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Review Article

APPLICATION OF MICRODIALYSIS IN PRE-CLINICAL DRUG RESEARCH AND DEVELOPMENT

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ABSTRACT

Microdialysis has been a valuable tool in drug research and development for the past several decades. It is an *in vivo* sampling technique that permits the continuous monitoring of local concentrations of drugs and metabolites in the extracellular space and provides temporal information about free concentrations at specific target sites in experimental animals and humans. The majority of microdialysis applications at the time it was developed were preclinical studies which focused on neurotransmitter release, reuptake, and metabolism. However, microdialysis was quickly adapted for the purpose of measuring drugs and metabolites to study pharmacokinetics and drug metabolism in blood and other tissues of living organs and thus lead to quantitative studies of drug distribution to the central nervous system (CNS), subcutaneous sites, liver, lung, muscle, adipose, and other target tissues. Soon thereafter, microdialysis was employed in clinical settings to monitor endogenous neurotransmitter levels in the human brain. The microdialysis technique can also be used to deliver drugs to a desired target tissue (termed as reverse microdialysis) and it has recently been used for the study of the local actions of drugs in specific tissues of interest in pharmacological and toxicological studies. This paper describes an overview of the general aspects of this *in vivo* sampling technique, followed by a survey of selected recent papers describing various applications in pre-clinical drug research and development. It can be concluded that microdialysis is a well-validated method and can provide valuable target site-specific information for a variety of target tissues.

Keywords: Microdialysis, drug research and development, drug disposition, pharmacokinetics.

INTRODUCTION

The cost of drug development in the pharmaceutical industry has risen remarkably in the past 30 years, due in part to the high failure rate of development candidates. As these escalating costs continue to threaten the development of new drugs, there is also an increasing pressure to improve the predictability of clinical outcomes for new chemical entities from preclinical studies[1]. Although all aspects of the drug discovery and development processes should be examined for potential cost savings, one approach would be a better understanding of the effect of a drug candidate at the site of action and improved knowledge of drug exposure at the appropriate site of action. Microdialysis is an *in vivo* methodology capable of providing rich target site-specific data and can therefore be an extremely useful tool in the pharmaceutical industry, to investigate pharmacokinetic-pharmacodynamic (PK/PD) profiles of drugs and to facilitate the selection of lead compounds and optimal dosing for subsequent clinical studies[2-3].

MICRODIALYSIS SAMPLING: AN INTRODUCTION AND OVERVIEW

Background and rationale

Traditionally, in clinical practice and in drug research and development processes, most decisions are made based on measurements of the concentrations of endogenous or exogenous biomolecules in the bloodstream[4]. This relies on the underlying assumption that there is a lasting equilibration of drug concentrations between blood and the target site[4].

Yet, most biochemical and pharmacological events take place in the tissues and target sites located far from the bloodstream. Because of this a complete and long lasting equilibration between blood and target tissues cannot always be assumed. Drug distribution processes may be characterized by high inter-tissue and inter-subject variabilities, resulting in target site drug levels substantially different from corresponding plasma levels[5]. Furthermore, most drugs, with only a few prominent exceptions, exert their pharmacological effects not within the bloodstream, but at defined target sites into which drugs have to be distributed from the central compartment in order to exert their pharmacological effects. Suboptimal target site concentrations can have important clinical implications, as this is a potential explanation for some therapeutic failures[5-6]. Therefore, evaluation of target site distribution of a drug has long been examined as a more rational way to provide clinically meaningful data, rather than simply obtaining indirect information from blood samples[5-7]. More specifically, it is often the interstitial tissue space that is most closely related to the site of action of a drug and microdialysis is currently the only clinical and experimental tool that provides data from the extracellular space. The advantage of this approach, compared to traditional blood sampling and tissue collections, is the ability to continuously monitor blood and extracellular fluid drug concentrations without repeated blood sampling and with minimal tissue damage, while minimizing animal use[2]. The first studies of human drug pharmacokinetics utilizing the microdialysis technique were published in the early 1990s. Today, the

FDA has approved the use of microdialysis catheters for humans and microdialysis can be performed in many tissues including brain, skeletal muscle, adipose tissue, liver, tumors, heart, and lung. Currently, there are more than 13,000 publications available on microdialysis, including more than 2,000 publications on its applications in humans.

