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Workshop Report

AAPS-FDA Workshop White Paper: Microdialysis Principles, Application and Regulatory Perspectives

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Abstract. Many decisions in drug development and medical practice are based on measuring blood concentrations of endogenous and exogenous molecules. Yet most biochemical and pharmacological events take place in the tissues. Also, most drugs with few notable exceptions exert their effects not within the bloodstream, but in defined target tissues into which drugs have to distribute from the central compartment. Assessing tissue drug chemistry has, thus, for long been viewed as a more rational way to provide clinically meaningful data rather than gaining information from blood samples. More specifically, it is often the extracellular (interstitial) tissue space that is most closely related to the site of action (biophase) of the drug. Currently microdialysis (μD) is the only tool available that explicitly provides data on the extracellular space. Although μD as a preclinical and clinical tool has been available for two decades, there is still uncertainty about the use of μD in drug research and development, both from a methodological and a regulatory point of view. In an attempt to reduce this uncertainty and to provide an overview of the principles and applications of μD in preclinical and clinical settings, an AAPS-FDA workshop took place in November 2005 in Nashville, TN, USA. Stakeholders from academia, industry and regulatory agencies presented their views on μD as a tool in drug research and development.

KEY WORDS: clinical pharmacology; microdialysis; recovery; regulatory aspects.

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INTRODUCTION

Background and Rationale

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endogenous and exogenous molecules. Yet most biochemical and pharmacological events take place in the tissues. Also, most drugs with few notable exceptions exert their effects not within the bloodstream, but in defined target tissues into which drugs have to distribute from the central compartment.

Assessing tissue drug chemistry has, thus, for long been viewed as a more rational way to provide clinically meaningful data rather than gaining information from blood samples. More specifically, it is often the extracellular (interstitial) tissue space that is most closely related to the site of action (biophase) of the drug. Currently microdialysis (μD) is the only tool available that explicitly provides data on the extracellular space.

Although μD as a preclinical and clinical tool has been available for two decades, there is still uncertainty about the use of μD in drug research and development, both from a methodological and a regulatory point of view. In an attempt to reduce this uncertainty and to provide an overview of the principles and applications of μD in preclinical and clinical settings, an AAPS-FDA workshop took place in November 2005 in Nashville, TN, USA. Stakeholders from academia, industry and regulatory agencies presented their views on μD as a tool in drug research and development.

Historical Development

The concept of μD goes back to the early 1960s, when push pull cannulas, dialysis sacs, and dialytrodes were inserted into animal tissues to directly study tissue biochemistry, notably transmitter release in the rodent brain. In 1974 Ungerstedt and Pycock (1) reported on the use of "hollow fibers," which were steadily improved and eventually resulted in the needle probe (Fig. 1). The probe is inserted as such or via a guide cannula into the tissue. While the majority of μD applications are preclinical studies on neurotransmitter release, μD was rapidly used also for pharmacokinetic studies in rodents, and further adopted for the clinical setting, initially with experiments on subcutaneous adipose tissue glucose levels in the mid-1980s. Soon thereafter μD was also employed to monitor endogenous metabolite and transmitter levels in the human brain. The first studies on human drug pharmacokinetics were published in the early 1990s. Today US Food and Drug Administration (FDA)—and European Union Conformite Europeene (CE)—approved μD-catheters are available for use in humans and μD can be performed in virtually every given human tissue, including myocardium, brain, lung and also human tumors. As of today there are more than 10,000 publications available on μD, including about 1,600 publications on its human applications. Besides other documents, and in light of the FDA's Critical Path initiative, the CDER (Center for Drug Evaluation and Research) Report to the Nation 2003 indicates a need for tools that enable the measurement of tissue concentrations by stating that the CDER continues to extend its "long-standing interest in the application of dose–response principles by viewing drugs and their actions directly at the level of the drug target, rather than indirectly via plasma concentrations."

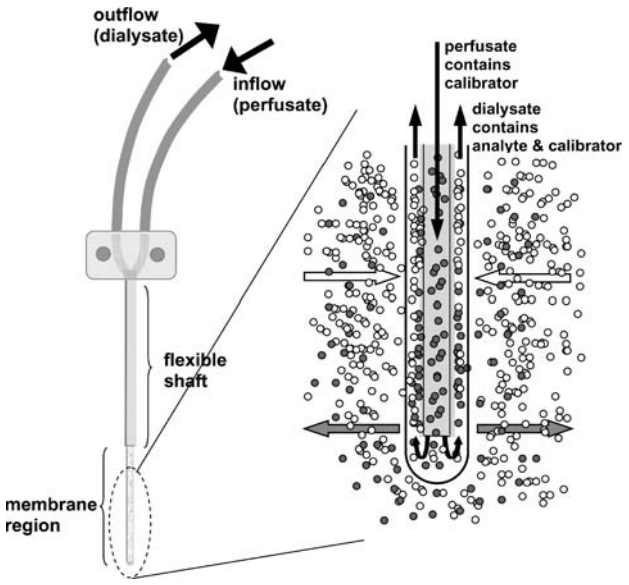

Fig. 1. A microdialysis probe of concentric design is shown. The *magnified membrane region* illustrates net diffusion of a compound (analyte) of interest (*open circles*) into the probe, and the net diffusion of the calibrator (*closed circles*) which has been added to the perfusate, from the probe to the extracellular space.

