REVIEW ARTICLE

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Microdialysis; an Overview

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Microdialysis is an *in vivo* technique that permits monitoring of local concentrations of drugs and metabolites at specific sites in the body. About a decade ago the microdialysis technique entered the field of pharmacokinetic research, in the brain, and later also in peripheral tissues and blood. Microdialysis has several characteristics, which makes it an attractive tool for pharmacokinetic research. The objective of this review is to survey the recent literature regarding the application of microdialysis in pharmacokinetic studies and facilitating many other studies and peripheral tissues, subcutaneous adipose tissue, heart, lung etc and in blood. Microdialysis measures the unbound concentrations in the peripheral tissue fluid which have been shown to be responsible for the pharmacological effects. With the increasing number of applications of microdialysis, it is obvious that this method will have an important place is studying drug pharmacokinetic and pharmacodynamics.

Physicians frequently face the challenge to select appropriate drugs on the basis of the presumed ability of a drug to reach the target site. Due to the limited availability of reliable techniques to measure the distribution process to the target site, however, the success of this approach was often hampered by the physician's uncertainty about actual target tissue concentrations. Fortunately, recent years have seen the development of novel methods, notably innovative imaging techniques[1-3] and *in vivo* tissue microdialysis[4-7] which enable us to follow the path of a drug within the human body[8,9]. Microdialysis is used extensively in biomedical research to sample the extracellular space for endogenous and exogenous compounds and has become a routine research tool in physiology, pharmacology and pathophysiology.

Microdialysis was originally developed to measure concentrations of endogenous substances in the extracellular fluid of normal brain[10], particularly neurotransmitters[11,12] and has since become an important tool to investigate disposition of many classes of drugs. Sampling of substances in the extracellular environment by dialysis is not a new idea[10,13] during the last 10-15 years the technique has become as an important research tool in the field of neuroscience. More recently the technique is widely used in the areas such as drug distribution into the CNS, other organs, tissues[14-16], transcutaneous absorption[17,18]. The use of microdialysis sampling in various tissues has been extensively employed for pharmacokinetic and pharmacodynamic studies[19]. One advantage to microdialysis sampling is the drug concentrations measured are in specific tissue location, which may be different from the systemic circulation and possibly more representative of the concentration at site of drug action. Therefore, it is easily seen how this technique can be utilized in studies designed to determine the tissue distribution and targeted drug delivery of many compounds. While there are several examples of the use of microdialysis as an actual drug delivery device (the tissue volume to which drug may be delivered via a single microdialysis probe is very small), the use of quantitative microdialysis as a powerful means to evaluate various drug delivery strategies to specific tissues is gaining popularity.

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Attainment of drug concentration measurements in tumors is not trivial, and accordingly has been somewhat limited. The most common technique has utilized rodent serial sacrifice studies in which tumors are homogenized and processed for total drug concentrations which represents an average of vascular, interstitial and intracellular drug amounts that invariably limit evaluation of site-specific phenomenon[20]. Another drawback of serial sacrifice studies is the considerable resources needed to conduct the studies. The most troublesome aspect of such investigations is that each animal only contributes one timed sample, leading to high inter animal variability and compromising interpretation of the data[21,22]. Alternatives to tissue homogenization analysis for measurements of drug concentrations include quantitative autoradiography (QAR)[23], positron emission topography (PET) and nuclear magnetic resonance (NMR) imaging studies[1,24]. Each of these methods possesses certain advantages and disadvantages, yet none of the methods are generally applicable in that radioligands or contrast agents are required. NMR has a limited sensitivity and low spatial resolution, where as radio ligand-dependent QAR or PET cannot distinguish between parent drug and metabolites.

Microdialysis avoids a number of the aforementioned pitfalls of the other techniques used to characterize drug disposition in tumors. Similar to the non-invasive imaging techniques, serial timed samples can be collected from each animal that should minimize interanimal variability. Since radio labeled compounds are unnecessary, microdialysis should be applicable to a broad range of low-molecular weight drugs, and can enable characterization of both parent drug metabolites. The fact that the unbound drug concentration is measured in a specific compartment should facilitate a clear pharmacological interpretation and readily support physiologically based pharmacokinetic and pharmacodynamic models.

