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Application of Microdialysis in Clinical Pharmacology

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Abstract: Microdialysis has been developed during the last 25 years by several authors primarily to study brain function and changes in levels of endogenous compounds such as neurotransmitters or metabolites in different laboratory animals. However, in the last ten years microdialysis sampling has been introduced as a versatile technique in the clinical setting. Although, microdialysis sampling has been extensively used for metabolic monitoring in patients, it was also employed for the study of distribution of different therapeutic agents especially anti-infective and antineoplasic drugs. In addition, clinical effect of drugs in patients could be also determined by means of microdialysis. So, this article reviewed the vast applications of the microdialysis technique for the study of pharmacokinetic and pharmacodynamic properties of drugs in the clinical setting.

Key Words: Microdialysis, clinical applications, therapeutic agents, pharmacokinetics, pharmacodynamics, metabolic studies.

INTRODUCTION

The microdialysis technique is an increasingly employed research tool for the study of pharmacokinetic and pharmacodynamic aspects of drugs in preclinical research, and more recently, in the clinical setting. The first published application of microdialysis in humans was a study on interstitial glucose in 1987 [1], and its use was initially confined to adipose tissue [1, 2]. This technique has the advantage of being minimally invasive, and it provides highly reproducible real-time chronological sampling with a short time resolution at the focus of interest (e.g. focus of infection). The tolerability of microdialysis sampling is high, and pharmacokinetic studies of up to 3 days duration have been performed in patients [3].

In vivo microdialysis has found important applications in the field of tissue pharmacokinetics, pharmacodynamics and pharmacokinetic-pharmacodynamic (PK-PD) modeling. The large body of work published (at least 1000 publications of studies in humans) underscores the importance of microdialysis sampling technique in clinical pharmacology.

A broad spectrum for new research in clinical and diagnostic applications opens due to the high degree of development of the microdialysis technique. So, the aim of this review is to discuss several aspects of the application of microdialysis to the study of clinical pharmacology.

PRINCIPLES OF MICRODIALYSIS

In the past twenty years the microdialysis technique has become a method of choice in the study of tissue concentrations of both endogenous and exogenous substances. The microdialysis sampling technique, as we know it today emerged from the neurosciences where it was originally used for measuring concentrations of neurotransmitters in rat brain [4].

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In this technique, a probe that is inserted into tissue mimics the function of a capillary blood vessel Fig. (**1**). The probe has a hollow fiber that is permeable to water and small molecules, and when it is perfused with a physiologic fluid, molecules are exchanged through the dialysis membrane by diffusion in both directions. Later, dialysate samples are analyzed using high sensitive techniques.

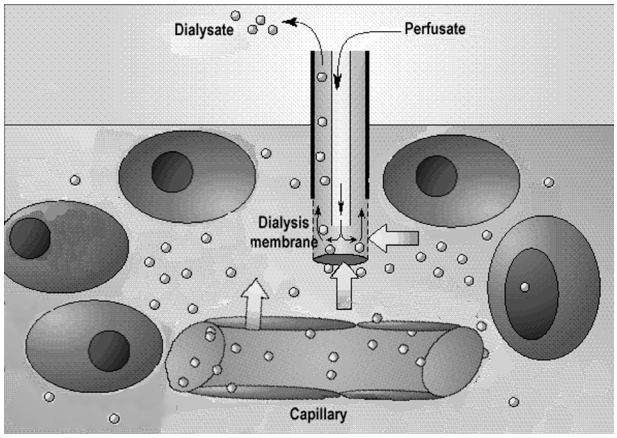

Fig. (1). Scheme of the microdialysis sampling principle. Adapted from [5].

The basic setup for a microdialysis experiment consists of a microdialysis probe, a perfusion pump, and an analytical method with the required sensitivity to quantify small concentrations of substances in small volumes of sample [6].

Probes used for microdialysis experiments can differ extensively in their shape and material, depending on where they are going to be used during the experiment. When they are implanted, a perfusion fluid enters the probe through the inlet tubing at a constant flow rate (generally 0.1–5 µl/min), passes the dialysis membrane and is then transported through the outlet tubing and collected in a microvial. Each probe has its own specific molecular weight cut-off determined by the pore size of the probe membrane but they are usually impermeable to large molecules, e.g. proteins.

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The perfusate solution usually mimics the composition of the surrounding medium of the probe. While the perfusate solution passes the dialysis membrane, molecules diffuse into (recovery) or out of (delivery) the perfusion fluid. The direction of the diffusion process is dependent on the concentration gradient. Thus, microdialysis can be used both for collecting a substance in the dialysate as well as delivering it into the periprobe fluid. The latter is referred to as reverse microdialysis [7].

The fact that the microdialysis technique provides protein free samples allows the analysis of the sample without pretreatment by liquid chromatography or capillary electrophoresis online coupling. Traditional blood sampling requires clean up procedures prior to analysis.

Taking into account that the microdialysis sampling is not performed under equilibrium conditions because the perfusate is constantly being pumped through the probe, the concentration of the drug in the sample is some fraction of that in the surrounding tissue. So, the limit of quantification of the analytical methods should be extremely low [6].

On the other hand, to obtain true tissue concentrations the factor by which dialysate concentrations are interrelated needs to be determined. This factor which is obtained during an *in vivo* or an *in vitro* calibration procedure is called relative recovery. Some of the variables that affect the relative recovery include membrane composition, effective membrane dialysis length, microdialysis probe design and dimensions, dialysate flow rate, diffusion characteristics of the analyte of interest and conditions of the medium and perfusion fluid such as temperature, composition and viscosity [8].

Microdialysis Catheters

The microdialysis probe is perhaps the nucleus of the microdialysis experiment. Many types are described and they are used in different experiments. The different geometry of microdialysis probes enables their use in virtually any tissue and any fluid of the body [9].

The microdialysis probe typically consists of a tubular dialysis fiber that is connected with an inlet and an outlet tube. The inlet and outlet tubes are connected with thin and flexible tubing with a perfusion pump and with a fraction collector, respectively.

In general, probes will either have a longitudinal, a semicircular or an I-shape design. Various designs have been described: concentric cannula probe, linear probe, shunt probe. It has also been reported several modified probes design such as: spinal loop dialysis catheter [10], flexible intravenous probe [11], and shunt intraarterial microdialysis probe [12], which are used in preclinical studies. For soft peripheral tissues flexible probes can be used.

Linear probes are useful for monitoring transdermal drug delivery. However, an increase in skin blood flow, skin color (redness) and erythema and skin thickness was demonstrated after insertion of a microdialysis probe [13]. Local anaesthesia prior to insertion reduced the vascular effects of needle insertion trauma and it was seen that probe depth did not have any influence on the needle insertion trauma.

Microdialysis in adipose tissue and resting skeletal muscle could be performed with an especially developed commercial catheter to achieve excellent diffusion characteristics. Insertion into the tissue is easily achieved with the help of a unique slit cannula introducer that leaves the catheter in place when withdrawn [14].

Another application is the hepatic microdialysis catheter, which allows the surgeon to continuously monitor hepatic tissue metabolism after liver transplantation. This catheter has a very long membrane (30 mm) and it is introduced into the abdominal cavity by using a special needle. Insertion into the liver is achieved with the help of introducer and after the insertion into the transplanted liver the catheter is then sutured by the surgeon. A similar catheter may be introduced into the intraperitoneal cavity [15].

Some probes can be rigid like the concentric cannula used intracerebrally. In this case, guide cannulas can also be implanted which opens up the possibility to insert the probe itself after surgical recovery, thereby decreasing effects of anesthesia.

The catheter tip of the CMA 70 Brain Microdialysis Catheter® (Solna, Sweden) has a gold tip, which makes it visible on a computed tomography scan in order to locate the exact position of the catheter in the brain [16].

Another catheter for the study of the brain is the CMA 71 High Cut-Off Brain Microdialysis Catheter® (Solna, Sweden). The membrane of the probe has a cut-off of around 100.000 Da [17], allowing diffusion of large molecules such as cytokines.

Sterilization of Microdialysis Probe

When microdialysis probes are implanted into a tissue it is necessary to keep asepsis in the surrounding area of the site of catheter insertion using surgical equipment.

A preliminary evaluation of several disinfection and sterilization techniques for use with experimental microdialysis probes was reported by Huff *et al*. [18]. These authors found that two disinfection methods (70% ethanol and a commercial contact lens solution) and two sterilization methods (hydrogen peroxide plasma and e-beam radiation) did not seriously affect the functionally of the probes although hydrogen peroxide plasma and contact lens solution groups reduced the extraction efficiency of microdialysis probe.

Gamma irradiation has also been used as method of sterilization [19]. Commercially available probes were sterilized by gamma irradiation (32 kGy) prior to use. Subsequent *in vitro* experiments confirmed that the irradiated probes behaved identically to native probes from the supplier. In this work, there was no sign of infection at the implantation sites, probably due to our strict attention to sterility during the probe implantation as well as to prophylactic treatment with a broad-spectrum antibiotic [19]. However, the most popular method to sterilize microdialysis probes is the gas sterilization method with ethylene oxide [20, 21].

Dialysis Membranes

Various materials can be used to construct the probes and the choice of the membrane type is an essential element to optimize the probe for a particular experiment (Table **1**).