BASIC PRINCIPLES

Technique

A number of reviews are available on methodological aspects of the μD technique (2–7). The technique involves the implantation of a small probe into a specific region of a tissue or fluid-filled space (8). Variety of probe designs have been used, including linear, U-shaped, or concentric geometries. Semi-permeable membrane materials used in probe construction range from low-to high-molecular weight cut off. During μD, a physiologically compatible perfusion fluid (perfusate) is delivered through the probe at a low and constant flow rate (typically ranging between 0.1 and 5.0 μl/min). Exchange of solutes occurs in both directions across the semi-permeable membrane of the probe (Fig. 1) depending on the orientation of the solute concentration gradients. Thus, the probe can be used in the delivery as well as in the sampling mode. For a perfusion fluid that is devoid of the compound of interest, the microdialysate concentration is usually a fraction of the tissue extracellular diffusible (unbound) level. This fraction is referred to as the *relative recovery*. In investigations that examine changes in the levels of endogenous compounds from their baseline values, it may not be necessary to determine the relative recovery, if it is reasonable to assume that recovery remains relatively constant throughout the experiment. These studies typically evaluate percent changes from a baseline level when an intervention (e.g., the administration of a drug) is introduced. However, in pharmacokinetic investigations without baseline values for exogenous substances, knowledge of the relative recovery becomes crucial for the determination of true extracellular tissue concentrations (9). Furthermore, recovery becomes time-dependent when the tissue extracellular concentrations vary during the course of the experiment (10).

Many experimental conditions affect probe recovery, including μD flow rate (perfusion), temperature, probe membrane composition and surface area, nature of the dialyzed tissue (which precludes the use of calibration *in vitro* as a sur-

rogate for calibration *in vivo*), physicochemical properties of the analyte of interest, and other factors that influence molecular diffusion characteristics. In general, the higher the perfusion flow rate, the lower the relative recovery. Higher temperatures and greater probe membrane areas usually result in increased recovery. The rate of solute clearance from the extracellular space is an important determinant of recovery. Consequently, probe recovery may vary during an experiment if the rate of solute metabolism, cellular uptake or loss to blood from the extracellular space is perturbed (11).

Usually, μD is a sampling technique that is "volume-neutral," i.e. there is little removal of fluid volumes from the perfused tissue. This is because the μD probe is continuously supplied with perfusate that picks up the compound of interest by diffusion without accompanying fluid loss or gain across the membrane. This eliminates the volume limitations that are present in small animal pharmacokinetic studies or in pediatric patients and allows data-rich study designs in these populations.

In experiments requiring only minimally invasive surgical access, especially those involving blood, muscle, skin, adipose, and certain regions of the brain, the experimental animals or subjects may be allowed to recover from anesthesia following probe implantation and remain conscious or even freely-moving during μD. These types of awake models are ideal for pharmacokinetic and pharmacodynamic evaluation because they are devoid of the effects of anesthetic agents. Normal physiological conditions are maintained so that μD can continue for many hours, or even days.

Development and refinement of the μD methodology over the past two decades has led to its increased acceptance in studies of drug distribution, metabolism, and pharmacodynamics (12). μD has found its most important role, perhaps, in the measurement of compounds in the brain. These studies have examined not only endogenous compounds, e.g., neurotransmitters, but in the past several years have included the investigation of drug distribution to specific regions of the central nervous system. In addition, μD has found application in studying the levels of endogenous compounds and drugs in a wide variety of other tissues (skeletal and heart muscle, skin, blood, bone, adipose, lung, liver, middle ear, spinal cord, eye, synovial fluid, gut lumen, intrathecal and ventricular cerebrospinal fluid, peritoneum, tumor) in numerous species of experimental animals (e.g., mice, rats, rhesus monkeys, rabbits, chinchillas, dogs) as well in as in humans (13–24). Thus, μD has permitted the investigation of the distribution kinetics and delivery of drugs to a wide variety of target tissues, allowing for the measurement of unbound, pharmacologically active drug levels over time in the relevant species and tissue.

CALIBRATION METHODS

Calibration methods are important in μD experiments when quantitative information on extracellular fluid (ECF) concentrations is desired. Although recovery may approach 100% for long probes and slow flow rates, the commonly used perfusate flow rate (around 1 μl/min) and membrane length (3–10 mm) usually results in recoveries that are much less than 100% in animal experiments. A generalized measure of the degree of equilibration between the dialysate and the tissue extracellular fluid is the extraction fraction (Eq. 1)

$$Extraction\ Fraction = \frac{C_{in} - C_{out}}{C_{in} - C_e} \quad (1)$$

where C_{in} is the perfusate concentration (inflow to probe), C_{out} is the dialysate concentration (outflow from probe), and C_e is the distant extracellular fluid concentration in the surrounding tissue. Relative recovery is the special case of extraction fraction for sampling, for which C_{in}=0.

In addition to perfusate flow rate and probe geometry, extraction fraction (and hence, recovery) is influenced by a number of solute and tissue related factors. Among these factors are the physico-chemical properties of the solute of interest and its diffusion coefficient in the tissue, the extracellular fluid (ECF) volume fraction and the processes for elimination from the tissue, including active transport mechanisms. Many of these factors have been incorporated as parameters in mathematical models (25–27). A principal objective in μD modeling is to explicitly relate these parameters to the measurable analyte concentrations in the inflowing perfusate, C_{in}, and the dialysate sample concentration, C_{out}. One of the advantages of μD is that the underlying principles are relatively well understood and can be incorporated in these mathematical models to aid in the planning and interpretation of experiments.

Mathematical models of steady-state and transient μD have been derived for linear behavior, i.e. for kinetic expressions in which the parameter values are independent of analyte concentration. For linear behavior, the models predict that the extraction fraction has the same value for all C_{in}, and hence is independent of whether the analyte is being sampled or delivered. This equality between sampling (gain) and delivery (loss) extraction fraction values provides the theoretical underpinning for most calibration techniques.

The most frequently used calibration methods are the low-flow-rate method, the no-net-flux (or zero-net-flux) method (28), the dynamic (or extended) no-net-flux method (29) and retrodialysis by drug or by calibrator methods (30). The general requirement of these methods is that the extraction fraction is the same whether the solute exchange across the membrane occurs by either loss or gain as predicted by the linear models. The low-flow-rate method is based on the assumption that recovery is close to 100% and is generally applied in clinical μD with longer probes and flow rates ≤0.3 μl/min. The no-net-flux method requires steady state concentration in the tissue, while perfusate concentrations are changed in several discrete steps within one experiment. The dynamic no-net-flux method allows for time-varying *in vivo* concentrations, but instead requires that probes be perfused with a different constant concentration in each of at least three subject groups.