Microdialysis is not without its limitations that in large part can be attributed to the invasiveness of the procedure. It was shown that time-dependent changes occurred in blood-brain barrier permeability due to the insertion of the microdialysis probe[25]. Certainly caution should be exercised when interpreting such data, particularly when the measurements are assumed to be an absolute indicator of drug transport. Proper study designs that allow adequate time for tissue damage to repair and incorporation of comparative treatments should provide at least a relative measure of drug transport.

PRINCIPLE OF MICRODIALYSIS

Microdialysis is based on the principle that solute diffusion between two compartments separated by a semi-permeable membrane results from the concentration gradient across the membrane (fig.1) Applied to an *in vivo* situation, these two compartments represent the tissue ECF and the artificial physiological perfusion fluid inside the microdialysis probe (fig.1). Therefore the technique may also be used to deliver drug to a desired target tissue in addition to monitoring the extracellular compartment concentration of analytes.

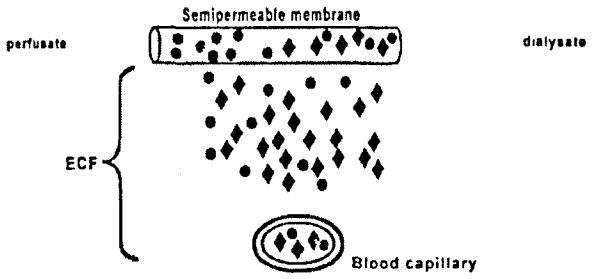

Fig. 1: Microdialysis process *in vivo*.

The compound being recovered from the surrounding ECF compartment in to the capillary (♦) and the compound being delivered to the surrounding ECF compartment from the capillary (●).

Microdialysis involves the insertion of a microdialysis probe into a selected tissue or (body) fluid. The probe consists of a small semipermeable hollow fiber membrane connected to an inlet and outlet tubing with a small diameter. The probe is continuously perfused with a physiological solution, the perfusate. The perfusate is an aqueous solution that must closely match the (ionic) composition of the (extracellular) fluid surrounding the probe in order to prevent unwanted changes in composition of periprobe fluid due to drainage or introduction of molecules. Molecules able to pass the semipermeable membrane will diffuse over the membrane down their concentration gradient into or out of perfusate. The solution that exits the probe, the dialysate, can be collected for analysis. The concentrations of the drug in the dialysate reflect the concentrations in the (extracellular) fluid around the semipermeable part of the probe. However, as the dialysis procedure is not performed under equilibrium conditions, the concentration in the dialysate will be different from that in the periprobe fluid. The term recovery is used to describe this relationship and should be determined by a suitable method for quantification of microdialysis data.

MICRODIALYSIS EXPERIMENTAL SETUP

A basic microdialysis setup consists of a microdialysis probe, a subject (an animal or human), a perfusion pump, inlet and outlet tubing, and a (refrigerated) microfraction collector. The length and inner diameter of the outlet tubing should be considered to minimize mixing of the dialysate and to prevent hydrostatic pressure build-up across the probe membrane. A syringe selector, an *in vitro* stand for the probes swivels for inlet and outlet connection tubing, and an on-line analysis system can extend the equipment.

The microdialysis probe can be home-made or purchased commercially. The perfusion pump should be able to provide an exact and pulse-free flow rate in the nl/min and ml/min range, while the microfraction collector should be able to collect volumes exactly according to pre-set volumes or pre-set time. Perfusate (inlet) tubing, the microdialysis probe and dialysate (outlet) tubing should not interact with the drug. A syringe selector accomplishes a change of perfusate syringes without interrupting the flow. An *in vitro* stand is useful for the safe storage of reusable probes and for testing *in vitro* recovery and may be home-made as well as commercially purchased. Swivels can be used to prevent tangling and twisting of the inlet and outlet tubing by the freely moving animal.

An on-line ejector enables direct collection and injection of the microdialysate when the analysis can be performed directly, for example by high-pressure liquid chromatography (HPLC) and an appropriate detection method. An example of an on-line experimental setup is given in fig. 2.

RECOVERY/EXTRACTION EFFICIENCY

The term recovery describes the relation between concentrations of the drug in the periprobe fluid and those in the dialysate. Those concentrations will differ from each other in case of a constant flow of the perfusete by which concentration equilibrium will never be reached. *In vitro*, a number of parameters influencing recovery can be investigated. Parameters include temperature, perfusate composition, perfusate flow rate, characteristics of the semipermeable membrane, probe geometry, surface of the semipermeable membrane, and the characteristics of the drug. The diffusion of the drug through the periprobe fluid may also influence recovery. *In vivo*, however, effective diffusion of the drug through the extracelluler fluid of a tissue will also be affected by uptake into cells, metabolic conversion rate, active transport across membranes, extent of tissue vascularization and blood flow. Special quantification methods are needed to determine the actual relation between dialysate concentrations and those in the extracellular fluid.