PRINCIPLE OF MICRODIALYSIS

The microdialysis sampling technique was invented as an animal research technique[8]; however, it has been gradually adapted for clinical use in human applications[8-9]. The technique is based on the fundamental principle of passive diffusion and dialysis (dialysis adapted from the Greek to separate), in which a small diameter probe containing a semi-permeable membrane (permeable to water and small molecular weight solutes) is introduced into a living tissue and perfused with an isotonic solution which mimics the ionic composition of the medium surrounding the probe[9]. As the perfusion fluid flows through the membrane, an equilibration process is initiated with the surrounding medium via diffusion in both directions (**Figure 1A**). A concentration gradient created across the membrane causes passive diffusion of low molecular weight substances from the outside of the membrane into the perfusion fluid, where they are carried by the constant flow of the perfusion fluid to the probe outlet [2, 9]. The continuous flow through the probe allows samples to be collected from tubing connected to the probe outlet for analysis of the analyte(s) of interest.
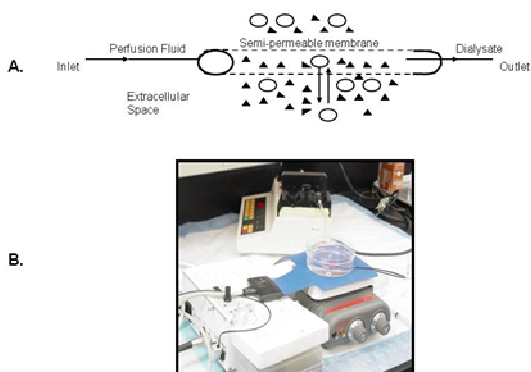

Figure 1. A. Illustration of the basic principle of microdialysis. B. Experimental set up of the *in vitro* recovery determination of MTX using a linear probe (CMA30).

Microdialysis probes

Microdialysis probes are commercially available in various sizes, designs, and materials. Each probe consists of an inlet tube, a semi-permeable membrane, and an outlet tube. All dialysis membranes are made of highly biocompatible and inert materials, such as cellulose acetate, regenerated cellulose, polycarbonate, polyacrylonitrile, and polyether sulfonate copolymer[10]. There are four basic designs of probe geometries: loop, side-by-side, linear, and concentric. Probe choice is usually based on the site of implantation and the characteristics of the tissue examined[10-11]. For instance, for sampling of moving soft tissues such as muscle, heart, skin, liver, tumor, and adipose tissue, linear and loop style

probes are typically most suitable[11]. The linear probe is more useful when implanted in peripheral tissues due to its flexibility. Linear probes easily enter and exit the tissue, allowing exact positioning of the dialysis window over a specific site of interest[11].

Probe characterization

Principle of recovery

There are several critical factors which must be considered in experiments utilizing microdialysis. Because microdialysis sampling does not produce samples directly from the fluid in which the probe is immersed and because the perfusion fluid flows through the dialysis probe at a rate which does not allow complete equilibration to be achieved, microdialysate samples contain only a fraction of the extracellular tissue diffusible (unbound) compound of interest. Therefore, one important question in microdialysis is how to relate the concentration measured in a dialysate to the actual tissue concentration outside the probe, within the tissue or extracellular fluid (ECF) in which the probe is inserted. This fraction, usually referred to as the relative recovery, is the factor by which the dialysate concentration is correlated to the ECF concentration[2, 9, 11].

In studies that investigate changes in levels of endogenous compounds from their baseline values, as for most studies which examine neurotransmitter level changes in the brain, it may not be necessary to determine the relative recovery. This is contingent on the assumption that there is a relatively constant recovery throughout the experiment. These types of studies typically evaluate percentage changes from a baseline level when an intervention such as a drug is administered or a physiological stress is introduced[2]. However, in pharmacokinetics and drug metabolism investigations in which there are no baseline values associated with exogenous substances, knowledge of relative recoveries becomes imperative for estimates of "true" extracellular tissue concentrations to be made[12]. Furthermore, in these types of studies recoveries may become time-dependent when the extracellular tissue concentrations vary during the course of an experiment[13].

Factors affecting probe recovery

There are many parameters related to experimental conditions which affect *in vitro* and *in vivo* probe recovery. These include perfusion flow rate, physico-chemical properties of the analyte of interest (such as size and charge of the molecule collected), perfusate composition, temperature, probe membrane composition, probe geometry, and various other factors that impact molecular diffusion characteristics of the compound of interest[2, 11, 14]. The average pore size of a dialysis probe typically has a 5-30 kD MW cutoff, to allow free diffusion of solute molecules but to resist the transport of proteins and other macromolecules[15-16]. Higher temperatures and larger probe membrane surface areas typically increase recoveries[15-16]. Among other

important factors are the diffusion coefficient of a solute in the tissue, the extracellular fluid volume fraction, and the processes involved in the elimination of a compound of interest by the tissue, including active transport mechanisms[2, 11]. Many of these factors have been incorporated as parameters in mathematical models [13-14].