The most common calibration method is retrodialysis by drug, which is performed prior to or after drug administration without the drug in the tissue. The advantage is that the solute of interest is measured; however possible changes in recovery over time are not measured. This method is not applicable to endogenous compounds as the requirement of no concentration in the tissue during the calibration interval cannot be met. Changes in recovery during the experiment

can be partially taken into account via retrodialysis by calibrator. It is crucial that the suitability of the calibrator be thoroughly documented, especially for compounds that are actively transported in membranes. A good calibrator should have similar diffusion, transport and metabolism properties as the solute of interest. The ideal calibrator is thus a deuterated or radioactive form of the test compound.

When using a calibrator, it is recommended to use moving average or an average of the whole experimental period if the recovery is constant with time (31). This is due to the fact that calculating a small loss from the perfusate by retrodialysis is generally more prone to mathematical errors than the gain of molecules from the tissue into the probe. As the dialysate concentration is subtracted from the perfusate concentration in the calculations of the retrodialysis extraction fraction (Eq. 1), the standard deviations from the chemical analysis will lead to large errors in estimated recovery. Therefore, relative recoveries of 20% or more are recommendable. However, this cannot always be obtained and it has to be accepted that retrodialysis is not feasible for some compounds. In some circumstances an endogenous substance can be used as an internal reference to compensate for differences in relative recovery between tissue sites. For example, urea has sometimes been used as an internal marker, as this molecule equilibrates in all body fluids (32), but the validity of its use should be verified as with any calibrator.

One of the key factors determining recovery of an analyte or a calibrator is the strong influence of processes in the tissue that remove the solute from the extracellular space. The clearance processes can be cellular uptake, chemical conversion or loss to blood through the microvasculature in the vicinity of the probe. ECF clearance processes produce the same effects when the probe is operated to deliver analyte from the perfusate as when the probe is sampling analyte from the tissue. Significantly, μD theory predicts that these features are not affected by the processes supplying analyte to the ECF, provided the rates of these processes are independent of the analyte concentration in the ECF (25). For example, efflux from the ECF to blood (clearance) depends upon the ECF concentration, whereas influx from blood to the ECF (supply) depends upon the free plasma concentration.

Almost all μD measurements are initially transient even if the tissue would be at steady state in the absence of the probe. Furthermore, most calibration procedures are designed for steady-state conditions. Hence, the time required to reach steady state can be an important consideration. The rate of analyte clearance can exert a dramatic influence on μD transients. In the absence of clearance mechanisms the time to approach steady state can be several hours, because diffusion is slow and both the volume of tissue and mass of analyte involved are relatively large (10). However, except for some situations such as hydrophilic drugs in tissues with slow transport across tight junction endothelial barriers, efflux to blood may occur at sufficient rates for steady state to be approachable on a practical time scale. With very rapid clearance rates, such as those for neurotransmitters in the brain, steady state may be achievable on a time scale of minutes.

For quantitative μD studies on a new compound it is advisable to perform *in vitro* experiments prior to animal or human use, checking for *in vitro* adsorption to tubing, time-delays in solute movement, and to compare solute gain and loss, all in order to obtain basic information on the feasibility of the method *in vivo* (Fig. 2). The *in vitro* experiment does not in general replace *in vivo* recovery determinations, because the effect of the diffusional resistance in the tissue on recovery can be much different than the diffusional resistance of the bathing medium *in vitro*.

In conclusion, it is important to take recovery and its time aspects into consideration when performing quantitative μD. The choice of calibration method will depend on the purpose of the experiment, but *in vitro* experiments are recommended initially when a new compound is studied. There is a trade-off between theoretical requirements and practical possibilities when performing μD, but it is important to keep in mind that potential practical problems of μD are far outweighed by the increased understanding of basic aspects of (patho)physiological processes, drug distribution and drug effects obtained with this method.

Tissue Damage

Insertion of μD probes has been shown to cause tissue trauma, which could influence the results of μD experiments (33–35). To allow "tissue equilibration," i.e. to provide time for the initial trauma to subside, probe perfusion for more than half an hour has shown to be sufficient before starting probe calibration, although longer recovery times (12–24 h) may be required. This is based on the finding that several markers of tissue trauma (e.g. thromboxane B2, adenosine triphosphate, adenosine, K^+, glucose, lactate, lactate/pyruvate ratio) are elevated after probe insertion and reach baseline or become undetectable within this time range. Furthermore, changes in local skin circulation after insertion of μD probes have been investigated using laser doppler perfusion imaging and found to return to baseline within 60 min. Histological studies have shown that the implantation of small diameter dialysis tubings does not induce foreign body reactions, if implantation times are kept below 24 h, and bleeding is usually not a problem. Significant edema can be a complication, at least for implantations in the brain (36,37). One of the controversial questions addressed in CNS μD research is the integrity of the blood-brain barrier (BBB) after probe placement. It was shown that chemical selectivity of the BBB is maintained with respect to mannitol (a marker with very low BBB permeability) and tritiated water (a marker that has rapid and complete equilibration between plasma and the brain interstitial fluid). Also other studies indicated a functional BBB in terms of passive and active transport mechanisms when using intracerebral microdialysis, provided optimal surgical and experimental conditions are employed (5,9).

