialysis and population pharmacokinetic modeling (109). The authors concluded that plasma concentrations of etoposide was not a good predictor for tumoral exposure, where the concentrations were significantly lower, stressing the importance of understanding intratumoral concentrations for successful understanding of effect relationships.

Another type of relationships was recently investigated in our group with microdialysis, regarding if liposomal delivery would quantitatively improve the brain uptake of methotrexate (110). It was revealed that liposomes based on

egg yolk phosphatidylcholine improved the uptake threefold, while liposomes based on hydrogenated soy phosphatidylcholine did not influence the uptake at the blood-brain barrier at all. The concentrations of methotrexate obtained in brain interstitial fluid with the egg yolk-based liposomes was even found to be high enough to be able to exert pharmacological effects on brain tumors in humans. Here, microdialysis is crucial in several aspects, (1) to separate unbound from total liposomal drug in plasma, (2) to quantify the blood-brain barrier transport of the drug with and without the liposomes, and (3) to measure resulting concentrations in the brain and thereby be able to draw conclusions on clinical relevance of the findings.

CONCLUSIONS

Microdialysis has proven to be a very important method for the understanding of unbound target site concentrations and their relationship to effects. The method has been instrumental in quantifying active transport of drugs, especially at the BBB and also in other tissues. Thus, the method is a very valuable contribution to quantitative systems pharmacology in its understanding of tissue distribution and concentration–effect relationships—in combination with modeling of the data. The full potential of the method for more complicated pharmacodynamic measurements, e.g., metabolomics fingerprints, has not yet been fully utilized although a few studies have been published. In conclusion, more and more studies are published with important contributions to the understanding of target-specific concentrations and their relationship to pharmacodynamic effects from a systems pharmacology perspective, aiding in the global understanding of drug effects.

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