Conventional microdialysis probes are constructed with 20 kDa molecular weight cut-off membranes enabling the measurement of small molecules such as glucose, lactate, pyruvate and glutamate. Common substances used as membrane materials are cuproamonic rayon, celluloses, polycarbonate, polyethersulfone or cuprophan and the effective membrane dialysis length ranges from 1 to 30 mm.

A 100 kDa molecular weight cut-off microdialysis catheter has recently been introduced to allow detection of larger molecules such as cytokines [17]. These probes can be constructed with polyethylenesulphone [23]. One of the technical problems encountered with the 100 kDa microdialysis membrane has been the issue of poor sample volume retrieval caused by pressure differences between the membrane and the surrounding tissue forcing the perfusate out of the catheter. A way of addressing this problem is to equalize the pressure differences between in-flowing and out-flowing perfusate at the membrane by increasing the colloidal pressure of the perfusate by using dextran [24].

Despite the molecular weight cut-off of the membrane, the molar mass of the substance of interest has to be taken into consideration. As we discussed previously, only substances with a molar mass lower than the weight cut-off are capable of passing the membrane. However, even if the molar mass falls below the molecular weight cutoff, an acceptable relative recovery will only be attained with substances having a molar mass lower than approximately one-fourth of the membrane cutoff [7].

The size of the microdialysis membrane can also influence the relative recovery. According to Fick's law the rate of diffusion across a membrane is proportional to its area. Therefore, increasing the length and thus the area of the microdialysis membrane will lead to an increase in relative recovery [7]. On the other hand, it has been reported that increasing the outer diameter of the inner cannula may enhance relative recovery of the probe [25, 26].

An important key is that the membrane does not interact in any way with the surrounding tissue or with the perfusate. Lower recoveries of acid aminoacids due to the presence of surface charge have been described [27]. Also recovery of neuropeptides can vary as much as 20% with different dialysis membranes [28].

Perfusion Solutions

There are several perfusion media used in microdialysis experiments and they vary widely in their composition and pH (Table **2**). The ideally composition, ion strength, osmotic value and pH of the perfusion solution should be as close as possible to those of the extracellular fluid of the dialyzed tissue.

In most experiments, the perfusate is composed by an aqueous solution of sodium and potassium salts and other ions in a minor proportion, sometimes with a very small concentration of protein.

In previous studies on skeletal muscle, an imbalance between the expected and the collected volume has been demonstrated at low perfusion flow rates [29, 30]. The addition of dextran to the perfusion solution prevents the fluid lost. Rosdahl *et al*. [24] demonstrated that the estimated concentrations of dextran in the perfusion solution at which no net loss of perfusion fluid occurred produces a colloid osmotic pressure similar to reported values for plasma. This implies that the plasma colloid osmotic pressure contributes to a mass transfer of fluid from microdialysis catheter to capillaries. In some cases, proteins should be added to the perfusion medium to prevent sticking of drugs to the microdialysis probe and tubing connection [31].

METHODOLOGICAL ASPECTS OF MICRODIALYSIS IN PHARMACOKINETIC AND PHARMACODYNAMIC STUDIES

Microdialysis offers a powerful investigative tool that is actually applied to clinical pharmacological studies. The microdialysis technique not only allows sampling of extracellular concentrations of drugs but also endogenous compounds such as neurotransmitters, metabolites, glucose, lactate and low molecular weight peptides. Therefore, this

Table 1. Microdialysis Membranes (from Levine and Powell [22] with kind Permission from Academic Press Inc.)

Membrane materials	Outer Diameter (mm)	Molecular Weight cut off (Da)
Cellulose	0.20	6,000
Cellulose	0.25	5,000
Cellulose	0.32	5,000
Copolymer (polyacetronitrile /sodium methallyl sulfonate)	0.31	15,000
Copolymer (polycarbonate / polyether)	0.50	20,000
Acrylic copolymer	0.30	50,000
Polyethylenesulphone	0.30	100,000

Table 2. Different Compositions of Perfusion Medium (Adapted from Höcht *et al*. [6])

Perfusion Medium	Composition
Distilled Water	
Saline	0.9% NaCl 0.9% NaCl; 0.5% bovine serum albumin
Ringer Solution	147mM NaCl; 1.3mM CaCl2; 4mM KCl (pH 7.2)
Modified Ringers solution	145mM NaCl; 1.2mM CaCl2 ; 2.7mM KCl, 1mM MgCl2; 0.2mM ascorbate (pH 7.4)
Buffered Ringers solution	147mM NaCl; 3.4mM CaCl2; 2.8mM KCl, 1.2mM MgCl2; 0.6mM K2HPO4; 114 mM ascorbate (pH 6.9)
Krebs Ringer solution	138mM NaCl; 1mM CaCl2; 5mM KCl; 1mM MgCl2; 11mM NaHCO3 ; 1mM Na2HPO4 , 11mM glucose (pH 7.5)
Krebs Ringer bicarbonate	122mM NaCl; 1.2mM CaCl2; 3mM KCl, 1.2mM MgSO4; 25mM NaHCO3; 0.4mM KH2PO4, (pH 7.4)
Krebs-Henseleit bicarbonate buffer	118mM NaCl; 2.5mM CaCl2; 4.7mM KCl, 0.6mM MgSO4; 25mM NaHCO3; 1.2mM NaH2PO4, 11mM glucose
Mock-cerebrospinal fluids	127mM NaCl; 1.1mM CaCl2; 2.4mM KCl; 0.85mM MgCl2; 28mM NaHCO3; 0.5mM KH2PO4, 0.5mM Na2SO4; 5.9mM glucose (pH 7.5)
Bile salt Ringer's	155mM NaCl; 5.5mM KCl; 2.3mM CaCl2; 20 mg.ml –1 bile salts.
Modified Krebs-Henseleit solution	118mM NaCl; 2.5mM CaCl2; 4.7mM KCl, 0.6mM MgSO4; 25mM NaHCO3; 1.2mM NaH2PO4, 11mM glucose, Dextran 70, 36g

technique is highly useful for the simultaneous study of the pharmacokinetic and some pharmacodynamic behaviors of drugs.

Microdialysis has a unique characteristic that made it very useful to its application in pharmacokinetic studies. The continuous sampling of a drug tissue concentration can provide a more thorough description of their rate and extent of tissue uptake and hence lead to more real pharmacokinetic profile. On the other hand, microdialysis sampling is much less useful for pharmacodynamic studies, because only the effects of drugs on endogenous compounds could be studied.

An important key in pharmacokinetic studies is the selection of an adequate analytical method for the drug concentration determination. Microdialysis generates small volume samples (1-10 µl), because of the need of slow perfusion rates (0.1-5 µl/min) to obtain high recoveries of the drug maintaining an adequate temporal resolution. On the other hand, microdialysis samples often contain low analyte concentration (pM-µM range) [6]. The analysis of the microdialysis samples can be made using a wide range of analytical methods but liquid chromatography and immunoassays are one of the most popular [32].

Assessment of *in vivo* recovery is an essential part of using microdialysis to study drug pharmacokinetics. *In vivo* recovery is generally less than the *in vitro* performance of the probe, because of reduced capacity for substrates and other molecules to diffuse in the extracellular space surrounding the membrane when compared with diffusing capacity in an aqueous solution [33]. I*n vivo* recovery of the microdialysis probe can be determined through different methods: the flow-rate or stop- flow method [34], the zero-net-flux method [1] and the retrodialysis method [35]. Because the zero-net-flux method and the flow rate method require that the study subject should be examined under steady state conditions prior to the experiment, total study time is extended limiting the application of these methods for pharmacokinetic purposes.

In the retrodialysis method, *in vivo* recovery of the compound of interest is determined before drug administration by perfusing the microdialysis probe with a solution of the compound of interest, taking the proportion of lost across the dialysis membrane as an estimate of the recovery. A shortcoming of this approach is that recovery changes resulting from the experiment are not detected. However, changes in recovery during the experiment are more common when studying endogenous than exogenous compounds, indicating that the recovery of the microdialysis probe generally remains constant in pharmacokinetic studies. The retrodialysis method is broadly used in pharmacokinetic experiments.

However, retrodialysis method is not suitable for the *in vivo* recovery determination of endogenous compounds. A recent description of an alternative calibration method [36, 37] involving isotopic perfusion allows an almost immediate estimation of microdialysis recovery for endogenous compounds. The technique is based on the principle that through the addition of a substrate in isotope form to the perfusate, the relative delivery of isotopic substrate from perfusate into local tissue extracellular fluid equals the relative recovery of cold substrate from tissue extracellular fluid into dialysate.

The process of probe implantation has been analyzed because it may produce a local altered blood flow and thus an altered recovery. A recent study, involving the coupling

of laser Doppler flowmetry as a measure of local blood flow with microdialysis, showed that insertion of the microdialysis catheter into skin only caused a short lived hyperaemic response which quickly resolved [38]. These results show that probe insertion would not produce an altered local blood flow and therefore an altered recovery.

On the other hand, studies conducted on animals and humans show that significative changes in blood flow affect the recovery of glucose in adipose and muscle tissue [30, 39]. However, Maggs *et al*. found no differences on the recovery of glucose in peripheral tissues when they measured extracellular glucose levels by two different methods [40]. The differences in the observed results may be assigned to the fact that some studies were made under non-steady state conditions.

