INTRODUCTION TO APPLICATIONS OF MICRODIALYSIS IN PHARMACEUTICAL SCIENCE

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Microdialysis is a very useful sampling tool that can be used in vivo to acquire concentration variations of protein-unbound molecules located in interstitial or extracellular spaces. This technique relies on the passive diffusion of substances across a dialysis membrane driven by a concentration gradient. After a microdialysis probe has been implanted in the target site for sampling, generally a blood vessel or tissue, a perfused solution consisting of physiological buffer solution flows slowly across the dialysis membrane, carrying away small molecules that come from the extracellular space on the other side of the dialysis membrane. The resulting dialysis solution can be analyzed to determine drug or target molecules in microdialysis samples by liquid chromatography or other suitable analytical techniques. In addition, it can be applied to introduce a substance into the extracellular space by the microdialysis probe, a technique referred to as reverse microdialysis. In this way, regional drug administration and simultaneous sampling of endogenous compounds in the extracellular compartments can be performed at the same time.

Initially, miniaturized microdialysis equipment was developed to monitor neurotransmitters continuously [1], and over the decades its use has extended to different fields, especially for drug discovery and clinical medicine. The main objectives in the early stages of drug development are to choose promising
candidates and to determine optimally safe and effective dosages. Pharmacokinetic (PK) simulation is concerned with the time course of drug concentration in the body, and pharmacodynamic (PD) simulation deals with the relationship of drug effect versus concentration. The method of PK–PD modeling can be used to determine the clinically relevant relationship between time and therapeutic effect. It also expedites drug development and helps make critical decisions, such as selecting the optimal dosage regimen and planning the costly clinical trials that are critical in determining the fate of a new compound [2–4]. The conventional concept for PK–PD evaluation of medicines is to measure total drug concentrations (including bound- and free-form drug molecules) in the blood circulation. However, only free-form drug molecules can reach specific tissues for therapeutic effect, and thus determining drug levels at the site of action is a more effective method of obtaining accurate PK–PD relationships of drugs.

The case of antibiotics serves as a good example to elucidate this concept. Most infections occur in peripheral tissues (extracellular fluid) but not in plasma, and the distribution of antibiotics to the target sites is a main determinant of clinical outcome [5]. Hence, the non-protein-bound (free-form) drug concentration at the infection site should be a better indicator for therapeutic efficacy of antibiotics than indices such as the time above the minimum inhibitory concentration (MIC), the maximum concentration of drug in serum ($C_{\text{max}}$/MIC), or the area under the curve over 24 h (AUC$_{24}$)/MIC derived from the total plasma concentration [6]. Recently, regulatory authorities, including the U.S. Food and Drug Administration, have also emphasized the value of human-tissue drug concentration data and support the use of clinical microdialysis to obtain this type of pharmacokinetic information [7], further indicating the significance of this technique.

This book focuses on the utilization of microdialysis in various organs and tissues for PK and PD studies, covering the range of current clinical uses for microdialysis. Topics include applications of this device for drug discovery, analytical consideration of samples, central neurological disease investigations, sampling at different organs, diabetes evaluations, tumor response estimations, and comparison of microdialysis with other image techniques. Special applications of microdialysis such as in vitro sampling for cell media, drug–drug interaction studies, and environmental monitoring are also included. Drug discovery and the role of microdialysis in drug development are described in Chapter 2. Due to the cost and time required for drug development, a more complete understanding of the pharmacokinetic, pharmacodynamic, and toxicological properties of leading drug candidates during the early stages of their development is fundamental to prevent failure. The use of microdialysis in early drug development involves the estimation of plasma protein binding, in vivo pharmacodynamic models, in vivo pharmacokinetics, and PK–PD relationships.