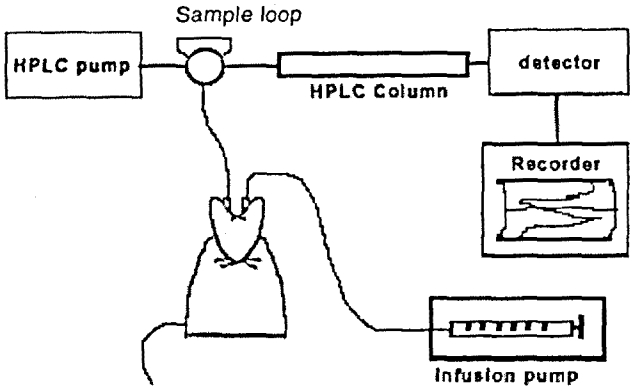

Fig. 2: On-line microdialysis experimental set up.

An infusion pump pumps the physiological solution via inlet tubing through the probe. The probe is present in the brain of the animal. The outlet tubing guides the dialysate into a loop of an HPLC system in which the sample can be collected during the sample interval. At the end of each sample interval the loop sample is loaded on to an HPLC column. The sample is separated, and subsequently the detector measures the content of the drug in each sample.

ADVANTAGES OF MICRODIALYSIS

Microdialysis has a number of advantages. Sampling can be performed continuou ly with this technique, without fluid loss. Thereby, one can obtain high-resolution concentration profiles of drugs and metabolites from (freely moving) individual subjects. This reduces the number of subjects needed for pharmacokinetic investigations. The probe is present at a certain location within the selected tissue whereby dialysate concentrations will reflect an extracellular concentration is a distinct region. With the dialysis principle providing protein free samples, which may be of special value from a pharmacological point of view, potential ex vivo enzymatic degradation is eliminated and clean up procedures for analysis will not be needed. Moreover *ex vivo* analysis of the dialysate sample permits the measurement of drug concentrations by virtually every analytical technique able to deal with the small dialysate volumes, which contributes to the selectivity and sensitivity.

LIMITATIONS OF MICRODIALYSIS

Implantation of probe will elicit tissue reactions that may

interfere with the system under investigation. Therefore, the valid use of the technique should be investigated for each application. The diluting effect of the dialysis procedure leads to lower concentration samples, which requires sensitive analytical methods. Another problem is associated with, mostly lipophilic drugs sticking to tubing and probe components, there by complicating the relation between dialysate and extracellular concentrations. The disadvantage of the technique is needed to determine *in vivo* recovery of the drug to calculate true concentrations in the extracellular fluid of the surrounding tissue. This may be time-consuming and partly counteract the advantage of the decrease of the number of subjects needed.

MICRODIALYSIS IN PERIPHERAL TISSUES

It has been suggested that the number of animals needed in preclinical pharmacokinetic studies can be substantially reduced by applying the microdialysis technique[6].

Muscle:

Endogenous compounds can be useful as biological markers, for example, glycerol is an indicator of lipolysis and lactate is a marker for glycolysis. Measurement of these compounds in peripheral tissues by microdialysis can be very useful and informative. The tissue penetration and distribution of antibiotics is of great importance, since most of the infections occur in the tissue. At the infection site, the free, unbound fraction of the antibiotic is responsible for the antiinfective effect. Microdialysis is a suitable method to evaluate unbound drug concentrations in the tissue.

Sarre *et al.* used microdialysis technique to determine the concentrations of L-dopa, dopamine, and their metabolites using α–methyldopa as internal standard (IS) in blood plasma and skeletal muscle extracellular fluid in anesthetized beagle dogs, after i.v. administration of L-dopa[26]. In a first calibration experiment, the *in vivo* relative losses (RL) of the compounds and the IS were determined. These were lower in skeletal muscle than in plasma. In a second step, the method was validated in blood plasma. The AUC_{0-3} values for the unbound L-dopa did not differ significantly between the dialysis and traditional whole blood sampling.