The site of the probe insertion is another important point to be considered. Jansson *et al*., using a catheter in the abdominal cavity to predict postoperative complications, have demonstrated that the precision of the measurements of endogenous substances and metabolites is dependent on the location of the catheter [41].

Moreover, data obtained from intracerebral microdialysis could be only correctly interpreted if the locations of the catheters as they relate to focal brain lesions are visualized. This is relevant because a "biochemical penumbra zone" surrounds focal traumatic brain lesion [42].

Microdialysis catheters have been inserted in a number of places within the brain parenchyma, for example, the frontal lobes, basal ganglia, hippocampus or adjacent to a tumor [43]. The probe can be inserted with other devices such as a ventriculostomy catheter or an intracranial pressure-monitoring device. Alterations in surrounding brain tissue after implantation of the probe have been noted, mainly caused by small haemorrhages into the catheter tract, mild astrogliosis, macrophage infiltration and vacuolization in an area surrounding the probe with a maximum diameter of 500 μm [43]. These alterations change over time and may influence the data obtained.

Ekstrøm *et al*. [19] using a microdialysis technique to assay intratumoral methotrexate studied the functional lifespan of the implanted microdialysis probes. Probes were inserted in a tumor in the medial femoral condyle, muscle and antecubital vein. Neither of the used probes showed any sign of malfunction during the initial 5 days after implantation, and the probe localized in the tumor appeared to be functional for a total of 11 days. The authors also observed a leakage in the venous and muscular probes probably caused by mechanical stress rather than local perturbations at the dialysis site [19].

Contrary to the pharmacokinetic studies, accurate calibration of the microdialysis probe is not necessary, because the desirable information is the relative change in concentration induced by drug administration. Therefore, only the concentration independence and stability of the recovery need to be known. These properties could be determined by *in vitro* calibration of the microdialysis probe [6].

A minimal lesion of the tissue surrounding the probe is produced by implantation of the microdialysis probe. So, basal concentrations of the endogenous substance of interest must be determined in approximately four microdialysis samples. Once these levels became stable, the experiment can be started and the change in the endogenous substance induced by a specific treatment (e.g. the administration of a drug) could be calculated as a percentage of the basal mean [6].

COMPARISON OF DIFFERENT TISSUE SAMPLING TECHNIQUES

Microdialysis is an alternative sampling technique used for the *in vivo* determination of tissue concentrations. Other techniques used for this purpose are biopsy, saliva sampling, skin blister, imaging techniques and ultrafiltration. In this section, microdialysis is compared to alternative methodologies for different clinical applications.

Biopsy

During the process of biopsy a tissue sample is taken and generally homogenized resulting in cellular lysis. Therefore, biopsy gives information about concentrations of analytes in the homogenate without distinguishing between blood, intra- or extracellular drug levels. Moreover, it is an invasive method associated with the risk of infection due to cross-contamination and scarring. As tissue has to be removing every time a sample is taken, biopsy is not suitable for the study of the concentration *versus* time course of a drug [7].

Advantages of biopsy with regards to microdialysis are that no special equipment is needed, there is no limitation with the size of the drug and no calibration is necessary [7].

Saliva

Saliva sampling represents an alternative method to blood collection for pharmacokinetic measurements of exogenous compounds. Advantages of saliva sampling are no fluid loss, minimal invasiveness, continuing sampling at the site of collection. However, the validity of estimation of plasma concentration by saliva sampling is controversial. It was found that saliva overestimates free plasma concentrations of paracetamol and teophylline [44, 45]. Therefore, saliva sampling must be validated for the pharmacokinetic study of each individual drug. On the other hand, saliva sampling did not allow the study of drug distribution in the target tissue. Moreover, gingivitis due to a greater dilution of the saliva could alter salivary pharmacokinetic behavior of drugs.

Skin Blister

Suction blisters have been an established method for more than 30 years for the study of pharmacokinetics in the dermis [46]. The method principle relies on the separation of the epidermis from the dermis along the lamina lucida due to the application of prolonged suction to the skin surface (suction blister technique) or due to the adverse reaction effect of cantharidin (cantharides blister technique). The fluid drawn into the separated epidermis can be sampled and analyzed for the content of either topically or systemically administered drug [7]. Skin blister technique has been

compared to microdialysis in several works with different tested drugs. Whilst studies with penciclovir [47], paracetamol [48] and acetylsalicylic acid [46] demonstrated similar results comparing skin blister and microdialysis; other authors found skin blister unsuitable for the study of dermal pharmacokinetics of fluconazole [49], theophilline [46] and moxifloxacin [50]. The authors concluded that blister concentrations might be more similar to total plasma levels due to accumulation of proteins [46]. Therefore, microdialysis is superior to skin blister sampling and skin blister seems to be valid only for the dermal study of low protein bound drugs.

Skin blister sampling produces a great discomfort in the patients and the technique is only applicable for studies at a certain time point not allowing continuous monitoring of drug skin penetration to the same test area.

Ultrafiltration

Ultrafiltration is an alternative membrane sampling technique to microdialysis. This technique collects a sample by the application of negative pressure as a driving force. The rate of fluid collection is determined by the amount of negative pressure applied, the membrane surface area and the hydraulic resistance [51]. The mayor advantage of ultrafiltration with regard to microdialysis is that *in vivo* calibration is not necessary because the *in vivo* recovery is greater than 95% for small molecules [51]. However, continuous tissue sampling with ultrafiltration depends on rapid replacement of interstitial fluid by the blood vessels. In tissue with limited flow rate and low replenishment of interstitial fluid, such as subcutaneous tissue, only low sampling rates are possible [51]. The brain is also unsuitable for ultrafiltration sampling because of the limited extracellular space. In addition, ultrafiltration did not allow the administration of a substance into the extracellular space through the probe [51].

The applicability of ultrafiltration has been demonstrated for drug kinetics studies and glucose and lactate monitoring [51]. However, the scope of application of ultrafiltration technique is limited in clinical pharmacology.

Imaging Techniques

Several imaging techniques such as planar γ-scintigraphy, single photon emission computed tomography, positron emission tomography, and magnetic resonance spectroscopy have been developed for the study of drug distribution in the clinical setting [52-54]. Although these techniques are non invasive, there are only applicable for a small group of compounds with special functional groups. Moreover, imaging techniques are very expensive and labor-intensive and therefore not suitable for clinical routine settings.

APPLICATION OF MICRODIALYSIS SAMPLING IN CLINICAL PHARMACOLOGY

In the following sections, the principal aspect of microdialysis application in clinical pharmacokinetic and pharmacodynamic studies of different therapeutic agents would be discussed. Although several articles have summarized the principles and applicability of microdialysis sampling for clinical studies [5, 7, 55-59], a review that summarized the broad range of application of microdialysis technique in clinical pharmacology is lacking. Therefore, the aim of this section is to review the more recently pharmacokinetic and pharmacodynamic features of this technique in the clinical setting.

ANTI-INFECTIVE AGENTS

Traditionally, pharmacokinetic assessment of antimicrobial agents was based on measuring of total plasma concentrations. However, use of plasma antibiotic levels is not ideal, because most infections occur in tissue sites, and therefore the ability of antibiotics to reach the target site is a key determinant of clinical outcome. Thus, free antimicrobial tissue concentrations are more relevant than serum levels in predicting therapeutic efficacy. In general it is assumed that total plasma concentrations and plasma protein binding can be used to predict free tissue levels of antibiotics, because it is believed that unbound plasma concentrations and free tissue levels are equal at equilibrium. However, many studies have shown lower tissue unbound levels than plasma concentrations [60-63]. Tissue distribution is also affected by anatomic barrier, such as blood-brain barrier, presence of active transport systems like P-glycoproteins and tissue metabolism. On the other hand, time to equilibrium between plasma and tissue concentrations of antibiotics may range from minutes to days [64].

Thus, measurement of unbound drug concentrations in the interstitial fluid of the target tissue should be considered a gold standard for improvement of antimicrobial therapy and dose adjustment.

Several techniques, such as skin blisters, saliva, microdialysis and imaging techniques, have been used to monitor free drug concentrations in interstitial fluid in human studies [64]. However, taking into account the advantages and drawbacks of the different techniques (see above); microdialysis seems to be the most adequate technique for the study of tissue concentrations time profile of antimicrobial agents.

Regulatory authorities encouraged the study of tissue distribution of antimicrobial agents in unaffected and infected target sites and the relationship of unbound drug concentrations at the site of action to the *in vitro* susceptibility of the infecting microorganism [65]. Moreover, the Food and Drug Administration (FDA) advisory committee considered that microdialysis is an attractive approach for clinical studies on tissue distribution of antibiotics [66]. So, microdialysis sampling has been extensively employed for the tissue distribution of anti-infective agents in healthy volunteers and patients.