CALIBRATION METHODS

In vitro calibration of the microdialysis probe

The objective of an *in vitro* calibration study is to characterize the diffusion of a compound of interest into the dialysis probe. Typically, this is determined by submerging a probe into a solution containing a known concentration of the compound of interest and perfusing the probe at a constant flow rate (normally less than 5 µL/min). The probe is examined to observe the effects of varying concentrations on the transfer rate of the compound across the dialysis membrane. Samples are collected from the outflow of the probe over specified time intervals and the concentration of analyte is determined. Subsequently, the relative recovery is calculated as the ratio of the outflow concentration of the analyte to the concentration of the analyte in the solution of known concentration outside the probe[2, 9, 11]. For instance, we investigated the *in vitro* recovery of Methotrexate (MTX, MW=454 g/mol) and its primary active metabolite, 7-OH-MTX, by placing a linear probe (CMA 30) in a petri dish containing different concentrations of MTX (1, 10, 100 µM) and 7-OH-MTX (**Figure 1B**). These *in vitro* recovery experiments demonstrated that a stable equilibrium condition was attained after 30 min, and that the relative recovery after equilibration was independent of the absolute concentrations of MTX and 7-OH-MTX in the surrounding medium[17]. The mean *in vitro* recovery of MTX after equilibration was determined to be approximately 39 ± 3.5%. Similar results were observed for 7-OH-MTX. These findings confirmed that the diffusion process across the linear probe is indeed independent of the drug concentration over a wide concentration range[17]. The validity of the *in vitro* calibration method depends on the assumed similarities of conditions to which the probe will be exposed when it is introduced into the living tissue of interest. This assumption may or may not be valid depending on the type of tissue into which the probe is inserted *in vivo*. In most cases, if the area of probe implantation is in a fluidic environment (e.g. blood), the *in vitro* calibration should be relatively valid. However, when the probe is implanted into a tissue which has a matrix constituting a more complicated microenvironment, the *in vivo* recovery will be altered in comparison to the *in vitro* recovery. Thus, the complex nature of living tissues excludes the use of *in vitro* calibration as a substitute for *in vivo* calibrations[2, 18].

In vivo calibration of the microdialysis probe

All parameters affecting *in vitro* recovery will also influence *in vivo* recovery. However, tissue characteristics *in vivo* have a crucial rule and ultimately determine recovery. A number of methods have been developed to attempt to measure true extracellular analyte concentrations [11, 18-19]. The most frequently used *in vivo* calibration methods are the low-flow rate, no-net-flux (NNF), dynamic no-net-flux (DNNF) methods, and retrodialysis (or reverse microdialysis) by either the drug or calibrator method[11, 18-19]. The premise of these methods is that the extracted fraction is the same in either direction across the membrane (isotropic), whether the exchange of solute across the membrane occurs by loss or gain. The most common calibration approach to determine *in vivo* recovery is retrodialysis using an analyte (e.g. a drug) which is performed either before or after analyte administration, when the tissue of interest is devoid of the analyte[2, 11, 18]. In this method, the analyte is added to the perfusate and its *in vivo* loss is used as an equivalent measure of *in vivo* recovery. This approach assumes that the relative loss of the analyte into the tissue, before the experiment, represents its recovery or relative gain from the tissue during the actual experiment. Although the retrodialysis method is relatively easy and useful, it requires validation. A disadvantage of this approach is the possibility of changes in recovery over time, which is not taken into consideration [11, 18]. This method is not applicable to study endogenous compounds as the requirement of zero 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 with a calibrator. In this case, an internal standard, or calibrator, is usually added to the perfusate during the course of the experiment [11, 18]. The internal standard should be chosen to match the characteristics of the drug as closely as possible, so that the concentration loss of the internal standard will most closely predict the concentration recovery, or gain, of the drug itself. This is especially critical for compounds that are actively transported through membranes. A good calibrator should have similar diffusion, transport, and metabolic properties to those of the solute of interest. The ideal calibrator is a deuterated or radioactive form of the test compound[11, 18].

IN VIVO MICRODIALYSIS SAMPLING FOR THE STUDY OF PK/PD AND DRUG METABOLISM

In the field of PK/PD evaluation of biomolecules or new chemical entities, the ultimate goal is to quantitatively determine the relationships between drug concentrations in the blood (systemic pharmacokinetics) and drug responses (pharmacodynamics) at the sites of action. In this regard, microdialysis has afforded new opportunities to study drug distribution, metabolism and PK/PD research, as it allows direct sampling of the ECF of tissues[3]. This usually produces more meaningful data than serum/plasma concentrations and therefore enables a better understanding of exposure-response relationships to aid the development of improved drug products.

One of the factors known to contribute to drug response variability is tissue drug distribution.