Microdialysis technique has also been applied for sampling of the free drug concentration at the receptor sites. Inadome *et al.* investigated the presence and subtypes of functionally prejunctional receptors in cholinergic nerve endings of rabbit detrusor smooth muscle strips using HPLC coupled with microdialysis procedure[27]. The effects of pretreatment with various drugs on acetylcholine release and contractile responses induced by electrical field stimulation were evaluated. The results indicate that the muscarinic receptors in the rabbit detrusor smooth muscle are heterogeneous, prejuctional facilitator (M_1 receptors), and inhibitory (M_2-receptors) for acetylcholine release, and post-junction M_3-receptors mediating contractile responses.

Subcutaneous adipose tissue:

Microdialysis has been widely used for sampling of endogenous compounds in subcutaneous adipose tissue. Doris et al. evaluated the chronic lipolytic effect of somatotropin on adipose tissue in sheep[28]. *In vivo* lipolytic rate in subcutaneous adipose tissue were determined by microdialysis. The results show that somatotropin treatment alters lipolytic regulation in sheep and this is characterized by changes in a number of proteins involved in this process. To investigate the short-term and long-term influences of estrogens on the *in vivo* lipolytic response of rat parametrial fat pads, an *in situ* microdialysis technique was used to determine the extracellular glycerol concentrations[29]. In another study, the microdialysis technique, used for measuring interstitial concentrations of glucose, lactate, and glycerol, was evaluated in an isolated autoperfused dog fat pad[30].

Heart:

There are numerous reports of interstitial fluid (ISF) and coronary venous adenosine measurements in isolated perfused hearts. A study was designed to simultaneously compare ISF and a coronary venous adenosine concentration during various interventions *in vivo* in porcine myocardium[31]. To determine whether protein kinase C mediated activation of ecto-5'-nucleotidase would increase interstitial adenosine concentrations in the rat heart *in vivo*. Sato *et al.* used the microdialysis technique to collect the samples from the left ventricular myocardium[32]. Another study investigated whether nitric oxide, a possible cardioprotective substance, can increase the production of interstitial adenosine in the ventricular myocardium[33]. Wei *et al.* evaluated the enzymatic mechanisms by which angiotensin II is generated in the ISF space of the dog heart *in vivo*[34]. Gilinski *et al.* proposed a useful method for routine study of myocardial norepinephrine[35]. A study was performed to determine the free concentration of propranolol in the left ventricular wall tissue of six anesthetized pigs by microdialysis[36]. Intracardiac microdialysis was also employed to study contrast agents in ischemic and non ischemic myocardium in pigs[37,38].

Lung:

Microdialysis technique was applied to the *in vivo*

intrabronchial pharmacokinetic measurement of tobramycin and gentamicin in the anesthetized rat[39]. The concentration versus time profiles of the drugs in the lung epithelial lining fluid following intravenous bolus administration was determined. The results demonstrated that this technique can be used for, intrabronchial pharmacokinetic measurement of drugs in the lung, either as an extension of the bronchoalveolar lavage technique or as a practical alternative to it.

Kidney:

A study examined the production of nitric oxide in the renal cortex and medulla through the use of an *in vivo* microdialysis technique[40]. This study has shown that nitric oxide concentrations are higher in medullar tissue than in the cortex.

Liver:

Microdialysis sampling of liver tissue was performed using probe geometry. The extent of tissue damage and response *in vivo* caused by implantation and indwelling of the probe was evacuated by histological examination of the tissue[41]. Linear probe, implanted using fused-silica tubing, was less damaging than other probe designs and implantation procedures tested.

Pancreas:

To evaluate the role of nitric oxide in leukocyte endothelial adherence in the pancreatic microcirculation after induction (cerulein) of acute pancreatitis, *in vivo* microdialysis was performed to detect pancreatic NO levels coupled with HPLC assay[42]. The results demonstrated a strong passivity correlation between numbers of adherent leukocytes and pancreatic nitric oxide level, suggesting that during the development of acute pancreatitis, nitric oxide could play an adverse role in microcirculation.

Blood:

The combination of blood microdialysis and HPLC or mass spectrometry has been used as an on-line method to follow the pharmacokinetics of the compounds directly in the blood-stream of animals[43-47]. Microdialysis sampling also provides potential use for *in vivo* determinations of plasma protein binding of compounds. Larger molecules, such as proteins and protein-bound drugs are excluded to give values for *in vitro* protein binding in plasma equivalent to those determined by ultrafiltration.