The applicability of microdialysis for the study of antimicrobial drugs has been previously reviewed [55]. Microdialysis has been used to measure various antimicrobial agents in human tissues, including aminoglycosides [67], penicillins [68], cephalosporines [69, 70], fosfomycin [71], fluoroquinolones [72, 50] and antiviral agents [47] in healthy volunteers and patients. A summary of recent studies of tissue distribution of anti-infective drugs by means of microdialysis is given in Table **3**. These studies have served to develop PK-PD models in peripheral tissues using the

Table 3. Overview of Recently Published Studies of Tissue Pharmacokinetics of Anti-Infective Drugs by Means of Microdialysis

Drug	Subject	Tissue	Probe	Calibration	RR (%)	Ref.
cefpirome	patients with pulmonary tumors	lung	concentric	In vitro recovery	78	[73]
cefpirome	heathy volunteers	skeletal muscle and subcutaneous adipose tissue	concentric	retrodialysis	DNS	[74]
cefpirome	healthy volunteers	skeletal muscle and subcutaneous adipose tissue	concentric	retrodialysis	18±8	[75]
ciprofloxacin	heathy volunteers	skeletal muscle and subcutaneous adipose tissue	concentric	retrodialysis	DNS	[76]
ciprofloxacin	Non-insulin dependent diabetes	Inflamed food lesions	concentric	retrodialysis	DNS	[77]
fluconazole	heathy volunteers	subcutaneous adipose tissue	Concentric	retrodialysis	57-67	[49]
fosfomycin	Uncomplicated cellulitis and diabetic foot	Upper subcutaneous layer of inflamed and noninflamed tissues	concentric	retrodialysis	DNS	[78]
fosfomycin	patients with sepsis	skeletal muscle	concentric	ND		[79]
gemifloxacin	healthy volunteers	skeletal muscle and subcutaneous adipose tissue	concentric	retrodialysis	34±14	[14]
gentamicin	heathy volunteers	Subcutaneous fat layer	Linear	Zero net flux	35±17	[67]
imipenem	healthy subject and critical ill patients	skin and muscle	concentric	retrodialysis	67±8	[80]
levofloxacin	patients with soft tissue infections	soft tissue	concentric	retrodialysis	35	[81]
levofloxacin	patients with soft tissue infections	skeletal muscle	concentric	retrodialysis	DNS	[82]
meropenem	patients with pneumonia	lung	concentric	retrodialysis	DNS	[83]
metronidazole	patients under elective gynaecological surgery	muscle	concentric	retrodialysis	50±10	[84]
metronidazole	heathy volunteers	skin	linear	retrodialysis	DNS	[85]
metronidazole	patients with septic shock	skeletal muscle	concentric	retrodialysis	55±20	[86]
moxifloxacin	Severe soft tissue infections	healthy subcutaneous adipose tissue and inflamed lesion	concentric	retrodialysis	DNS	[87]
ofloxacin	healthy volunteers	skin	linear	retrodialysis	DNS	[88]
penciclovir	healthy volunteers	skin	linear	ND		[47]
piperacillin	healthy patients undergoing aortic valve replacement	subcutaneous adipose layer	concentric	retrodialysis	DNS	[68]
rifampin	Patients undergoing craniotomy	brain	concentric	No net flux	60±14	[89]
telithromycin	healthy volunteers	skeletal muscle and subcutaneous adipose tissue	concentric	retrodialysis	62±3	[90]

DNS: data not shown, ND: not determined, RR: relative recovery, Ref.: references.

same parameters calculated in plasma: time (T) above the minimum inhibitory concentration (MIC) (T>MIC), the ratio of the maximum concentration of drug in serum (Cmax) to the MIC (Cmax/MIC), the area under the inhibitory curve or the area under the curve (AUC)/MIC ratio. The most relevant findings of these studies are discussed below.

One advantage of microdialysis is that direct measurements of free drug fraction may be performed in different tissues. It is well known that only the unbound drug fraction exert anti-infective efficacy. The relevance of protein binding in therapeutic failure was observed by Wise *et al.* [91], in patients with gonorrhea treated with fusidic acid or ceftriaxone. In this study, a relationship was also found between the failure in cefoperazone treatment in serious illness and the degree of drug bounded to proteins [91].

The pharmacokinetic profile of two cephalosporin, cefpodoxime and cefixime, in muscle was studied in six healthy male volunteers [92]. Cefpodoxime, with lower protein binding than cefixime (25 *vs* 65%) showed higher peak concentration and tissue penetration than cefixime [92]. This greater tissue penetration suggests favorable efficacy of cefpodoxime, and this is supported by clinical trial data provided by a study in paediatric acute otitis media [93].

The lung penetration of meropenem was determined in seven patients with pneumonia and metapneumonic pleural emphysema [83]. Microdialysis probes were inserted into pneumonic lung tissues and healthy skeletal muscle. Although meropenem rapidly penetrated infected lung tissue, interstitial lung fluid and muscle tissue levels were lower than serum concentrations. Furthermore, meropenem concentrations were maintained above the MIC90 threshold for many clinically relevant pathogens for up to 6 hours [83].

Tissue penetration of the fluorquinolone levofloxacin appeared to be unaffected by local inflammation. Bellman *et al.* [81] observed that administration of a standard dose of levofloxacin produced adequate levels at target site, although the extent of tissue penetration showed a high interindividual variability. Therefore, some cases of clinical failure may be explained by low tissue penetration of this fluorquinolone [81].

In summary, several microdialysis studies have demonstrated that sub-inhibitory concentrations of some anti-infective may be present at the target site, despite effective concentrations being attained in plasma. Therefore, employment of microdialysis in clinical drug development and in critically ill patients should enhance knowledge on adequate antibiotic drug dosing and improve patient's outcome.

ANTINEOPLASIC DRUGS

Tumor drug exposure, a marker linked to clinical outcome, may be dramatically reduced due to diffusion barriers in solid tumors. Physiological diffusion barriers, such as alterations in local blood flow, tumor vessels development, and modification of interstitial matrix with large extracellular space, alter drug intratumoral distribution [56]. Therefore, plasma anticancer drug profiles are frequently inappropriate for predicting outcome in oncology. This condition makes it difficult to reproduce tumor tissue *in vitro*. Thus, microdialysis appeared as a valuable minimally invasive tool that allows *in vivo* investigations. The utility of microdialysis sampling for the study of antineoplasic agents drugs has been reviewed [56].

In vivo drug uptake into tumors has been described by indirect measures of exposure to drugs according with pharmacokinetic profiles in plasma. Direct measurements may be feasible by different approaches included biopsy for single point measurements, or several non-invasive techniques such as magnetic resonance imaging and positron emission tomography [94, 95], although the application of these techniques is limited by the high cost and availability.

Differences in tumor drug distribution do not allow to predict the antineoplastic response from plasma profiles [94], thus measurements of drug exposure into tumor interstitium by microdialysis may help to develop clinical PK-PD models with the aim of individualize drug therapy.

Microdialysis has been employed for the characterization of different antineoplastic drugs. Methotrexate [19, 96], cisplatin [97], capecitabine [98], 5-fluorouracil [99], dacarbacine [100] and melphalan [101] have been measured into the tumor using clinical microdialysis in several types of malignancies such as breast cancer [98, 99], melanoma [100, 101], osteosarcoma [19, 101] and malignant fibrous histiocitoma [101]. Table **4** describes recent studies of tumoral pharmacokinetics of antineoplasic drugs by means of microdialysis. The most relevant findings of these studies are discussed below.

The antifolate methotrexate is one of the most widely used cytotoxic drug. High doses of methotrexate have been employed in the treatment of leukemia and various solid tumors such as osteosarcoma. Measurements of plasma drug concentration have been employed during high-dose methotrexate treatment [103]. Although this practice has demonstrated to improve outcome in children with acute lymphoblastic leukemia, it assumed a relationship between pharmacological response and circulating levels of drug. Müller *et al.* [96] measured interstitial tumor pharmacokinetics and plasma-to-tumor transfer rates of methotrexate in breast cancer patients. Microdialysis probes were inserted into the primary tumor and the periumbilical subcutaneous adipose layer of previously chemotherapy-naive breast cancer patients. The authors showed no correlation between plasma AUC and the AUC in the interstitial space of tumor tissue together with a high interindividual variability in transendothelial methotrexate transfer, concluding that plasma levels of methotrexate were not predictive of intratumor levels.

Interstitial tumor 5-fluorouracil (5-FU) pharmacokinetics and 5-FU transfer rates from plasma into the tumor interstitium were studied in breast cancer patients after intravenous bolus administration [99]. Although plasma or subcutaneous AUC of 5-FU failed to predict tumor response, a high interstitial tumor AUC of 5-FU was associated with increased tumor response. This information may explain drug resistance in some patients and help to optimize dosing and administration schedules [99].