Drug responses are often mediated by interactions with varied target structures such as receptor proteins, enzymes, and transporters, which are frequently located far from the bloodstream. Consequently, the dose-effect relationship depends upon distribution of the active drug fraction to the binding sites, which leads to the concept of the target site concentration directly linked to therapeutic drug effect as a means of optimizing individualized drug therapy [20]. Most new chemical entities often fail during drug development processes because of an inadequate distribution at the target site[1]. While it has been acknowledged that most drugs do not distribute uniformly in the body but rather attain varying concentrations in different tissues[5], and that tissue concentrations are more predictive of clinical outcome than plasma concentrations in some cases[6, 21], the assessment of drug distribution and target site pharmacokinetics has long been treated as a "forgotten relative" in clinical pharmacokinetics[22]. The primary reason for this has been a lack of appropriate methods to provide *in vivo* access to target sites, and therefore PK research has long been restricted to drug concentration measurements from biological specimens that are relatively easy to access (e.g. tissue biopsies, urine and saliva sampling, or skin blister fluid measurements) or to indirect modeling of tissue concentrations from plasma concentration curves [4, 20].

During the past several years, a number of novel techniques for the assessment of drug distribution and target tissue PK in humans have become available; these include *in vivo* microdialysis, magnetic resonance spectroscopy (MRS)[23-25] and positron emission tomography (PET)[26]. The cost of microdialysis experiments is relatively low compared to those of MRS and PET techniques; however, microdialysis is a semi-invasive technique which has limited access to organs such as brain, lung or liver without surgery. Non-invasive imaging techniques, i.e. PET or MRS, allow drug concentration measurements in essentially all human organs. Spatial resolution of MRS imaging, however, is low, and although PET enables monitoring of regional drug concentration differences with a spatial resolution of a few millimetres, the discrimination between bound and unbound drugs or parent compounds and metabolites is difficult in this technique as PET measures primarily total tissue concentrations [27]. Furthermore, limited numbers of drugs are eligible for labeling, and radiotracer development of these drugs is time and labor intensive and requires special expertise and radiation exposure, in addition to high costs[27].

HIGHLIGHTS OF STUDIES DURING THE PAST 10 YEARS

While an exhaustive examination of the microdialysis literature is beyond the scope of this review, the application of microdialysis technique in pharmacokinetic and drug metabolism studies has been extensively reviewed in several places [28-31]. In the following overview, we have focused on selected examples from the past 10 years including our own recent investigations, to illustrate the broad range of possibilities for the use of microdialysis in drug research and development.

The primary application of microdialysis sampling has been in the examination of blood-brain barrier (BBB) transport and brain distribution of drugs. A requirement for this application is that BBB transport characteristics should not be considerably influenced by the implantation of a microdialysis probe into the brain. Whether or not the integrity of the BBB had been compromised during microdialysis experiments was initially of concern since the insertion of microdialysis probes has been shown to cause tissue trauma, which can impact the results of a microdialysis experiment[32-34]. However, a series of studies was performed to validate the effectiveness of intracerebral microdialysis in measurements of passive and active BBB transport [12, 35-38]. These investigations have shown that integrity of the BBB appears to hold during microdialysis experiments, provided that this technique is used in well controlled surgical and experimental conditions[39-40]. To provide sufficient time for initial trauma to subside, probes should be perfused for at least 30 min prior to the start of probe calibration, although often longer recovery times, 12-24 hours, may be recommended. This is based on the finding that several markers of tissue trauma such as glucose, lactate, lactate/pyruvate ratio, thromboxane B2, adenosine triphosphate, adenosine, and K^+ are elevated after probe insertion and reach baseline levels or become undetectable within this time range [41]. Additionally, changes in local skin circulation after insertion of microdialysis probes have been shown to return to baseline conditions within 60 minutes [41-42]. Histological studies have shown that the implantation of small-diameter dialysis tubing does not induce bodily rejection of foreign objects if implantation times are kept below 24 hours [41, 42].

Brain microdialysis has been applied to determine the distributional pharmacokinetics of CNS-acting drugs such as L-DOPA, amphetamine, cocaine, morphine, acetaminophen, analgesic peptides, anticonvulsants, in addition to antibacterial, antifungal, and antiviral agents[28]. Microdialysis has also been used in the study of CNS pharmacokinetics for potentially central-acting antihypertensive drugs, such as atenolol or irbesartan, to clarify their mechanisms of action [43-45].