Other peripheral tissues:

Besides the tissue discussed above, microdialysis technique, also makes it feasible, to study compounds in the extracellular space of other tissues, such as cutaneous tissue[48,49], eye[16,50], reproductive system[51,52] and mucous membranes *in vivo*[53,54].

Clinical studies:

Microdialysis has become more popular in the studies, of drug concentrations in peripheral tissues is human. This technique allows the monitoring of metabolites and small molecules from the extracellular compartment as well as local delivery of metabolically active agents to this compartment.

Skeletal muscle:

The tissue kinetics of key metabolites of ischemic and postischemic tissue damage was studied in the intercellular space of human skeletal muscle by microdialysis[55]. Muller *et al.* used microdialysis to selectively assess the effect of the paracrine renin-angiotensin system (RAS) on interstitial glucose and lactase concentration profiles in skeletal muscle of healthy volunteers during basal and insulin-simulated conditions[56]. Paracrine RAS was selectively inhibited by local retrodialysis with enalaprilate. Interstitial lactate concentrations slightly increased during basal as well as during clamp conditions. Selective inhibition of paracrine muscle angiotensin converting enzyme increases interstitial glucose and lactate concentrations and decreases the serum interstitial gradient in muscle by facilitating Trans capillary glucose transport.

To study the relationship between blood flow rate and muscle metabolism, muscle microdialysis was performed in nine human subjects after an oral glucose load (75 g)[57]. Two microdialysis probes were inserted in to the medial femoral muscle for estimation of glucose and lactate concentrations in the interstitial fluid. After subjects fasted overnight, their glucose concentration in arterial plasma and interstitial fluid was 4.6±0.13 Vs 3.8±0.23 mM, and the corresponding lactate concentrations were 0.60±0.07 Vs 0.83±0.07 mM.
It has been reported that interstitial, insulin concentrations in lymph and adipose tissue are approximately 40% lower than in plasma. A microdialysis study was performed to measure measure insulin concentration is human muscle interstitial fluid[58].

Subcutaneous adipose tissue:

Microdialysis has been recently applied in investiga-

tion of metabolism of adipose tissue in humans. The method has been used to determine the interstitial concentrations of metabolites (glycerol, glucose and lactate) in adipose tissue and to investigate the regulation of lipolysis and of carbohydrate metabolism in situ in subcutaneous adipose tissue[59].

The correlations between the concentrations of topiramate (TPM), a novel antiepileptic drug, in subcutaneous extracellular fluid, plasma and subdural cerebrospinal fluid (CSF) were studied using microdialysis[60]. In a single case study of a patient with drug resistant partial epilepsy, the correlation between unbound TPM concentrations in subdural CSF and abdominal subcutaneous ECF was good.

Recently it has been demonstrated that microdialysis allows monitoring of free concentrations of antibiotics in extracellular fluid of muscle and subcutaneous tissue in humans. In one study, microdialysis was applied to investigate the peripheral compartment pharmacokinetics of selected antibiotics in humans[61]. The authors suggest that the concept of pharmacokinetic/pharmacodynamic surrogate markers for evaluation of antibiotic regimens originally developed for serum pharmacokinetics can be extended to peripheral tissue pharmacokinetics by means of microdialysis. This novel information may be useful for the rational development of dosage schedules and may improve predictions regarding therapeutic outcome.

Lung:

Currently no method is available for measurement of mediator release from intact human lung. A study was performed by using a microdialysis technique to measure histamine release from mast cells in human lung tissue *ex vivo*[62]. The authors concluded that the microdialysis technique allowed measurements of histamine release from must cells in intact lung *ex vivo*. It was mentioned that this method may be particularly useful since a number of experiments can be performed in a few hours in intact lung tissue without any dispersion or enzymatic treatment.

Breast tissue:

Previously it has been impossible to perform studies of human breast tissue *in vivo*. Dabrosin *et al.*, investigated whether the microdialysis technique is applicable in human breast tissue and whether the concentrations of amino acids, lactate and pyruvate change during the menstrual cycle[63]. Microdialysis was performed twice during the menstrual cycle in eight healthy women, in the breast and subcutaneous fat. Amino acids, lactate and pyruvate were analyzed by HPLC. None of the women showed any complications, such as bleeding or infection, after the experiments. The concentrations of aspartic acid, asparagine, serine, glycine, threonine, tyrosine and ornithine were decreased in the breast late in the menstrual cycle. This study has shown that microdialysis is a safe technique and is suitable for investigations of human breast tissue.