In another report Mader *et al.* [98] investigated the intratumoral transcapillary transfer of capecitabine, an oral prodrug of 5-FU, and its metabolites in patients with skin metastases from breast cancer. Capecitabine has been designed to achieve selective tumor accumulation of 5-FU exploiting increased enzymatic activation in tumoral cells. Microdialysis probes were inserted into a cutaneous metastasis and subcutaneous connective tissue. The authors

Table 4. Overview of Recently Published Studies of Tumoral Pharmacokinetics of Antineoplasic Drugs by Means of Microdialysis

Drug	Subject	Tissue	Probe	Calibration	RR (%)	Ref.
capecitabine	patients with breast cancer	Suitable cutaneous metastasis and healthy subcutaneous connective tissue	concentric	retrodialysis	11-65	[98]
cisplatin	primary squamous cell carcinoma of the oral cavity	tumor	concentric	In vitro calibration	96	[97]
Delta-aminolevulinic	basal cell carcinoma	skin	Linear	endogenous reference calibration with urea	NI	[102]
melphalan	patients with melanoma, malignant fibrous histiocytoma, osteosarcoma or Merkel cell tumour	Normal and tumour tissue	concentric	NI		[101]
methotrexate	Patient with malignant fibrous histiocytoma	Tumor in the medial femoral condyle	concentric	ND		[19]

ND: not determinded, NI: not informed, Ref.: reference

concluded that capecitabine and its metabolites distribute extensively into the interstitium of malignant tissue [98]. Moreover, 5-FU concentrations were low in blood and in the interstitial space of malignant and healthy tissue, which might explain the mild side effects profile of capecitabine.

Thompson *et al*. [101] studied the clinical and biochemical responses to the time course of melphalan in the subcutaneous interstitial space and in tumor tissue from patients with various limb malignancies. The authors showed a significant correlation between the melphalan mean concentration in subcutaneous microdialysate and tumour response [101].

In summary, a key finding of these studies was a high interindividual variability in intratumoral drug distribution, indicating a lack of correlation between plasma concentration of antineoplasic drugs and interstitial tumoral levels. Therefore, plasma measurements do not serve as surrogates for intratumoral concentration and microdialysis may help to design optimal treatment schedules and to select appropriate drug, doses and dosing intervals for antineoplasic agents. Surprisingly, in the last three years, there is a lack of new studies of intratumoral distribution of anticancer drugs using microdialysis sampling in the clinical setting. A possible explanation is that microdialysis in cancer patients must be conducted in strict compliance with regulatory demands and need to be based on appropriate ethical conditions. On the other hand, puncture of solid tumors by microdialysis catheter implantation may induce metastasis [104]. However, it is expected that the incidence of metastasis by puncture ranged from 0.003% to 0.005 % and there is no evidence that puncture of tumor lesions affected the course or prognosis of the underlying disease [104].

Another limitation of the applicability of microdialysis sampling in oncology is the fact that the majority of the antineoplasic drugs act within cells. The relationship between extracellular drug concentrations and intracellular drug levels remains unknown. Moreover, antineoplasic drugs such as 5-FU requires intracellular enzymatic conversion in order to exert its cytotoxic activity. In addition, other aspects like tumor location and accessibility for microdialysis probe implantation, and the possibility of variation in interstitial concentrations of cytotoxic drugs in different metastases in a patient restrict the utility of microdialysis for studies of antineoplasic drug distribution.

Finally, the extracellular space is the bioactive site for the majority of growth factors and increased knowledge of protein activation in this compartment is of importance for comprehension of tumor biology. Microdialysis was used to determine the local levels of insulin-like growth factor-1 (IGF-1) in normal human breast tissue in healthy female volunteers during the menstrual cycle [105]. The results showed that the extracellular levels of IGF-1 locally in the breast were doubled in the luteal phase, when estradiol and progesterone levels were elevated, compared with the follicular phase. The increased local levels of the free form of IGF-1 may promote proliferation in the breast epithelium and this could be important in sex steroid dependent breast cancer development [105].

DRUGS ACTING IN THE CENTRAL NERVOUS SYSTEM

An important issue for drugs acting in the central nervous system is the knowledge of the distribution into the brain. The concentration *versus* time profile of the drug in the central nervous system determines the intensity and duration of the effects of a drug with a central action. Measurement of brain concentrations of the drug is highly restricted in the clinical setting; therefore, the alternative determination of lumbar or ventricular cerebrospinal fluid (CSF) is sometimes used. However, determination of drug concentrations in CSF provides only limited information with respect to drug distribution into the brain, because of brain distribution is

determined by multiple factors such as active metabolism, active transport at the blood brain barrier and intracellular-extracellular exchange [106]. Although brain distribution could be determined by non-invasive imaging techniques, these methods have important limitations. Intracerebral microdialysis would be a significant improvement, because this technique allows the study of the pharmacokinetic profile of the unbound drug fraction at a specific region within the brain. However, microdialysis is an invasive technique and may therefore bear risks. Microdialysis sampling was used for the study of brain penetration of drugs in the early phase of clinical trials. A phase II trial of topiramate in severe traumatic brain injury demonstrated different free-drug concentrations in the extracellular space from that measured in CSF, the traditional method of establishing brain penetration [107].

An interesting field of intracerebral microdialysis in the clinical setting is the study of the brain distribution of anticonvulsivant agents. There is increasing evidence that an over-expression of multidrug transporters such as P-glycoprotein is involved in the generation of pharmaco-resistance to antiepileptic drugs because of a greater efflux from the brain of the central acting agent [108]. Therefore, monitoring of the target site concentration of the anticonvul-sivant drugs by means of intracerebral microdialysis could help to pharmacoresistance of antiepileptic drugs in intractable epilepsy.

Lindberger *et al*. [109] by means of intracerebral micro-dialysis have demonstrated that CSF concentration of valproic acid were slightly lower than plasma and subcutaneous extracellular fluid levels, suggesting that valproic acid may be substrate for an energy-dependent carrier transport out of the central nervous system. The hippocampal extracellular levels of phenytoin were determined in two patients with intractable epilepsy using the microdialysis technique [110]. The authors found that brain phenytoin levels were slightly lower than unbound plasma levels. No differences were observed in the extracellular levels of phenytoin between different sites within the brain [110]. Scheyer *et al*. [111] also found that carbamazepine and carbamazepine-10, 11-epoxide concentrations in the extracellular brain fluid closely mirror their unbound serum concentrations [111]. In conclu-sion, the intracerebral microdialysis study of anticonvul-sivant agents demonstrated that brain concentrations of the drugs are in general only slightly lower than unbound plasma levels.

Another application of the microdialysis technique for the study of antiepileptic drugs is the monitoring of subcutaneous extracellular levels. Microdialysis was validated for the sampling of subcutaneous extracellular levels of valproic acid [112]. After a single dose administration of the antiepileptic, the authors found that the subcutaneous extracellular levels of valproic acid were similar to unbound plasma levels suggesting that the microdialysis can be used to sample unbound concentrations of valproic acid in the clinical setting. Moreover, no severe side effect was observed in the study and microdialysis only produces some discomfort and disturbed sleep during the first night [112]. In another report Linderger *et al*. [113] have demonstrated that the subcutaneous extracellular levels of valproic acid were consistently lower than unbound plasma levels at steady state. The authors hypothesized that subcutaneous extra-cellular levels determined by microdialysis differs from unbound plasma at steady state because of ex vivo changes in plasma samples due to metabolites of valproic acid. Therefore, the microdialysis technique seems to be a more accurate method for the determination of unbound levels of valproic acid in patients at steady state [113]. The fact that the authors did not measure central levels of antiepileptic to demonstrate a correlation between subcutaneous drug levels and target site concentrations limited the relevance of these studies.

Intracerebral microdialysis was also used for the determination of brain concentration of other central acting drugs. Ederoth el al [114] studied the blood-brain barrier transport of morphine in patients with severe brain trauma by simultaneous microdialysis of morphine levels in "better" and "worse" brain tissue. The authors found that unbound brain morphine levels were lower than plasma concentrations suggesting the existence of an efflux transport system for morphine across the human blood-brain barrier. In addition, the results suggested an increase of blood-brain barrier permeability to morphine in the "worse" brain tissue [114].

ANALGESIC AND ANTI-INFLAMMATORY DRUGS

Microdialysis technique allows the study of different pharmacological aspects of nonsteroidal anti-inflammatory drugs (NSAIDs) such as penetration of the skin barrier after topical administration, mechanism of action and *in vivo* ciclooxygenase (COX)-2 selectivity of therapeutic agents.

One approach to reducing the systemic adverse effects of orally administered NSAIDs has been to apply the drug to the skin overlying affected joints and muscles. Therefore, effectiveness of the topical administration of NSAIDs depends on the ability to penetrate into deep tissues underneath the site of application in therapeutically amounts. There are conflicting results about the penetration depth and quantity of topically delivered NSAIDs. The lack of appropriate methods for the assessment of *in vivo* drug penetration through the skin contributed to this conflicting data [115, 116]. Microdialysis sampling provided concen-tration *versus* time profiles with a high temporal resolution, and therefore has been shown to be a suitable method for the characterization of *in vivo* transdermal drug transport. In several works, microdialysis was employed to evaluate tissue concentrations after topically administration of different NSAIDs. Tegeder *et al*. [117] have demonstrated that unbound ibuprofen concentrations were greatly enhanced in tissue relative to plasma after topical administration. The authors also concluded that patients with pain caused by dermal or subcutaneous tissue damage will likely experience greater pain relief after topically application of ibuprofen because of higher subcutaneous tissue concentrations [117]. Transdermal penetration of diclofenac was also studied [118]. Transdermal penetration of diclofenac after multiple as well as after single application of the present formulation was highly variable, because of factors influencing the transdermal penetration process, as well as dose and mode of administration of topical NSAIDs administration [118].