Microdialysis has also been successfully employed in studies of drug equilibration across the BBB. The rate and extent of BBB transport is an important determinant of the time course and intensity of drug effects on the CNS[46-47]. The assumption that hydrophilic drugs reach equilibrium across the BBB more slowly than hydrophobic drugs has been shown not to be valid, and this equilibrium may be more dependent upon the transport processes utilized by many drugs. These results are

supported by findings of the presence of p-glycoprotein and other transport mechanisms at the BBB, and non-passive transport across the BBB seems to be the case for most drugs studied using microdialysis thus far[46]. The presence of p-glycoprotein in the BBB could be an important factor in the development of pharmacotherapeutic resistance to centrally-acting drugs such as antiepileptic agents[48]. Therefore, another utility of brain microdialysis is the study of p-glycoprotein drug efflux pump involvement in the central distribution of various drugs.

There has been a growing interest in recent decades in the application of microdialysis methodology to evaluate the disposition of anticancer agents in tumors, tissues of interest, and in patients with accessible tumors[49]. Microdialysis is considered to be a suitable technique to obtain drug concentration-time profiles in the interstitial fluid of solid tumors. Therapeutic failures in cancer patients often occur for various reasons that in some cases can be attributed to PK and PD failures, such as inadequate tumor drug concentrations and the development of drug resistance [50]. The microenvironment within a tumor is often significantly different from that of normal tissues, and many factors such as heterogeneous tumor blood flow, vascular permeability, cell density, and increased interstitial pressure can hinder the delivery and penetration of drugs from plasma to a tumor and can affect the overall distribution within the tumor [49-50]. This leads to complex relationships between concentrations in plasma, interstitial space, and ultimately at the target site (neoplastic cells). Therefore, given the complexities of drug accumulation in tumors, it is likely that tumor-specific drug concentration measurements will be of greater value than plasma drug concentrations as indicators of drug action and clinical response. This approach will facilitate the collection of meaningful data that might help compensate for the inherent nonhomogeneities in tumors and lead to accurate depictions of drug disposition in such tumors[49]. Preliminary investigations in breast cancer patients have suggested that concentrations of chemotherapeutic agents in a tumor may correlate with the clinical response to chemotherapy [7].
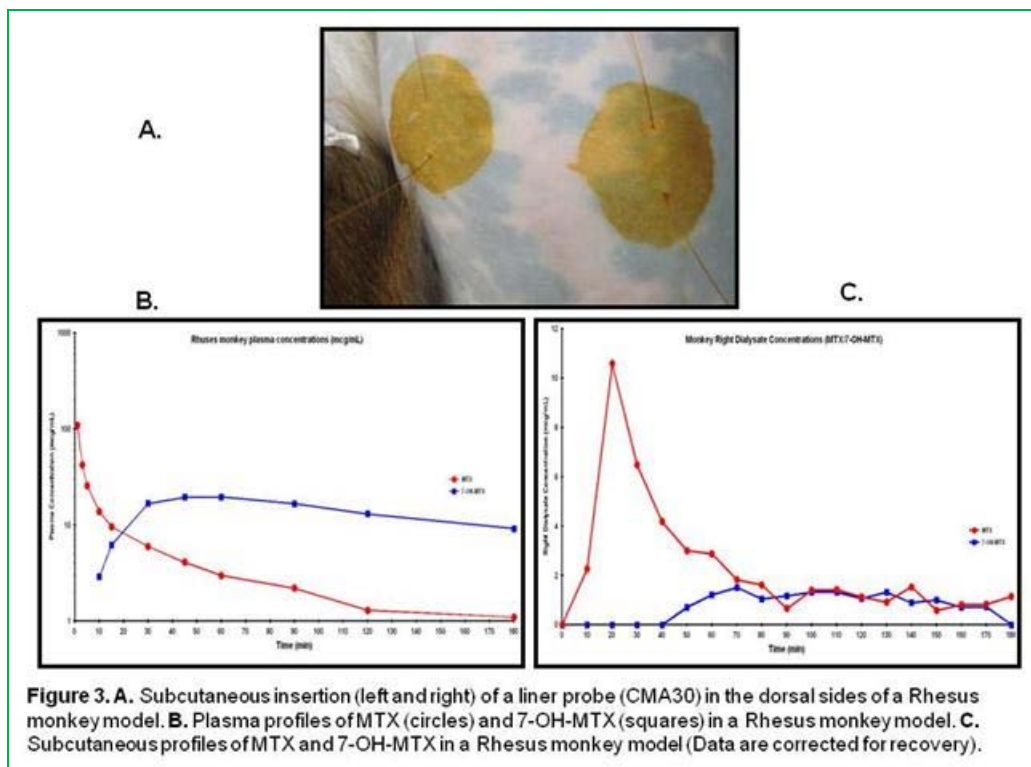

