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Review Article

Microdialysis: A Real-Time Sampling Technique for More Effective Pharmacokinetic-Pharmacodynamic Studies in Drug Research and Development

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Abstract

Microdialysis has been a valuable research tool in drug discovery 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 real-time free concentration profile at specific target sites in 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 organs and thus led to quantitative studies of drug distribution to the central nervous system, 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 review provides an overview of the general aspects of in vivo microdialysis sampling technique, and a survey of selected recent papers describing various applications in drug research and development, followed by an editorial perspective on the future implication of the technique. 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.

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 real-time target site-specific data and can therefore be an extremely useful tool in investigating pharmacokinetic-pharmacodynamic (PK/PD) profiles of drugs and to facilitate the selection of lead compounds and optimal dosing schedules for subsequent clinical studies [2-3].

MICRODIALYSIS

Traditionally, in clinical practice and in drug research and

development processes, most decisions are made based on the 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. As a result, 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]. Suboptimal target site concentrations can have important clinical implications, as this is a potential explanation for some therapeutic failures [5,6]. Conversely, abnormally high concentrations in the target site would promote drug-induced toxicity. Therefore, drug distribution in the target site has long been estimated by more reliable methods to provide clinically meaningful results, rather than simple, indirect information based on blood concentrations [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 human studies in 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 muscles, adipose tissues, liver, tumors, heart, and lungs. Currently, there are more than 13,000 publications available on microdialysis, including more than 2,000 publications on its applications in humans.

IN VIVO MICRODIALYSIS SAMPLING FOR THE STUDY OF PK/PD

In the field of PK/PD evaluation of 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 toxicity since it allows direct sampling of the extracellular fluid (ECF) of tissues [3]. This usually produces more meaningful data than methods for 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 [8]. 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 [8]. 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 [6-9], the assessment of drug distribution and target site pharmacokinetics has long been treated as a "forgotten relative" in clinical pharmacokinetics [10]. 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 traditionally been restricted to drug concentration measurements from easily accessible specimens (e.g. tissue biopsies, urine and saliva sampling, or skin blister fluid measurements) or to indirect modeling of tissue concentrations from plasma concentration curves [4,8].

During the past several years, a number of novel techniques for the assessment of drug distribution and target tissue PK have become available; these include in vivo microdialysis, magnetic resonance spectroscopy (MRS) [11-13] and positron emission tomography (PET) [14]. 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 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 is difficult in this technique since PET measures primarily total tissue concentrations [15]. 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 [15]. It should also be noted that imaging techniques frequently do not distinguish a parent drug from its metabolites, which could result in misinterpretation of pharmacokinetics and drug metabolism.

RECENT HIGHLIGHTS IN MICRODIALYSIS PK/PD

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 [16-19]. In the following section, 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 focused on 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. 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 [16]. 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 [20-22].

Microdialysis has also been successfully employed in studies of drug equilibration across the BBB. The rate and extent of BBB transport are important determinants of the time course and intensity of drug effects on the CNS [23,24]. The assumption that hydrophilic drugs reach equilibrium across the BBB more slowly than hydrophobic drugs has been shown to be invalid, and instead, 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 [23]. 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 [25]. Therefore, another utility of brain microdialysis is the study of p-glycoprotein drug efflux pump involved in the CNS 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 [26,27]. 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 [27]. 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 [26,27]. 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 the measurements of tumor-specific drug concentrations will be of greater value than those of plasma drug concentrations as indicators of drug action and clinical response. This approach will facilitate the collection of meaningful data that could help compensate for the inherent nonhomogeneities in tumors and lead to accurate depictions of drug disposition in such tumors [26]. 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].

Our recent investigations using subcutaneous applications of linear probes in animal models indicate that microdialysis can be successfully implemented to determine the biodistribution profiles of methotrexate (MTX) in both peripheral tissues and in tumors [28,29]. For example, normal and tumor-bearing mice were intravenously injected with MTX and the plasma concentration-time profiles were obtained from normal mice, while the microdialysis technique was employed to characterize the time course of MTX in tumor core. Disposition profiles of plasma and tumor were analyzed by two modeling approaches: (a) compartmental and (b) hybrid physiologically-based pharmacokinetic (PBPK) using SAAM II® (Simulation, Analysis, and Modeling) software. Both mechanism-based compartmental and hybrid PBPK models were developed to characterize atypical transport of MTX in tumors, indicating that microdialysis is a valuable tool to study tumor biodistribution of drugs. This information can ultimately aid in the development of anticancer drugs with improved PK profiles [29].

PERSPECTIVES: APPLICATIONS AND IMPLICATIONS

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, 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, since 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 a considerable amount 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. These include type of probes 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, time needed to reach steady-state conditions, and tissue 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 to perfusion fluids. The presence of albumin in perfusates will usually increase the

uptake of a drug across the membrane, leading to an 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. Large molecules, 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 (1-20 μL). Additionally, microdialysis samples often contain very low concentrations of analytes, 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 appears to overcome these issues and microdialysis can be used with a wide variety of analytical techniques. 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 over 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, pharmacogenomics, drug distribution and metabolism, and toxicity. 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 for regulatory approvals.

CONCLUSIONS

The utility of the microdialysis technique to monitor unbound extracellular levels of both endogenous and exogenous compounds has led to extensive use of the 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 and toxicological effects. These types of experiments could help to elucidate mechanisms of action and toxicity of various drugs and to optimize dosing regimens.

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