Pharmacodynamics of NSAIDs were also evaluated by means of microdialysis. Gordon *et al.* [119] studied the time course of the prostanoids prostaglandin E_2 (PGE_2) and thromboxane B_2 (TxB_2) and their relationship to clinical pain and NSAID analgesia. After intramuscular and submucosal administration of ketorolac, the authors found an early analgesic onset without changes of PGE_2 levels in the site of inflammation, suggesting an initial analgesic effect mediated by an action presumably in the central nervous system. Moreover, no differences were found in onset of pain relief according to route of administration [119]. In another report, diclofenac exerted a higher antihyperalgesic effect after oral administration with regards to topical application at comparable tissue concentrations [120]. These results suggested that not only peripheral but also central mechanisms are involved in the analgesic effects of systemically administered diclofenac [120].

In vivo selectivity of NSAIDs in an oral surgery model was studied using the microdialysis technique [121]. It is well known that PGE_2 is a product of COX-1 and COX-2, whereas TxB_2 served as an indicator of COX-1 activity. Therefore, the measurement of PGE_2 and TxB_2 concentrations in dialysate after NSAID administration allows the determination of *in vivo* selectivity of NSAIDs for the COX-2 isoform [121]. Khan *et al*. have demonstrated that oral administration of celecoxib suppressed PGE_2 but not TxB_2 dialysate concentrations suggesting a relative selective *in vivo* COX-2 inhibition by celecoxib [121].

Microdialysis sampling has also been employed for the study of peripheral antinoceptive effects of opiod analgesic drugs [122]. It was found that subcutaneous tissue levels of morphine-6-glucuronide assessed with microdialysis were about half those of the corresponding plasma concentrations. These tissue levels are sufficient to produce antihyperalgesic effects in inflammatory pain through activation of peripheral opiod receptors without a central effect [122].

LOCAL ANESTHETICS

Microdialysis sampling was also employed for the study of tissue pharmacokinetics of local anesthetics in order to improve local anesthesia and to study the mechanism of local anesthetic nerve block potentiation by adrenergic drugs. The effect of concomitant administration of adrenergic drugs on lidocaine tissue pharmacokinetics was studied by means of microdialysis sampling. Bernards *et al.* [123] studied the effects of concomitant administration of epinephrine on lidocaine clearance by implanting microdialysis catheters adjacent to the superficial peroneal nerve in both feet. The authors found that the mechanism for prolonging the duration of local anesthetic block by epinephrine rests on its ability to decrease local anesthetic clearance and not on a pharmacodynamically mediated potentiation of local anesthetic effect [123]. In another study it was found that clonidine prolongs local anesthetic block partly by a pharmacokinetic mechanism due to slowing lidocaine tissue elimination by inhibition of lidocaine vasodilatation [124].

The pharmacokinetic and safety of lidocaine in liposuction were also evaluated by means of subcutaneous microdialysis [125]. Although lidocaine plasma concentrations remain within the therapeutic range, prudence should be exercised with regard to the recommended maximum lidocaine dose because of the high interindividual pharmacokinetic variation [125].

Kopacz *et al.* [126] studied a model to evaluate the pharmacokinetic and pharmacodynamic variables of bupivacaine-loaded microcapsules by *in vivo* tissue microdialysis. The authors simultaneously determined tissue and plasma bupivacaine levels, anesthesia and adverse effects, finding that subcutaneous injection of dexamethasone-containing bupivacaine microcapsules produces a prolonged duration of skin anesthesia as a result of sustained release of the local anesthetic. On the other hand, adverse effects occurred much later than peak tissue bupivacaine levels, suggesting that they are caused by the copolymer matrix of the formulation [126].

MICRODIALYSIS IN METABOLIC STUDIES

Microdialysis technique not only allows the recovery of exogenous compounds and the study of tissue pharmacokinetics, but also permits the sampling of endogenous compounds such as neurotransmitters, hormones, autacoids and different metabolites. Therefore, *in vivo* pharmacodynamic of drugs could be studied by means of microdialysis sampling. In the next section, the applicability of the microdialysis technique in different metabolic studies is reviewed.

Microdialysis in Neurosurgical Intensive Care

The applicability of microdialysis technique in neurosurgical intensive care has been reviewed recently [57]. As brain damage resulting from acute traumatic or neurovascular brain injury is partly mediated by secondary delayed ischemia, the purpose of the microdialysis technique is to obtain early warning signals of ischemia by means of monitoring biomarkers such as glucose, glycerol, pyruvate, lactate and neurotransmitters like glutamate [57]. It was demonstrated that biochemical changes detected by intracerebral microdialysis appear before significant increases in intracranial pressure [127]. Microdialysis also allows the study of the beneficial effects of different pharmacological treatments of intracranial hypertension. However, microdialysis technique has several methodological limitations. It is an invasive technique causing tissue damage that may influence the measurement and interpretation of the results. Data from clinical studies indicated that dialysate metabolite levels normalized in approximately 30 to 150 min [128]. On the other hand, as gliosis around the microdialysis catheter could cause a diffusion barrier and blunt neuronal chemical responses, the validity of the microdialysis technique for long-term measurement must be determined [129]. Microdialysis catheter implantation could produce bleeding and infection as adverse effects, but these side effects do not seem to present much of a problem [129].

An important drawback for the application of the microdialysis technique in metabolic studies is the need of *in vivo* recovery determination of the interest compounds [128]. Moreover, the *in vivo* recovery of the catheter may vary during the experiment because of change in blood-brain barrier permeability, interstitial volume and intracranial

pressure, temperature fluctuations and gliosis. Ronne-Engström *et al*. [130] have demonstrated the validity of the use of urea as an endogenous compound for the *in vivo* calibration of microdialysis catheters. Another approach to eliminate the interference of *in vivo* recovery fluctuations is the use of ratios such as the lactate/pyruvate ratio or the lactate/glucose ratio because the ratio is independent from the *in vivo* recovery [129].

However, the most important limitation of the microdialysis technique is the fact that the technique only monitors metabolic changes in the vicinity of the catheter. Unless the catheter is placed in an area where secondary insults are affecting neurons, normal concentrations will be measured [131].

Recently a consensus meeting on microdialysis in neurointensive care was published [131]. The consensus recommended the use of glutamate and lactate/pyruvate ratio as chemical markers for the development of ischemia. It was also remained that due to insertion artifacts, there is a period of unreliable values for at least 1 h after catheter insertion [131].

Intracerebral microdialysis can be used for the evaluation of the efficacy of treatments used for reduction of cerebral perfusion pressure. Ståhl *et al*. [132] have demonstrated that reduction of intracranial pressure by administration of metoprolol and clonidine was associated with a normalization of cerebral metabolism. Low doses of prostacyclin normalized lactate levels and lactate/pyruvate ratio in the injured area of the brain of patients with severe brain trauma [133]. Johnston *et al*. [134] have demonstrated that propofol did not change ischemic markers in the injured brain tissue, suggesting that propofol does not appear to be a useful therapeutic tool in reducing the level of ischemia in the short-term brain trauma.

Lipolytic Drugs

Microdialysis was extensively employed for the study of lipolytic and anti-lipolytic drugs. Although lipolysis could be studied by *in vitro* and *in vivo* methods, *in vivo* microdialysis sampling has several advantages. *In vitro* methodologies such as isolated adipocytes undergo treatment by proteolytic enzymes and are removed from their natural surroundings [135]. Therefore, one should be cautious when drawing conclusions from *in vitro* studies regarding physiological situations. However, Kolehmainen *et al*. [135] determined that *in vivo* microdialysis and *in vitro* lipolysis assay with isolated adipocytes provide concordant and complementary information of adipose tissue metabolism in the same individual.

Microdialysis has some advantages with regard to *in vitro* lipolysis studies such as a particular study reflects the metabolism of adipose tissue in its natural environment and microdialysis offers the possibility to perform local manipulation and simultaneously study metabolism [135]. However, *in vivo* microdialysis recovery is dependent on local tissue blood flow. To eliminate this variability, blood flow must be simultaneously measured using ethanol dilution technique [135]. Ethanol is added to the perfusion media and ethanol concentrations were analyzed from the perfusate and dialysate. The changes in the concentrations of ethanol expressed as dialysate/perfusate ratio describe the changes in the local blood flow [135]. Lipolytic effects are determined by the measurement of glycerol dialysate concentrations previously and during administration of the lipolytic or anti-lipolytic drug. In different reports, the lipolytic actions of diverse drugs such as phosphodiesterase 3 inhibitors [136], atrial natriuretic peptides [137], nicotine [138], endotoxin [139], growth hormone [140] and β-adrenergic agonists [141, 142]. The antilipolytic effects of metformin [143], acipimox [144] and α_2-adrenergic agonist [145] were also measured by means of *in situ* microdialysis. Recently published reports of the effects of drugs on lipolysis are summarized in Table **5**.

Muscle Metabolism

Microdialysis technique was used for the recovery of different endogenous compounds such as potassium, glucose, lactate, prostanoids, and thromboxane in the muscle interstitium mainly during exercise. However, there are some reports of the effects of therapeutic drugs on muscle metabolism. So, the local effect of the insulin-mimetic agent vanadate on glucose metabolism in human skeletal muscle was studied using the microdialysis technique [149]. Vanadate was locally administered through the microdialysis catheter and simultaneously glucose and lactate were collected in the dialysate. It was found that vanadate decreased interstitial glucose concentrations and increased lactate levels, in the vicinity of the microdialysis catheter, indicating that vanadate mimics the effect of insulin in human skeletal muscle *in vivo* [149].