Limitations

As μD probes are usually perfused with aqueous solutions (physiological Ringer's solution or phosphate buffered saline), the technique is conceptually limited to the study of water-soluble drugs. Several attempts to measure highly lipophilic compounds have more or less failed, but, there are some reports on the successful measurement of

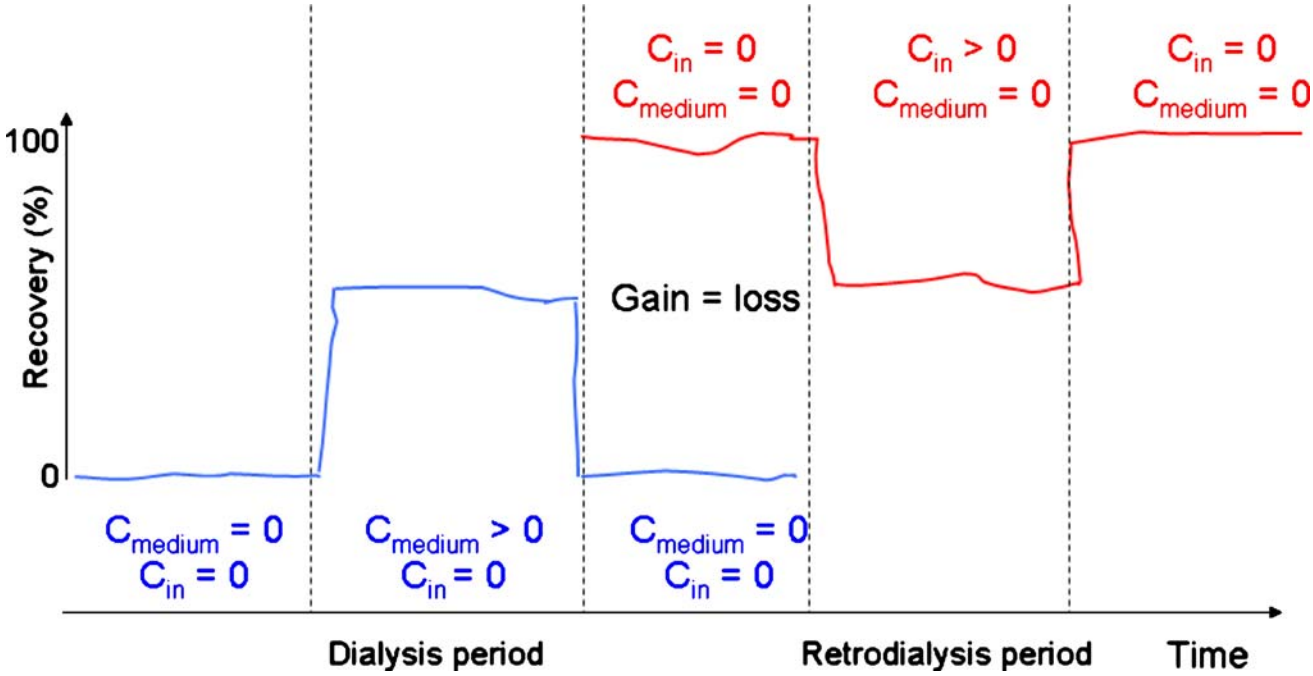

Fig. 2. An example of *in vitro* preparations for a microdialysis experiment to check if the solute movement across the membrane is fast and symmetrical in both directions. This is a prerequisite for the use of retrodialysis recovery methods and determination of transient solute concentrations *in vivo*. The experiment is performed in two parts where the solute is shifted to be present either in the surrounding medium ("gain") or in the incoming dialysate (retrodialysis, "loss") with time in between with no solute. The *dashed lines* represent changes of concentrations.

lipophilic compounds. In order to enable the measurement of lipophilic compounds routinely, *in vitro* experiments have demonstrated the usefulness of lipid emulsion as perfusate instead of aqueous solution.

Low sensitivity of conventional analytical methods is often the bottleneck for the successful detection of low drug concentrations in the small dialysate sample volumes, which are in the micro liter range depending on the drug and the desired temporal resolution. However, the introduction and improvement of analytical methods such as high-performance liquid chromatography (HPLC), microbore/capillary LC methods, mass spectrometry and biosensors are likely to overcome this issue (6).

As opposed to imaging techniques, which allow simultaneous drug distribution studies in several organs and tissues, μD provides focal information on tissue PK from a limited number of sites or organs.

Analytical Considerations

Several technical issues in the analysis of μD samples, notably the detection limits, required sample volume and analytical interferences need to be considered carefully (6). For a successful overall experiment, the sampling and analysis parts must be designed in conjunction with each other. The typical total microdialysate volumes of a few μL or less and analyte concentrations in the pM or nM range present tremendous challenges for the analytical chemist. Thus, it is important to optimize the flow rate as a function of the analytical system to be used. For example, volume sensitive methods require larger sample volume, i.e. higher μD flow rates, with consequent decrease in extraction efficiency. As a result, the limits of detection of the analytical method must be improved. On the other hand, if the flow rate is decreased the temporal resolution of the μD experiment may be compromised.

A particular advantage of μD is that it can be used with a wide variety of analytical techniques. The specific analytical method employed can be optimized based on the requirements of the physiological experiment and the particular analytes of interest. By far the most common analytical method used in conjunction with μD is HPLC. This is because, microdialysate samples consist primarily of relatively small hydrophilic analytes in highly ionic aqueous samples. Reversed-phase and ion-exchange are the modes of liquid chromatography that are most compatible with direct injection of aqueous μD samples. The mode of chromatography chosen for analysis is dependent on the physiochemical properties of the analyte and the type of column is determined by the sampling interval desired and the required sensitivity.

In general, the volume of a μD sample is considerably less than eluting peak volume. For this reason, sample preconcentration does not result in improved detection limits and is therefore not used. On the other hand, because μD samples are generally protein-free, no sample preparation is needed before HPLC analysis. This has led to the development of many systems where μD is directly coupled to an HPLC or LC. Capillary electrophoresis (CE) is another separation method that can be combined with μD due to the low sample volume requirement. One disadvantage of CE, however, is its incompatibility with high ionic strength samples.

MICRODIALYSIS IN DRUG DISCOVERY AND DEVELOPMENT

Rationale for Microdialysis in Drug Development

As drug development costs continue to escalate, partially because of the high attrition rate of development candidates, there is increasing pressure to improve the predictability of clinical outcomes from preclinical studies for a new medical entity. Better knowledge of the exposure in the appropriate biophase, as well as the effect of a drug candidate at the site of action, would greatly facilitate and improve selection of the best compound and optimal doses Fig. 3 for subsequent clinical studies. As a practical, data-rich, animal sparing *in vivo* method, μD is thus an extremely useful tool that is increasingly applied in the pharmaceutical industry to investigate the pharmacokinetic and pharmacodynamic profiles of drugs.