Chapter 3 presents general considerations for microdialysis sampling and microdialysis sample analysis. The homogeneity or heterogeneity of a sampling
site must be considered initially, and selecting the appropriate microdialysis probe and sampling parameters helps improve the spatial resolution within a specific region. Moreover, optimization of testing parameters, such as perfusion flow rate and modification of perfusion solutions, increases the extraction efficiency for more reproducible results. In addition, the advancement of analytical methodology supports a wider use of microdialysis, because highly sensitive detection instruments are capable of detecting trace analytes contained in the very small volume samples.

Microdialysis applications for several nervous system diseases, such as dopamine-related disorders, glutamate- and γ-aminobutyric acid (GABA)-linked neurobiological events, as well as the neurobiological mechanisms of seizures and antiepileptic drug action, are discussed in detail in Chapters 4 to 6. Dopamine is a neurotransmitter with multiple functions, and abnormal concentrations in the body have been known to lead to movement, cognitive, motivational, and learning deficits [8,9]. In the central nervous system, glutamic acid and aspartic acid are the chief excitatory amino acid neurotransmitters, while GABA and glycine are the main inhibitory transmitters. One of the chronic neurological diseases associated with these neurotransmitters is epilepsy, so GABA neurotransmission is a target for the design and development of drugs to treat epilepsy. In addition, cerebral microdialysis can help clarify the mechanisms of action of psychostimulants, addictive drugs, and analgesics, as well as contributing to studies on the control of amino acid-related neurons by receptors. A combination of microdialysis with brain imaging and immunological detection methods can further confirm and correct the results from those investigations. Microdialysis allows experiments to be performed in animals while conscious and with minimal movement restrictions, so that seizure-related behavioral changes can be both determined more accurately and correlated more closely with the fluctuation of neurotransmitters observed. As mentioned above, microdialysis is the method of choice for pharmacokinetic evaluations, because it samples the pharmacodynamically active free-form drug molecules. Microdialysis also permits the disposition and transport across the blood–brain barrier of antiepileptic drugs to be assessed. In short, microdialysis is an indispensable tool for the evaluation of neurotransmitters and thereby contributes to understanding the pathophysiology of neurological illnesses.

The range of current applications of microdialysis for clinical evaluation and basic research on different organs is presented in Chapters 7 to 14. Chapter 7 cover microdialysis in the lung for monitoring exogenous and endogenous compounds. Implanting a microdialysis probe in interstitial lung tissue is much more complex than is implanting probe in other peripheral tissues (e.g., skin, muscle, or adipose), because the lung has a protected anatomical position and is a highly vulnerable organ. Clinically, thoracotomy is generally required to avoid the risk from the abnormal presence of air in the pleural cavity, which results in collapse of the lung in clinical studies, thus limiting lung microdialysis experiments in patients with elective thoracic surgery. Due to the clinical
significance of infections in the lower respiratory tract, studies have focused on the pharmacokinetics of antimicrobial agents in lung tissue and the epithelial lining fluid to understand the amount of drugs that penetrate to the infection site. Another vital organ, the liver, is not only responsible for many metabolic processes but also produces bile, which contains surfactant-like components that facilitate digestive processes. Chapter 8 demonstrates how microdialysis offers an alternative way to monitor drug metabolism in the rat liver. By using microdialysis to investigate drug metabolism, the integrity and physiological conditions of the animal can be maintained, and more of the actual metabolic processes of xenobiotic compounds can be observed than with hepatocyte culture systems and in vitro enzymatic reactions. In the field of organ transplants, microdialysis combined with an enzymatic analyzer has been employed successfully to determine glucose, pyruvate, lactate, and glycerol to monitor tissue metabolism after liver transplants in humans, as discussed in Chapter 9.