Other peripheral tissues:

Microdialysis has also been explored for measurement of substances in the interstitial space in intact or infected human skin[64-67]. Furthermore, microdialysis technique was applied for sampling around the superficial peroneal nerve in human feet[68]. Several studies were performed to measure the drug concentrations in tumors using microdialysis technique[69, 70].

BLOOD MICRODIALAYSIS

The microdialysis sampling technique is well suited to perform pharmacokinetic studies in small laboratory animal such as mice and rats. Since microdialysis probes can be implanted in almost any organ or tissue, including blood vessels, it offers a number of important advantages over the classical approaches to carry out pharmacokinetic studies. Because there is no net fluid exchange, continuous sampling for long periods of time is possible, without interfering with the process that governs the pharmacokinetic behaviour of the drug. This results in drug microdialysate concentration-time curves with a high time resolution thus facilitating pharmacokinetic analysis. A fundamental difference between serial blood sampling and microdialysis sampling is that the former is concerned with analyte concntrations measured at discrete time points while microdialysis samples are obtained during a certain time interval and therefore reflect the average concentration of analyte during that collection interval. This should be considered in the description of the concentration-time profiles[71-73].

Blood microdialysis in mice:

Repetitive blood sampling to carryout a full pharmacokinetic study in a single mouse is not possible because of its limited total blood volume. When using the mouse for pharmacokinetic studies, therefore, a number of animals are euthanized at certain times following drug administration and thus each animal only provides one blood concentration on the concentration-time profile. Microdialysis sampling which is not associated with fluid loss is therefore the only practical method to carryout pharmacokinetic studies in the mouse. The most challenging aspect of carrying out blood

microdialysis in the unanesthetized mouse is the implantation of the probe in a suitable blood vessel. Evrard *et al.* described a surgical implantation technique to implant a flexible homemade specific microdialysis probe in the inferior vena cava of the rat and subsequently applied exactly the same technique to the mouse[74]. The applicability of the technique was shown by measuring flurbiprofen concentrations in blood microdialysis during a 6 h period after i.v. bolus administration of flurbiprofen (20 mg/kg) to the mouse. Application of microdialysis in the mouse to carryout pharmacokinetic studies not only leads to a marked increase in the quality of the data but also significantly reduces the number of animals needed to characterize the pharmacokinetic behavior of a substance in this animal species.

Blood microdialysis in humans:

Microdialysis sampling in humans is mostly carried out by implanting the probe subcutaneously, intramuscularly, in adipose tissue or also intracerebrally in critical care patients and during operations[75-77]. The application of blood microdialysis in man is limited because the risk associated with implanting a probe into a blood vessel is still higher compared with the risk involved in probe implantation into subcutaneous , intramuscular or adipose tissue. However, with the recent development of flexible and robust probes that can be easily sterilized and that are introduced in arteries/veins of patients using standard clinical procedures, blood microdialysis in man has become feasible and a few reports demonstrating the use of blood microdialysis sampling in patients have recently been published. Patsalos *et al.*, have studied the pharmacokinetics of various antiepileptic drugs (carbamazepine, phenytoin, primidone and phenobarbital) in patients with intractable epilepsy[78]. It was observed that microdialysate pharmacokinetic profiles were comparable to those obtained by direct blood sampling. Paez and Hernandez developed a blood microdialysis probe and used it to monitor glucose levels after administration of an oral glucose load in healthy volunteers[79]. They showed that the procedure is easy and safe and that it would be possible to monitor blood glucose levels in an individual for several days. Simultaneous microdialysate and whole blood sampling from the same vein area may be very interesting when studying the plasma protein binding of drugs *in vivo*.

DRUG DELIVERY TO THE EYE

Microdialysis experimentation in ocular regions is a relatively recent event (late 1980s). Implantation in brain and neck precede ocular sampling by over 20 years[10]. Requirements such as sight preservation, development of ways to circumvent surgically related artifacts, and needed improvements in probe design contributed to the relatively recent start for exploring the microdialysis approach for ocular sampling. The anatomical size of the eye presents a number of difficulties for appropriate examination of both normal and pathologic conditions. Inherent in these difficulties is the sensitivity of the eye to immune protective cascades following manipulation[80,81]. Protection of visual function is also a major concern for any procedures proposed for observation of ocular pathophysiology or ocular pharmacokinetic/ pharmacodynamic experimentation. The anterior-posterior diameter of the normal adult globe is 24.5 mm[82]. The anterior chamber aqueous humor volume is 200 µl, the posterior contains 55 µl similar to the rabbit[83]. The depth is 3.5 mm. The vitreous chamber contains 2 ml of vitreous humor[84]. The chamber depth is much larger than that in the aqueous chambers. Differences in the dynamics of aqueous versus vitreous humor turnover provide clues to the challenges for targeted drug delivery to these sites.