Figure 2. A. Subcutaneous insertion of a liner probe (CMA30) in the dorsal side of a rat model.
B. Plasma profile of MTX in a rat model following an intravenous dose of 200 mg/kg MTX C. Subcutaneous dialysate profile of MTX in a rat model [Data are corrected for *in vivo* recovery and expressed as the mean of the observed data; circles (●), versus the model predicted line (-)]
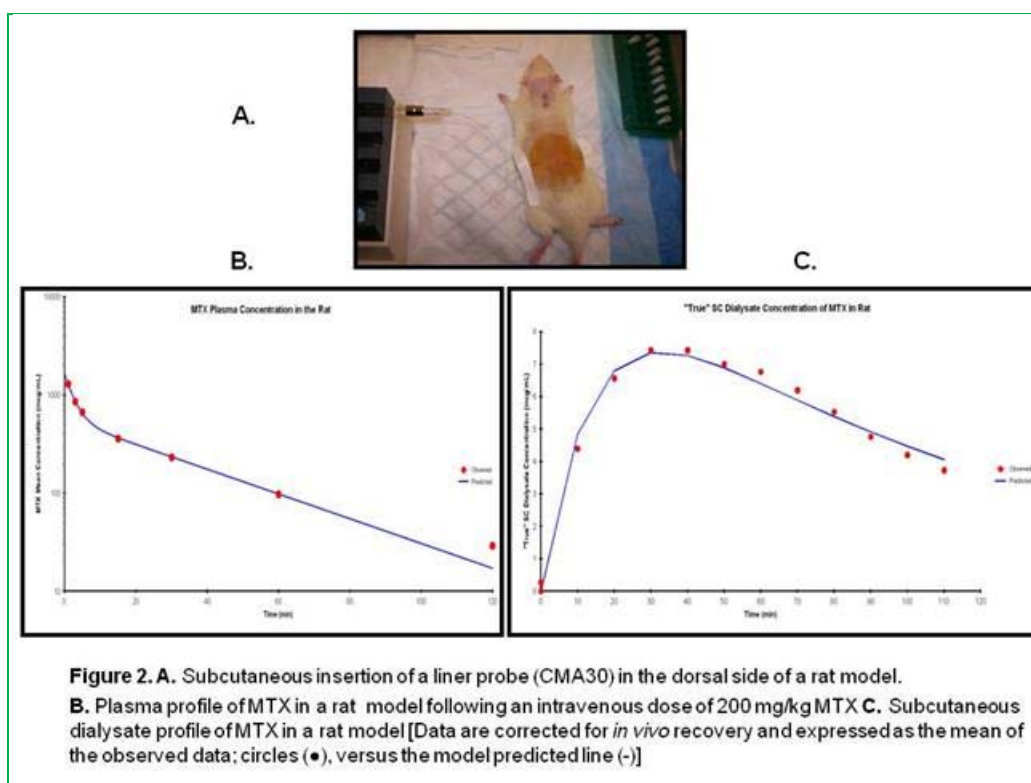
Our recent investigations using subcutaneous applications of linear probes in animal models (**Figure 2A, 3A**) indicate that microdialysis can be successfully implemented to determine the biodistribution profiles of MTX and 7-OH-MTX in both peripheral tissues and in tumors [17]. For example, in a rat model, the plasma and dialysate concentration-time profiles of MTX (**Figure 2B**, **2C**) were obtained to determine the pharmacokinetic parameters of the drug. 7-OH-MTX was not detected in plasma or dialysate, but was detected in excreted bile. The mean subcutaneous dialysate concentration of the drug reached a peak with a delay of 40 min after intravenous administration and exhibited a prolonged elimination half-life at its terminal phase (**Figure 2C**). Additionally, there was a significantly lower subcutaneous drug level compared to the plasma level. A similar study was carried out in a Rhesus monkey model following intravenous administration of MTX. Interestingly, in the monkey the metabolite was detectable in plasma (**Figure 3B**) although the dose of MTX administered to the monkey was 20 times less than that administered to rats. The elimination half-life of 7-OH-MTX in the terminal phase was clearly

very prolonged (more than 3-fold) compared to that of the parent compound. The right-side and left-side subcutaneous dialysate concentrations reached a peak with a delay of 20 min, (**Figure 3C**) and the subcutaneous drug level was significantly lower than the plasma level.

The calculated $AUC_{dialysate}/AUC_{plasma}$ ratios for right and left sides indicated a non-uniform distribution of the drug between the two dorsal subcutaneous sides of the monkey [unpublished data] (**Figure 3C**).

Figure 3. **A.** Subcutaneous insertion (left and right) of a liner probe (CMA30) in the dorsal sides of a Rhesus monkey model. **B.** Plasma profiles of MTX (circles) and 7-OH-MTX (squares) in a Rhesus monkey model. **C.** Subcutaneous profiles of MTX and 7-OH-MTX in a Rhesus monkey model (Data are corrected for recovery).