Applications

Since μD is labor-intensive and requires specialized skills, it is not suitable for high throughput screening of large numbers of compounds rather; it is used to address specific questions from early in discovery to development efforts (Table I). In early target characterization phase, μD is frequently used for PK/PD correlation to establish target validity and to provide an early proof of concept *in vivo*. For CNS targets, high throughput *in vitro* assays and *in silico* tools may be used as a Tier 1 screen at the onset of the lead optimization phase to rapidly select potential candidates from large series of compounds with the desired pharmacological profile for the disease target. Molecules that clear these hurdles may advance to the Tier 2 screen, which generally involves *in vivo* studies. A typical screen includes harvesting the brain to obtain the homogenate and to normalize whole brain levels to plasma levels. Such data should be used with caution as it provides information on the levels that cross the blood–brain barrier (BBB), but not on the free fraction that is the efficacious level available to the target of interest, especially if the molecule is highly bound to brain tissue. The measurement of drug levels in the CSF is commonly used as a surrogate for free fractions in the brain, which is only relevant if transporters do not play a major role in the blood brain exchange, as the expression and function of transporters on the BBB and the blood-CSF barrier has been demonstrated to be diverse.

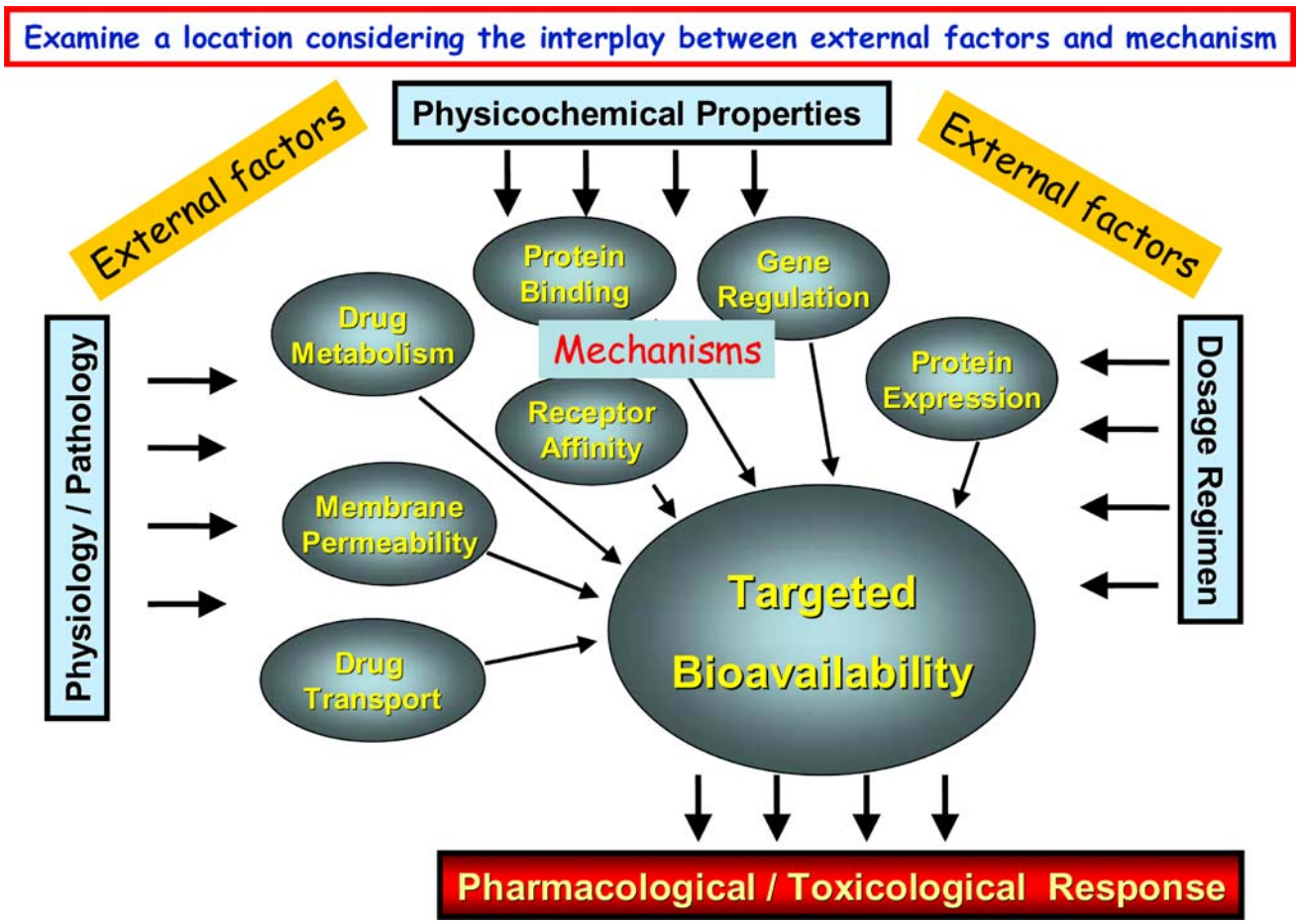

Fig. 3. Illustration showing the interplay between the body, the compound, and the dose, considering the various mechanisms that influence the delivery of a compound to its site of action. The resulting targeted bioavailability generates the observed pharmacological response. Microdialysis can be useful in the information balance between pharmacokinetics and pharmacodynamics by helping in determining the influence of a mechanism on drug delivery at each location.