Vitreous humor microdialysis sampling:

Earlier studies in the eye with microdialysis sampling were conducted in a rabbit[85] and cat[86] model. Adachi and coworkers[87,88], used a rather unusual species, the pigeon. In all the above mentioned studies vitreous humor was the site of examination. For many of these studies, microdialysis probes were positioned in close proximity to the retina in the vitreous humor in order to determine the time course of exogenously administered solutes and to characterize the disposition of endogenous substrates. Gunnarson and colleagues reported the earliest use of microdialysis in the eye. These authors examined the rate of appearance of radiolabeled water versus mannitol in vitreous following intra-carotid injection in rabiits[89]. Waga and colleagues provided a crucial examination of possible long-term placement of microdialysis probes in vitreous chamber of rabbits[90-92]. These authors were the first to develop a viable surgical procedure and to characterize carefully the pathophysiology of probe placement in the vitreous for upto 161 days. Louzada-Junior *et al.*[93] and Stempels *et al.*[94] examined the effect of ischemia on the health of the retina using microdialysis sampling of vitreous humor. Anesthetized rabbits were used for this animal model. Probes were placed adjacent to retina for sampling of endogenous amino acid markers such as glutamate and o-phosphoserine in vitreous humor. Studies such as these demonstrate the power of microdialysis approach for mechanistic investigations in the eye as well as other sites in the body.

Aqueous humor microdialysis sampling:

The dynamics of homeostatic regulation of intraocular pressure or aqueous humor formation rate, for example, directly impact on intraocular drug distribution characteristics. Due to these regulatory mechanisms, the eye is sensitive to any instructions to the anterior segment. Prior techniques such as paracentesis sampling resulted in disruption of this delicate regulatory balance that could precipitate appreciable alterations to ocular pharmacokinetics or pharmacodynamics of topically administered drugs. A major component in this disruption is compromise to the blood-aqueous barrier[81]. Other issues involved in the effects of the natural cascade of events in aqueous humor, such as fibrin formation and increased aqueous humor protein influx that may impact on available substrate concentration in aqueous humor[81]. Fukuda *et al.*[95] and Sato *et al.*[96] were the first to examine the utility of microdialysis sampling of anterior chamber aqueous humor. In their studies, probes inserted into the temporal cornea through the anterior chamber and exteriorized out of the nasal cornea were used to examine intraocular disposition of fluoroquinolones in the anesthetized rabbit. In later experiments Ohtori *et al.*[97] examined the ocular pharmacokinetics of timolol and carteolol in rabbits shortly after recovery from anesthesia.

CONCLUSIONS

The application of microdialysis sampling to pharmacokinetic studies in laboratory animals and man has seen a rapid development during the last 10 years. Employment of microdialysis in clinical drug development will enhance our knowledge on proper drug dosing and may help improve the design of pivotal studies in clinical drug development. Microdialysis provides an important advance in the assessment of intraocular disposition of drugs; substantially reduced populations are required for examination and realistic pharmacokinetic parameters devoid of confounding effects of anesthesia and protein binding have been obtained. Regions of the eye that have proven to be challenging for characterizing drug pharmacokinetics (vitreous humor) have been successfully examined using this technique.

For many years, pharmacokinetics was limited to blood and plasma concentration measurements. Although it was always well known that the site of action for most drugs is in the peripheral tissues, vascular concentrations were measured and therapeutically interpreted, simply because they were easy to obtain. Total tissue concentrations based on homogenized tissue biopsies further confused the situation. These concentrations which are quite commonly obtained in clinical toxicology studies are not very helpful since they reflect hybrid concentrations of free and bound drug and cannot easily be interpreted correctly. Microdialysis measurements in peripheral tissues has solved this ambiguity and opened the window to direct measurement of unbound drug concentrations of interest at the site of action. It can be expected that this technique will be further refined during the next years and will find a standard place in the arsenal of pharmacokinetic evaluation tools.

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