In drug metabolism studies, microdialysis probes have been implanted directly into multiple sites in the liver, simultaneously with a probe in the blood, to study concentration-time profiles of metabolites in the liver, and to determine whether regional differences in metabolite concentrations result from regional differences in enzyme activity or in substrate delivery [28, 51]. These studies often yield more metabolic and pharmacokinetic data and provide information about the heterogeneous distributions of drug metabolizing enzymes in different organs.

Another application of microdialysis is to examine the binding of drugs to plasma proteins *in vitro*, and more interestingly to evaluate the parameters affecting plasma protein binding *in vivo*. In this application, microdialysis is used to determine unbound concentrations of an analyte in the circulation with continuous sampling. This, in combination with traditional blood sampling techniques to measure the total concentration of the analyte in blood, will help characterize *in vivo* binding parameters [52-53]. This method more accurately determines protein binding than *in vitro* determinations since *in vitro* determinations systematically underestimate the unbound fraction of a drug [54].

Microdialysis sampling has recently been used to investigate dermal bioavailability and bioequivalence of topically administered products, since the site of drug

action in these cases is in tissue rather than in blood [55-56]. For instance, dermal microdialysis has recently been utilized to evaluate the bioavailabilities of a ketoprofen topical gel formulation and of topical metronidazole creams [57-58]. Cutaneous microdialysis data has been analyzed to investigate the potential to improve dermal drug delivery of hydrophilic and lipophilic substances by various formulations in microemulsion vehicles [59-60]. The relationship between dermal microdialysis sampling and a dermatopharmacokinetics method employed simultaneously has been evaluated in a bioequivalence study of topical formulations in humans [61]. In this study topical lidocaine cream and ointment were investigated in healthy human volunteers and dermal microdialysis was proven effective in bioequivalence studies in as few as 18 subjects[61].

APPLICABILITY OF REVERSE MICRODIALYSIS TO PHARMACOLOGICAL AND TOXICOLOGICAL STUDIES

In addition to monitoring extracellular fluid compartment analyte concentrations, the microdialysis technique can also be used to deliver drugs to a desired target tissue. This procedure is termed reverse microdialysis. Recently, reverse microdialysis has been used to study local actions of drugs in specific tissues of interest, primarily for pharmacological and toxicological studies. One advantage is that a substance is introduced into the extracellular space via the microdialysis probe selectively and in a

concentrated amount. The inclusion of a higher amount of drug in the perfusate allows the drug to diffuse through the microdialysis membrane and into the tissue. Reverse microdialysis not only permits local administration of a substance, but it also allows simultaneous sampling of extracellular endogenous compound levels. In this area, local effects of exogenous compounds have been studied in the CNS[62], hepatic tissue, dermis, and heart of experimental animals [63-64]. Although the species most often employed in reverse microdialysis investigations is the rat, experiments have also been conducted with other species such as hamsters, monkeys, mice, and chicks [65-68].

In CNS studies, reverse microdialysis has been used extensively to study effects on neurotransmission at different central nuclei of diverse pharmacological and toxicological agents such as antidepressants, antipsychotics, anti-parkinson drugs, hallucinogens, drugs of abuse, and many experimental drugs[45].

Studies of mechanisms of action of diverse therapeutic agents on the CNS is another application of reverse microdialysis. Reverse microdialysis experiments also permit the simultaneous study of other drug effects with a method that is independent of the standard microdialysis technique. In this sense, it is possible to monitor seizure activity, the cardiovascular response, the electrical activity at the site of microdialysis, and body temperature, and behavioral responses [69-73].

The possibility to use microdialysis for dynamic monitoring of hepatic metabolic function has been demonstrated by implanting a microdialysis probe into the middle lobe of the liver, perfusing the probe with a lidocaine-containing solution and determining the concentrations of tissue metabolites simultaneously with observations of altered physiological phenomena [74].

EXPERT OPINION

Microdialysis in a wide variety of applications has undergone significant development, improvement and validation during the last decade and has proved to be a well-validated, versatile, and valuable tool in drug research and development. The newer applications of microdialysis to pharmacokinetic and drug metabolism studies are now being utilized with much greater frequency than they were used in the past. The microdialysis technique, like other techniques, has limitations when it is compared to traditional sampling methods. The use of an appropriate calibration technique is crucial in pharmacokinetic studies to estimate actual interstitial drug concentrations, and perhaps this is still the most enigmatic difficulty in methodological aspects of microdialysis, as the relationships between dialysate concentrations and those at the local vicinity of a probe are uncertain. Additionally, there are still questions about the inter-study variabilities in recovery and the time-dependency of recoveries within an experiment. For optimal results in microdialysis experiments, an investigator must invest considerable amounts of time to

determine optimal experimental conditions to improve the outcomes of sampling. It is imperative to optimize the flow rate of microdialysis sampling as a function of the analytical system used (in general, slower perfusion rates improve recoveries but affect temporal resolution). For example, larger sample volumes (higher flow rates) will be required for volume-sensitive analytical methods with consequent decreases in recovery, which might necessitate improved limits of detection of the analytical methods used.