The ability of microdialysis to measure free drug concentrations at the site of drug action makes it an excellent tool for bioavailability and bioequivalence assessment. Therefore, it has been used to determine bioequivalence of topical dermatological products according to industry and regulatory recommendations [10]. Chapter 10 reviews microdialysis applications to skin and soft tissues and their impact on clinical drug development. White adipose tissue is generally considered to be the main site for lipid storage in the human body. However, it is now also viewed as an active and important organ involved in various metabolic processes by secreting several hormones and a variety of substances called adipokines. Practical considerations and applications of microdialysis on adipose tissue in humans are detailed further in Chapter 11. Microdialysis has been used to observe the regulation of lipolysis in human adipose tissue by determining the extracellular concentrations of glycerol as an indicator. Disturbances of adipose tissue metabolism may lead to illness, and obesity has been determined as a major risk factor for hyperlipidemia, cardiovascular diseases, and type 2 diabetes [11]. Diabetes is a metabolic disorder in which the body produces insufficient insulin (type 1 diabetes) or where there is insulin resistance (type 2 diabetes). Long-term metabolic control in diabetic patients is crucial, and the microdialysis system is a suitable technique for continuous measurement of glucose concentrations. Chapter 12 describes the application of microdialysis to diabetes-related events in patients, including the diabetic patient’s metabolic state and the monitoring of antibiotic therapies for the feet of diabetics.

Cancer affects people worldwide and is the leading cause of death in modern societies, and chemotherapy research is pursuing more specific antineoplastic agents to reduce adverse drug effects in patients. Chapter 13 focuses on the PK–PD evaluation of anticancer drugs by microdialysis and describes its recent employment to evaluate drug disposition and response in solid tumors. In addition to microdialysis, advanced imaging techniques such as positron-emission tomography and magnetic resonance spectroscopy
have also become available to assess drug distribution, and Chapter 14 compares microdialysis with imaging approaches for evaluating in vivo drug distribution. Their advantages and drawbacks are reviewed, and their values as translational tools for clinical decisions and drug development are discussed.

Chapters 15 to 17 introduce special applications of microdialysis in studies of cell culture assays, drug–drug interactions, and environmental monitoring. Cell-based assays are essential in the preclinical phase of drug development, because these in vitro systems can speed up the processes of screening lead compounds, assessing metabolic stability, and evaluating permeation across membranes such as the gastrointestinal tract and the blood–brain barrier. Microdialysis sampling of cell culture systems, enzyme kinetics, and protein-binding assays are discussed in Chapter 15. Drug interaction is an important topic for clinical pharmacy, especially since the incidence of drug interactions is expected to increase with the increasing number of new drugs brought to the market. Exploring the relevance and mechanisms of drug interactions will assist clinicians in avoiding these often serious events. Herbal products, dietary supplements, and foods can also induce drug interactions. The reduced concentration of a free-form drug can cause treatment failure, while side effects or toxicity may occur when the drug level increases. In Chapter 16, the use of microdialysis as a tool to evaluate drug–drug or food–drug interactions is described. Recent pharmacokinetic and pharmacodynamic reports of drug–drug interactions are reviewed. Chapter 17 illustrates microdialysis as an in situ sample system by providing to the experimenter simultaneous sampling, cleanup, and real-time monitoring of targeted analytes for monitoring aqueous or solid environmental compartments or plant tissues. Although the designs of microdialysis probes for in vivo sampling are similar, modifications for monitoring particular environments can be made to enhance extraction efficiency by manipulating membrane materials, effective length of dialysis membrane, and perfusate composition. Several practical examples for environmental monitoring are also presented.

Compared with other methods of sampling intact tissue or body fluids, microdialysis offers several advantages for the experimenter. It provides the free fraction of drug molecules, which is the bioactive portion, so that more accurate PK–PD relationships can be constructed to help drug development and clinical therapeutic regimens. In addition, temporal resolution of data is improved dramatically by its continuous sampling, which can be used to observe, almost in real time, in vivo and in vitro enzymatic processes and reactions. Furthermore, the in situ measurement and sample preparation characteristics of microdialysis provide relatively clear dialysate that is ready for analysis; and sample contamination and dilution can be avoided when further treatments and extraction are performed. In sum, a broad range of studies applying microdialysis have been realized, as shown by the various topics presented in this book, making microdialysis an indispensable tool for pharmaceutical studies.
REFERENCES