Hedenberg-Magnusson *et al.* [150] studied the relationship between prostaglandin E2 and leukotriene B4 levels in masseter muscle during local glucocorticoid administration and pain relief. The authors found that reduction of masseter level of prostaglandin E2 after intra-muscular glucocorticoid administration is associated with a decrease of resting pain in patients with fibromyalgia [150].

The mechanism of insulin sensitivity improvement by angiotensin-converting enzyme inhibitors was studied by means of microdialysis in skeletal muscle [151]. Selective inhibition of paracrine angiotensin converting-enzyme activity by enalapril increases glucose and lactate interstitial concentrations and decrease serum interstitial gradient of glucose in muscle by facilitating transcapillary glucose transport explaining the improved insulin sensitivity to enalapril [151].

Continuous Glucose Monitoring

Self-monitoring of blood glucose became a basic pillar in assessing diabetic control by allowing hour to hour measurement and therefore preventing severe hypoglycemia. However, self monitoring of blood glucose has several limitations such as inconvenience and discomfort for the patient, the information obtained is intermittent and depends on patient's initiative. Although self-monitoring of blood glucose provides several points in a day, blood glucose varies continuously and unpredictable. Therefore, continuous glucose monitoring will be a major improvement in diabetic self-care [152].

Table 5. Overview of Recently Published Studies of *In Situ* Drug Effects on Lipolysis by Means of Microdialysis

Drug	Route of administration	Probe	Calibration	Perfusion fluid	Ref.
atrial natriuretic peptide	Reverse microdialysis	Concentric	Zero flow method	Ringer solution with ethanol	[137]
CGP 12177, terbutaline	Reverse microdialysis	Concentric	ND	Ringer solution with ethanol	[141]
clonidine	Reverse microdialysis	Concentric	In vitro recovery	Ringer solution	[145]
cortisol	intravenous infusion	Concentric	internal reference method with [3H]glycerol	Ringer solution	[146]
growth hormone	intravenous bolus injection	Concentric	ND	physiological perfusion fluid	[146]
insulin	intravenous bolus injection	Concentric	ND	Ringer solution with ethanol	[147]
Isoprenaline dobutamine salbutamol	Reverse microdialysis	Concentric	Zero flow method	Ringer solution with ethanol	[142]
metformin	Reverse microdialysis	Concentric	ND	Ringer solution with ethanol	[143]
phosphodiesterase inhibitors	Reverse microdialysis	Concentric	ND	Ringer solution with ethanol	[136]
rosiglitazone	oral administration	Concentric	ND	Ringer solution with ethanol	[148]

ND: not determined, Ref.: reference

Continuous glucose monitoring could help to avoid severe hyperglycemia and hypoglycemia, identify patterns in diabetic control, and finally, control an insulin delivery system. Currently there are three available glucose sensor for continuous monitoring, one of them (GlucoDay /Menarini Diagnostics, Florence, Italy) consists of a microdialysis catheter inserted subcutaneously and perfused at a rate of 10 µl/min [152]. Glucose concentration in the dialysate is measured by an electrochemical sensor with immobilized glucose oxidase enzymes. The device is calibrated at 2 hours after probe insertion and acquires glucose level every second with an average glucose value stored every 3 min. The duration of the device is of approximately 2 days. The utility of the GlucoDay system was studied in a multicenter trial showing promising results [153].

Another device, named Subcutaneous Continuous Glucose Monitoring System 1 (Roche Diagnostic GmbH, Mannheim, Germany), uses the microdialysis technique coupled to an external amperometric glucose sensor and is approaching clinical availability. The most important difference with the GlucoDay device is the low flow rate (0.3 µl/min) of perfusion of the microdialysis catheter. Because of the low perfusate pump rate, the device has some limitations including a warm-up time of about 12 hours and a lag time of 31 min [154]. However, promising results were reported in a study of this device in 23 ambulatory patients [155].

Microdialysis catheters have been introduced in these systems as an interface between the biological matrix and the biosensor to prevent problems like fouling. Because the microdialysis membrane excluded cells and large molecules, a relative clean matrix is delivered for biosensor analysis [156].

An important issue in continuous glucose monitoring is that the device samples subcutaneous glucose levels rather than blood concentration. However, the relation between subcutaneous and intravenous glucose levels is unclear. There are reports indicating that subcutaneous glucose levels did not rapidly follow changes in blood. It was found a lower content of glucose in dialysate of the adipose tissue in the subcutaneous space compared to blood with relative low correlation [157]. However, when the microdialysis catheter was placed in the loose connective tissue, subcutaneous glucose concentrations matched plasma levels closely [158]. These results suggested that the loose connective tissue is a very good site for subcutaneous probe placement.

In conclusion, microdialysis facilitates the field of continuous glucose monitoring in diabetes care and contributes to the optimization of the therapeutic interventions.

BLOOD PHARMACOKINETICS

Microdialysis sampling in blood do not appear to be of much interest as there is always the possibility to sample blood directly. Moreover, blood microdialysis in man is limited because the risk associated with probe implantation into a blood vessel. However, with the recent development of flexible microdialysis catheters that can be easily sterilized and that are introduced in veins of patients using standard clinical procedures, blood microdialysis in man has become feasible. Microdialysis also allows obtaining of free protein samples stopping enzymatic degradation and making sample preparation redundant [59]. Moreover, in some populations, e.g. neonates or small animals, frequent plasma sampling is generally very limited due to the small plasma volume [159]. Patients undergoing blood microdialysis studies found

microdialysis catheter highly acceptable and preferable to conventional blood sampling. However, tolerability of microdialysis probes in pediatric patients may become a problem because of the possibility of probe removal.

Microdialysis is also well suited for the determination of protein binding of a drug. The microdialysis technique allows the determination of *in vivo* protein binding using microdialysis sampling in blood and simultaneous blood sampling [12]. The *in vivo* determination of protein binding using the microdialysis method permits a more accurate determination of protein binding, because it was found that the *in vitro* determination systematically underestimated the unbound fraction [11]. In addition, microdialysis permits the determination of the temporal course of protein binding in the same animal in order to determine saturation of the plasma protein binding [12].

Although, to date there are few reports of blood microdialysis in humans, pharmacokinetics of antiepileptic drugs such as carbamazepine [160], phenytoin [160], primidone and phenobarbital [160], levodopa [161] and sotalol [162] were studied by means of microdialysis.

Elshoff *et al*. [162] developed an *in vitro* and *in vivo* method to produce a workable intravenous microdialysis for human use. The pharmacokinetic analysis revealed that sotalol concentrations from microdialysate were not different from conventional plasma samples resulting in comparable pharmacokinetic parameters. The authors concluded that blood microdialysis would simplify pharmacokinetic studies in special populations, e.g. in small children, in order to improve drug treatment [162].

Traditionally serum protein binding studies of drugs have typically been performed using equilibrium dialysis or ultrafiltration. Whilst equilibrium dialysis requires a long equilibrium procedure and could be complicated by complex equilibrium in the system, ultrafiltration can be complicated by reequilibration as the protein and drug concentrations change during the filtration and the sample temperature during the centrifugation could change [163]. Several reports demonstrated that the microdialysis technique is an alternative technique for the study of drug-protein interactions [163, 164]. Advantages of the microdialysis technique with regards to other methodologies are that problems of nonspecific adsorption are less severe because of the smaller surface area of the membrane and the sample temperature could be controlled more precisely because of the absence of centrifugation [163].

CUTANEOUS MICRODIALYSIS

The implantation of microdialysis catheters in the human dermis has been employed for the evaluation of drug penetration from topically applied agents and skin adverse reaction of systemic administered drugs.

Evaluation of Percutaneous Penetration of Drugs by Microdialysis

The skin is an important absorption area of the body for topically applied drugs. To date the most applied technique for the measurement of percutaneous penetration of agents are *in vitro* determination by diffusion chambers and *in vivo* experiments by biomonitoring in animals. However, animal skin is often more permeable that human dermis and therefore percutaneous absorption of topically applied drugs must be tested in human volunteers *in vivo* [165]. For these studies, although invasive techniques such as suction blister sampling or biopsies are available, they are unsuitable for percutaneous absorption studies because they are traumatic and non-specific. Cutaneous microdialysis is a suitable method for the measurement of percutaneous penetration of topically applied agents. The applicability of microdialysis technique in percutaneous penetration studies has been reviewed recently [165, 166]. The validity of microdialysis sampling for cutaneous absorption has been determined in the last years. For cutaneous sampling studies two different probes such as home-made linear probe and commercial concentric catheter are used [165]. Although there is an insertion trauma, tissue damage due to probe implantation is minimal and an equilibration period of approximately 60 min is necessary [165].

On the other hand, there are controversial results regarding the influence of the probe position on the recovery. Therefore, as the influence of probe position in the dermis cannot be excluded, probe implantation should be measured by ultrasound technique [165].