There are numerous other factors which must be evaluated before performing any microdialysis experiment, such as: type of probe most suitable for the intended purpose of the experiment (e.g., concentric or linear, flexible or stiff), length of the membrane (a longer membrane improves recovery but affects spatial resolution), composition of the perfusion fluid (preferably to mimic the composition of ECF), molecular cutoff, use of awake or anesthetized animals as anesthesia could alter pharmacological responses to drugs, and time needed to reach a steady state subsequent to damage and adverse tissue reactions caused by probe implantation, which could influence analyte measurements and subsequent data interpretation. One approach to minimize tissue damage is to provide a recovery period to the animals following probe insertion, allowing an interval of a few hours to several days before an experiment, to re-establish a stable tissue condition.

The monitoring of every compound is not feasible with this technique. Highly lipophilic drugs can stick to the probe membrane and create technical difficulties. Sometimes, these problems can be addressed by adding albumin (HSA) to perfusion fluids. The presence of albumin in perfusates will usually increase the uptake of a drug across the membrane, leading to enhanced recovery. For lipophilic drugs, therefore, investigators should follow procedures to check the membrane and tubing adsorption of a compound by measuring the outlet concentrations at specified time intervals prior to *in vivo* experiments. Molecules with large structures and high molecular weights, such as proteins and peptides, cannot readily diffuse through dialysis probes. In the case of a drug with a high percentage of protein binding, only a small fraction of the drug will be recovered and sample analysis will require a very sensitive analytical method with an extremely low limit of detection. In fact, one of the challenges to the use of microdialysis in pharmacokinetic studies is the selection of an analytical method capable to determine drug concentrations. Because of the need to obtain acceptable recovery levels, perfusion rates are often slow (0.1-2 µL/min) and generate very small sample volumes (1-20µL). Additionally, microdialysis samples often contain very low concentrations of an analyte, in the pM-µM range. Thus, the analytical method selected should be very sensitive and should have a limit of detection less than the lowest expected *in vivo* concentration. However, the rapid evolution of analytical methods seems to overcome these

issues and microdialysis can be used with a wide variety of analytical techniques. Rapid-separation analytical systems can be coupled on-line with microdialysis to provide nearly real-time data[75]. By combining microdialysis sampling with a suitable separation technique such as liquid chromatography (LC), microbar/capillary LC, or capillary electrophoretic separation with a highly sensitive detection method (e.g., immunoassay, UV absorbance, fluorescence, laser induced florescence, electrochemical detection or mass spectrometry) a 'separation-based sensor' can be developed. Such sensors have been successfully applied to investigate drugs and endogenous analytes [75].

Many applications of microdialysis, particularly in newly implemented fields, are, therefore, still in developmental stages and continuous efforts will be required to improve quantitative aspects associated with the microdialysis technique. In contrast to imaging techniques, which allow simultaneous drug distribution studies in several organs and tissues, microdialysis provides focal information on tissue PK at a limited number of sites. Despite these difficulties, if the microdialysis technique is used and its basic principles are understood, and if careful attention is paid to its technical aspects, it will continue to be a valuable tool to advance studies of pharmacokinetics, pharmacodynamics, drug distribution, and drug metabolism. In the future, we expect to see further expansion and validation of the use of microdialysis in experimental and clinical settings as well as the optimization of the method to be applied in regulatory affairs.

CONCLUSION

The utility of the microdialysis technique to monitor unbound extracellular levels of both endogenous and exogenous compounds has led to extensive use of the microdialysis technique in research of PK and PD properties of both old and new drugs. In PK studies, a notable advantage of this technique is the time course determination of levels of bioactive agents in target tissues, especially of centrally acting, antimicrobial, and antineoplastic drugs. The simultaneous recoveries of endogenous compounds through the microdialysis probe allow studies of drug effects on physiological events. This permits the simultaneous determination of a local drug level and its pharmacological effects and the study of a PK/PD correlation. Finally, introduction of a drug to the microdialysis perfusate allows the study of locally related pharmacological effects. These types of experiments could help to elucidate mechanisms of action of various drugs and to optimize dosing regimens.

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