Table I. Stages of the CNS Drug Discovery and Development Process Where Microdialysis can be Applied (Indicated with [a])

Identification of Lead Compounds
In vitro potency and selectivity (binding affinities)
Tier 1
In silico (MW, PSA, cLogP)
Permeability (CACO2, efflux ratio, MDCK)
Plasma protein binding
Tier 2
In vivo CSF and brain homogenate (use with caution)
In vivo pharmacodynamic models [a]
Tier 3
In vivo PK: free drug concentrations at target [a]
In vivo PD: pharmacology [a]
Tier 4
Brain-plasma ratio in KO mouse models[a]
Co-administration of specific transport inhibitors[a]
Pharmacology studies[a]
Compounds in Development or Marketed
Special in vivo PK/PD studies [a]

If a molecule possesses desirable pharmacokinetic properties, μD studies will help profile lead candidates by bridging ECF concentration to target receptor occupancy in PK/PD studies. In special cases, transgenic or knockout models are used to elucidate mechanisms of transport of CNS drugs across the BBB, but compensatory pathways that do not exist in wild type animals may confound results from transgenic models. μD in wild type animals using specific inhibitors and substrates of transporters is a promising alternative approach. At this stage of the development process, μD is also frequently used, in particular for CNS targets, to study in detail the *in vivo* pharmacological properties and the mechanisms of action of the drug. Finally, clinical observations may raise mechanistic questions, which sometimes can be addressed by pre-clinical μD studies.

In preclinical drug discovery, μD is a valuable and useful tool for the pharmacokinetic profiling of library templates, with the goal of selecting the best chemical series to design around or the candidate to move forward into development. μD samples the unbound fraction of the drug (f_u) in the interstitial space which is in equilibrium with biophase membrane barriers and receptors. This makes the link between *in vivo* pharmacokinetic and pharmacodynamic analysis from μD studies to *in vitro* transport and biology models highly relevant. As pharmacologically active biophase concentrations and the distribution relationship between blood to biophase are established in preclinical models, it becomes possible to predict clinical doses designed to achieve therapeutically relevant concentrations in the biophase (12). This approach may expedite "go/no-go" decisions in clinical development and improve the efficiency of the drug development process.

In CNS drug discovery and development in particular, a key question is whether a compound reaches the intended site of action and at what concentration pharmacological responses occur. There are two advantages with using μD for answering these questions. First, the f_u measured in μD is comparable to the unbound concentration used for *in vitro* transport and efficacy models, thus making the *in vitro–in vivo* correlation with μD more relevant. Secondly, the steady-state equilibrium ratio of f_u between brain to blood serves as a guide to clinical dosing. While conducting a μD study for every compound within a discovery series is impractical, the data gained from selective compounds are often enough to establish a meaningful correlation with *in vitro* transport models.

Since effects on transmitters is one of the key *in vivo* endpoints in the evaluation of compounds acting on the central nervous system, intracerebral μD is increasingly used in the pharmaceutical industry for the *in vivo* evaluation of novel CNS drugs, mainly to study the mechanism of action and to estimate *in vivo* potency and selectivity in comparison with pharmacological standards or marketed compounds.

MICRODIALYSIS IN PK/PD EVALUATION

As mentioned, one of the necessary components to quantitatively determine the connections between drug concentrations in the blood (systemic pharmacokinetics) and a drug response (pharmacodynamics) is the ability to measure the drug compound in the "biophase" or tissue that is closer to the actual site of action of the drug. In that regard, μD has brought new opportunities to pharmacokinetic and pharmacodynamic research that will allow better understanding of exposure-response relationships and that will ultimately help to develop better drug products.

μD allows direct access to the ECF of the tissues and therefore creates much more meaningful data than serum or plasma concentrations. This is probably most apparent in the field of anti-infective agents, because most infections are located in the ECF which is easily accessible to μD. In this field, μD has been employed to measure drug concentrations in a variety of animal tissues including muscle, lung and middle ear fluid. The determination of these local concentrations allows one to assess if the administered dosing regimen is appropriate to obtain sufficiently high drug concentrations at the site of the infection. Integration of μD-derived tissue PK data to PK-PD kill curve approaches is an ideal approach to gain information on rational dosing of anti-infective agents.

Complex relationships between dose and response are governed by mechanisms that may vary: in rate and extent, in time, by subject, by disease condition, by genetic background, by age etc. Often a complex relationship between dose and response exists in particular for CNS drugs. This suggests that there is a need for studies designed to unravel the underlying mechanisms and interspecies similarities and differences. Mechanism-based pharmacokinetic-pharmacodynamic studies of CNS drugs, in which the μD technique plays a unique role, take these mechanisms into account, leading to better predictions of CNS drug effects. A number of key mechanisms govern the dose–response relationship of CNS drugs, including plasma pharmacokinetics, BBB transport, within brain distribution, pathological conditions, drug-target interaction and signal transduction. μD is well-suited to determine passive and active membrane transport mechanisms, such as those of the BBB and of the brain parenchyma cells. Brain chemical intercellular communication occurs via the ECF and often is modified by the drug. An added value of the μD technique,

is extracellular biomarkers of drug response and of disease progression can also be sought and monitored.

CLINICAL MICRODIALYSIS

Clinical μD has been shown to be a safe, reproducible, ethically acceptable and relatively inexpensive technique for studying tissue biochemistry and drug distribution in humans (1,38–40). Today μD is becoming an established clinical technology that can be applied to most organs in appropriate clinical situations (39,41). Clinical μD data are mostly derived from four different fields, notably (1) intensive care research, (2) clinical pharmacology and drug development (3) dermatology and (4) metabolic—and endocrinological research. In addition, μD has occasionally been employed in other fields, e.g. CNS research as a tool to gain insight into the mechanism of epileptic seizures.

Microdialysis Monitoring of Human Organ Chemistry During Intensive Care

By monitoring the ECF in the brain and in peripheral tissues it is possible to get crucial information about cell pathology and how seriously cells are affected by ischemia, hyperemia, trauma, hemorrhage, and vasospasm, as well as various physiological, pharmacological and surgical interventions during intensive care. The use of μD in intensive care has focused on markers of ischemia, a condition of critical concern for the survival of the organ. The lactate/pyruvate ratio is a well-known marker of changes in the redox state of cells caused by ischemia. The use of a ratio of two analytes abolishes the influence of catheter recovery, which influences lactate and pyruvate to a similar degree. Therefore the lactate/pyruvate ratio may be used to compare the redox state of different tissues as well as different individuals. Lactate alone is a less reliable marker of the redox state of the cells as an increase in lactate may be due to hypoxia, ischemia as well as hyper-metabolism.

The interpretation of μD data depends upon the position of the catheter in relation to the existing pathology. The position of a brain catheter can be rendered visible in CT by use of a gold tip. This is of great importance for the interpretation of bedside μD data. Only then it is possible to use μD (Table II) data effectively to provide an early warning of secondary insults and to evaluate the result of various clinical interventions aimed at improving the condition of brain tissue during neurointensive care. The principles of how to position the catheters were outlined in a consensus paper (42).

In the liver and in microsurgical flaps the positioning of the catheter is not as crucial as in the brain, since ischemia is liable to affect an entire liver lobe or surgical flap. However, in the peritoneal cavity the detection of ischemia is delayed if the catheter is positioned far away from the ischemic area. The catheter is therefore usually placed close to the intestinal anastomosis. The existence of complete mesenteric ischemia will be detected regardless of the placement of the catheter. During intensive care, organ chemistry often changes profoundly in the patient. With the present state of our knowledge it is impossible to interpret every change, however, pathological states manifest themselves as dramatic increases or decreases of the chemical markers that are easily related to clinical events observed at bedside.

Table II. Advantages and Limitations of Microdialysis

Advantages	Limitations
Highly dynamic continuous sampling	Requires training of skills
High-resolution real-time sampling	Insertion of probes
Both drug and metabolites in one sample	Probe manufacturing
Minimally invasive	Needs sensitive analysis
Multiple sites in one animal/person	Drug-specific problems
Sampling and/or delivery via the probe	Lipophilic drugs
Purified samples (protein free)	Highly protein-bound drugs
Highly reproducible	Absolute tissue levels more difficult to estimate
Allows simultaneous use of auxiliary techniques	Recovery is tissue and time dependent

The introduction of CE-and FDA-approved μD catheters intended for human use and bedside measuring equipment has enabled the start of routine monitoring of patients with severe head injuries (43) and subarachnoid haemorrhage. The most important findings have been that the lactate/pyruvate ratio provides an early warning of vasospasm after subarachnoid hemorrhage preceding clinical signs by an average of 11 h and that μD gives an early warning of secondary damage to the penumbra region in patients with traumatic brain injury, enabling the clinician to individualize the therapeutic interventions.

μD also provides the opportunity for simple, continuous monitoring of metabolic changes in the transplanted liver before they are detected in peripheral blood chemistry. A normal postoperative course results in decreasing lactate/pyruvate ratio. Complications such as peritonitis, bowel ischemia, anastomosis leakage and urinary fistula are preceded by two to four days of increasing lactate/pyruvate ratio as an early marker of intraperitoneal ischemia.

μD is also a reliable technique for early detection of ischemia in surgical flaps. In a follow-up of 80 consecutive microvascular flaps no flap was lost due to a delay in the diagnosis of secondary ischemia, when on-line μD monitoring was available.

Clinical Pharmacology

Because μD measures unbound, pharmacologically active drug concentrations in the interstitium, which is the target site for many bacterial infections, the technique has led to a reappraisal of concepts of "tissue-penetration" by antimicrobial drugs (39,44,45). μD data indicate that in healthy people interstitial concentrations of beta-lactams are in the range of free serum concentrations, whereas interstitial levels of quinolones and macrolides are considerably lower than those predicted from biopsies. For several conditions, notably septicaemia and septic shock, tissue concentrations of antibiotics such as piperacillin may be

subinhibitory, even though effective concentrations are attained in serum.

In oncology (46), μD studies in patients with breast cancer and melanoma revealed no association between serum concentrations of anticancer drugs and tumor exposure to the drugs. However, there is preliminary evidence that the concentrations of cytotoxic drugs in a tumor may correlate with response to chemotherapy. These findings cast doubt on the use of serum drug concentrations to predict response and corroborate previous findings of a high variability of drug penetration into tumors. μD may therefore prove to be a good method for selecting compounds with favorable penetration characteristics and may help to identify patients who are unlikely to benefit from chemotherapy because of poor drug penetration.

Dermal Microdialysis

Topical application of drugs is an attractive way to circumvent systemic drug administration and the associated side effects, but it is often not clear whether adequate drug concentrations are reached in the tissue. μD permits this issue to be addressed and to identify formulations and doses of topically applied non-steroidal anti-inflammatory drugs that produce effective local concentrations. The use of μD in topical drug research may thus lead to a critical reappraisal of cost benefit ratios of topically administered drugs administered for local effect.

μD methodology for assessment of topical drug penetration has been considered promising, but in need of validation, by the regulatory authorities (47). The μD method has received increased recognition based on an initial bioequivalence study in humans, where lidocaine delivery from two different vehicles was compared by dermal μD sampling and pharmacodynamic assessment of the pain-relieving effect of the formulations (48). μD methodology in the skin is easily combined with other methods such as laser doppler flowmetry, laser doppler perfusion imaging, transepidermal water loss (TEWL), colorimetry, high resolution ultrasound scanning, or scoring of itch and pain (49). The effect of barrier perturbation on cutaneous drug penetration (50) and the topical penetration of several components of topical analgesics has been demonstrated by μD (51).

A recent investigation compared dermatopharmacokinetic and dermal μD methodology for a topical lidocaine cream and ointment (52). The results showed the two methods to be in agreement, with both finding a 3–5-fold higher lidocaine penetration from cream formulation than from ointment. Analysis of variance identified that intersubject variability accounted for 61% of the variability in μD data. Statistical calculations indicate that with dermal μD methodology, bioequivalence (BE) studies with 90% confidence intervals and 80–125% BE limits can be conducted in 27 subjects, using two probes in each test area, or 18 subjects using three probes per formulation application site. This approach is thus far better and more cost-effective than clinical trials currently conducted for generic drug approvals, which may require as many as 300 patients. It has been estimated that between 40 and 50 subjects are needed for a BE study employing the dermatopharmacokinetic method.

Paracrine Endocrinology and Metabolism

μD has been widely applied in metabolic studies across individual tissues in humans *in vivo*, in particular with regard to adipose tissue and skeletal muscle metabolism under different experimental conditions (53,54). The tissue metabolite concentration, however, may be influenced by several factors, such as local blood flow, local uptake and/or release, as well as delivery of the compound of interest from other sources via the arterial circulation. Hence, the net arterial-interstitial concentration difference, rather than the interstitial level alone, should preferably be investigated when regional tissue metabolism is assessed.

Variations in local blood flow may substantially affect the substrate levels in the tissue, and several hormones regulate both local tissue metabolism and circulation. To distinguish between metabolic and vascular effects, a metabolically inert blood flow marker can be added to the perfusion medium. When the local blood flow is increased, the transfer of the marker across the microdialysis membrane is enhanced. Ethanol is usually used for this purpose, which provides a qualitative measure of major blood flow variations. Alternatively, μD may be combined with the ^{133}Xe clearance technique, which is well validated for measurements of the local blood flow rate. With this combined approach, absolute rates of metabolite fluxes across the tissue can be estimated under steady-state conditions, according to the Fick's, principle (net tissue uptake or release = blood flow x arterio-venous concentration difference). This equation has formed the basis for the arterio-venous balance technique, but is also applicable for μD where the (absolute) interstitial tissue concentration can be recalculated to equal the venous concentration.

A limitation with the Fick's, principle is that it only quantifies the net substrate flux across the tissue bed, whereas it cannot separate simultaneous uptake and release of the metabolite in the tissue. Recently, this distinction has been accomplished with the use of stable, non-radioactive isotopes of the metabolites of interest. The stable isotope is administered systemically, with measurement of the enrichment of the isotope in the tissue interstitial fluid sampled by microdialysis. Together with determination of local blood flow, the rate of tissue uptake and/or release can then be calculated (55).

By applying a protocol with *in situ* perfusion of various target cell receptor agonists and antagonists, the regulation of cellular metabolism may be investigated at the molecular level. The *in situ* perfusion technique can also be performed in conjunction with systemic administration of hormones or regulating compounds, or under experimental conditions where the release of endogenous hormones is stimulated.

The μD technique has been utilized clinically for continuous glucose monitoring in subcutaneous adipose tissue in patients with diabetes (56). By combining a microdialysis device with an extracorporeal electro-chemical glucose sensor, a real time glucose monitoring system has been developed which is commercially available (57,58). Real time glucose monitoring is primarily intended for patients with diabetes, but may also prove useful in the surveillance of critically ill patients.

The combination of μD and laser doppler flowmetry (LDF) provides an ideal system for the *in vivo* study of human physiological and pathophysiologic processes within the vasculature. LDF permits monitoring of blood flow in discrete areas of skin, while simultaneous μD allows direct administration of vasoactive substances and/or sampling of cutaneous ECF from the same areas. In this way, miniscule amounts of vasoactive agents can be delivered into minute areas of human skin to produce significant local drug effects. Because of the small amounts of drug used, there is no risk of systemic effects as is the case with intra-arterial or other types of systemic drug administration. This approach was used to demonstrate that nitric oxide (NO) generation is involved in the human thermoregulation. In animals and humans, the combination of LDF with *in vivo* μD and oxyhemoglobin (OxyHb) trapping has been used to measure the relationship between bioavailable NO concentrations and blood flow (59). This could not have been achieved in any other way.

REGULATORY ASPECTS

Bioavailability assessment is critical in early phases of drug development. As defined in the FDA CFR 320.1(a), bioavailability (BA) refers to the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and "*becomes available at the site of action*." Thus, a potential drawback of the current approach of relating plasma concentration with therapeutic response is inadequate prediction of tissue drug concentrations leading to poor therapeutic intervention and/or drug toxicity. Typical examples are (1) sub-therapeutic dose response in certain anti-infective therapy, potentially contributing toward drug resistance; and (2) toxicity associated with high doses of anticancer drugs, primarily due to poor drug-penetration at the site of action. Keeping in mind that the current drug discovery and development process has become lengthy, inefficient and very costly, and that the vast majority of investigational drugs fail after entering clinical trials, there is a need to focus on therapeutic strategies and drug development based on drug concentrations at the active site. μD has been used to measure *in vivo* tissue concentrations of endogenous compounds and to assess drug concentrations closest to the site of action in various human tissues, such as peripheral tissue or skin, in both healthy volunteers and patients. Consequently, μD is gaining recognition as a tool in drug development to select an appropriate compound for further development and to optimize dosing regimens. Evidences for this are drug development plans using μD techniques in support of clinical data assessment. In addition, the FDA Center for Devices and Radiological Health has recently approved two devices utilizing the principle of μD—a Cerebral Tissue Monitoring System to monitor biochemical markers of ischemia in the brain (60), and a device to measure glucose through the skin (58). While the FDA does not require μD studies at this point in time, the agency is receptive to μD data as part of an overall pre-clinical and clinical pharmacology package of drug development. For example, pre-clinical μD data have been accepted in support of the mechanism of action of the anti-seizure drug zonisamide (61). Another example is an Investigational New Drug Application submission providing pre-clinical *in vivo* evidence to evaluate the drug CPP-109 in the treatment of cocaine addiction (62). Applications of μD are being explored to address specific safety issues related to systemic drug delivery (63), while the European agencies have specified the use of μD for preclinical abuse liability studies (64). μD data are likely to become an important part of new drug submissions, and thus may potentially contribute to the FDA Critical Path Initiative (65) to facilitate innovation in drug development.

Ultimately the acceptance of μD as a regulatory tool will be dependent upon the correlation of the results from μD with clinical response. Thus, validation will be the key to regulatory acceptance of the methodology. As μD migrates from the research laboratory into a clearer role in drug development early involvement of the regulatory agencies in its use will be critical to the successful use of μD To this end the aforementioned FDA Critical Path Initiative charges the FDA with bringing scientific advances to the medical product development process and to modernize regulatory standards to reflect the best science. As regulatory policy evolves so will the role of μD in drug selection, development and ultimately clinical benefit to the patient.

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