However, the application of the microdialysis technique for percutaneous absorption studies is limited by some difficulties. In first place, measurement of lipophylic or highly protein-bound compounds presents special problems because of the low recovery of such substances [165]. Müller *et al.* [167] have determined estradiol cutaneous levels after topical application and found that in eight of ten experiments estradiol was undetectable. In second place, duration of microdialysis sampling is limited to a few hours because of the induction of an inflammatory response of the tissue to the membrane [165].

An overview of the more recently percutaneous drug penetration studies using microdialysis in humans is presented in Table **6**. Cutaneous pharmacokinetics of fast penetrating drugs such as nicotine [172], non-steroidal anti-inflammatory drugs [117, 170, 173, 175] and local anesthetics [171] have been studied by microdialysis. Lipophylic drugs have been scantly investigated by microdialysis sampling because of the low recovery of the catheter.

Cutaneous microdialysis was also used for the study of the effect of dermis perturbation on drug penetration. Benfeldt *et al.* [175] studied the effect of barrier perturbation on cutaneous salicylic acid penetration by means of microdialysis, finding a highly increased and differentiated cutaneous penetration of salicylic acid in barrier-perturbed skin [175].

Microdialysis also allows the *in vivo* evaluation of enhancers of drug transdermal absorption. It was found that the essential oil of Magnolia fargesii increased the *in vivo* skin deposition of cianidanol but not theophylline. On the other hand, *in vivo* microdialysis showed a higher subcutaneous theophylline amount after essential oil treatment [176].

Table 6. Overview of Skin Penetration Studies of Topical Applied Drugs Using the Microdialysis Technique

Drug	Probe	Calibration	RR (%)	Perfusion fluid	Ref.
8-methoxypsoralen	concentric	In vitro determination	25	sterile water	[168]
betamethasone-17-valerate	Linear	In vitro determination	38	saline solution with glucose	[169]
Delta-aminolevulinic	Linear	endogenous reference calibration with urea	NI	saline solution with glucose	[102]
estradiol	concentric	retrodialysis	1-3	Ringer with albumin 7%	[167]
fusidic acid	Linear	In vitro determination	44	saline solution with glucose	[169]
ibuprofen	concentric	zero no net flux retrodialysis	70±2 92±6	saline solution	[117]
ketoprofen	concentric	zero net flux	59±13	saline solution	[170]
lidocaine	concentric	retrodialysis	56-95	saline solution	[171]
methylnicotinate	concentric	In vitro determination	65	saline solution	[172]
methylsalicylate	concentric	In vitro determination	31±4	saline solution	[173]
propranolol	Linear	retrodialysis	13-72	lactate Ringer´s solution	[174]
salicylic acid	Linear	Retrodialysis	24±4	phosphate buffer with glucose	[175]

NI: not informed, Ref.: reference

Finally, the applicability of cutaneous microdialysis to study the bioavailability of topical formulation was also demonstrated. Kreilgaard [171] compared pharmacokinetic profile of lidocaine from a microemulsion vehicle and a commercially available oil-water (o/w) emulsion (Xylocain 5%) in eight subjects by means of cutaneous microdialysis sampling. The author has demonstrated a significant increase of lidocaine penetration form the microemulsion formulation with regards to the o/w emulsion.

Skin Adverse Drug Reactions

Adverse effects of drugs on the dermis could also be studied by cutaneous microdialysis sampling of endogenous compounds like histamine, prostaglandins, tryptase and bradykinin. It was demonstrated that absolute skin histamine levels could not be calculated by the no net method, because the data did not meet the theoretic assumptions of this method [177]. However, absolute assessment of skin histamine concentrations can be made by microdialysis by the flow rate method [177].

It is well known that rocuronium and vecuronium can induce burning sensations associated with withdrawal reactions during administration. So, dermal microdialysis in human was used to elucidate the underlying mechanisms of pain induction [178]. Microdialysis catheters were inserted intradermally and perfused with rocuronium and vecuronium. Dialysate were analyzed for protein, histamine, tryptase and bradykinin content. Rocuronium induced sharp burning pain, whereas vecuronium given in the usual clinical concentration induced only minor pain sensations. No correlations were found between pain rating and mediator release, concluding that the algogenic effect of neuromuscular blocking drugs is not related to release of algesic mediators [178].

Nielsen *et al*. have monitored extracellular levels of histamine, tryptase, PGD_2, total protein, eosinophilic cationic protein, leukotriene B_4 and myeloperoxidase by means of cutaneous microdialysis [179]. The authors found that cetirizine, a potent H_1-receptor antagonist, significantly reduced the late-phase skin induration to allergen, but did not reduce inflammatory mediators, indicating that cetirizine has no effect on mast cell activation [179].

Moreover, microdialysis sampling of histamine in the skin of patients with mastocytosis could be used as a tool to investigate the effects of dermal mast-cell histamine release in different kinds of treatment regimen. Petersen *et al*. [180] have demonstrated by means of microdialysis sampling that a chronic treatment with ranitidine normalized histamine levels in psoriatic patients, suggesting that histamine may be involved in the pathophysiology of psoriasis.

Finally, a comparison of intradermal injections with an atraumatic intraprobe drug delivery system for codeine-induced histamine release in intact human skin was made [181]. Although, peak histamine release was found within the first 4 min after skin challenge by intraprobe drug delivery system and intracutaneous injections of codeine, the coefficient of variation on peak histamine release was lower with intraprobe drug delivery compared to intracutanous injection. Therefore, the authors concluded that codeine

could be atraumatically administered to the skin by intraprobe delivery and it would be possible to deliver immunopharmacologically active drugs to the skin by intraprobe delivery [181].

PERSPECTIVES

Microdialysis will become available for a broad range of diagnostic applications, drug distribution studies and monitoring of the therapeutic effect of different pharmacological agents. Miniaturization and online atomization of analytical assays will ultimately allow "bedsides microdialysis" for these applications.

One of the most promising perspectives of microdialysis is the use of this technique as a therapeutic tool. A more recent application of microdialysis is the introduction of a substance into the extracellular space *via* the microdialysis probe. Therefore, microdialysis allows local administration of a drug without inducing systemic side effects. Microdialysis also allows the simultaneous measurement of the corresponding tissue response, such as lipolysis. Ronquist *et al.* [182] successfully employed reverse microdialysis of antineoplasic drugs for site specific drug delivery in the treatment of gliomas. On the other hand, Nicolaidis [183] proposed the use of microdialysis in deep brain structures for disclosing the neurochemical changes in the depression and the corresponding reverse dialysis as a method of neurochemical treatment of depression.

Another perspective of microdialysis is the use of microdialysis based glucose sensors for continuous monitoring coupled to an insulin pump as an "artificial pancreas" [152]. The device consists of a sensor, a delivery system, and programming to link the two components. Preliminary studies demonstrated that an implantable insulin pump linked to an intravenous glucose sensor for fully automated 48 hour closed-loop insulin delivery improved diabetic treatment by reducing the hypoglycemic and hyperglycemic events in patients [152].

FUTURE DIRECTIONS IN THE DEVELOPMENT OF MICRODIALYSIS AS A TECHNIQUE FOR CLINICAL PHARMACOLOGY STUDIES

Many technical challenges still remain to be resolved for this relatively new sampling technique. The standardization of probe depth insertion is still a problem in cutaneous microdialysis for the evaluation of penetration through the human skin barrier. Probe site insertion must be validated for the use of microdialysis in neurointensive care. The recent edition of a consensus statement of microdialysis in neurointensive care is an important attempt in this way [131].

It is also important to improve the microdialysis sampling of lipophylic drugs and high protein bound drugs. For high protein-bound drugs, the low recovery could be solved by use of new microdialysis membranes with high molecular weight cut-off. On the other hand, the addition of solubilizers to the perfusate could improve the recovery of lipophylic drugs [165]. Development of high sensitive analytical methods may also provide a significant progress in the use of microdialysis in the clinical setting.

Feasibility of chronic microdialysis sampling remains to be elucidated. The importance of tissue response to chronic probe implantation must be determined. Also the effects of tissue response like gliosis on microdialysis catheter recovery need to be evaluated.

Finally, the applicability of microdialysis sampling in bioequivalence needs to be established. An important advantage of microdialysis in this field is the possibility of the determination of target site concentrations or the pharmacodynamics of the drug instead of measurement of plasma concentrations.

CONCLUSIONS

The applicability of the microdialysis technique in the study of pharmacokinetic and pharmacodynamic properties of old and new drugs in the clinical setting is supported in the ability to monitor extracellular unbound levels of both endogenous and exogenous compounds. The most interesting clinical properties of pharmacological drug groups that can be determined through the microdialysis technique are shown in Table **7**.

In pharmacokinetic terms an important advantage of this technique is the time course determination of the bioactive levels in the interstitial fluid of the target tissue, especially for antimicrobial drugs.

The simultaneous recovery of endogenous compounds through the microdialysis probe allows the study of drug

Table 7. Principal Microdialysis Applications in Clinical Pharmacology

Pharmacokinetic applications	Pharmacodynamic applications
• Tissue distribution of anti-infective drugs in healthy volunteers and patients with infections. • In vivo PK- in vitro PD models of anti-infective drugs. • Tumoral distribution of antineoplasic drugs. • Target site distribution of central acting drugs. • Therapeutic drug monitoring of anticonvulsivant drugs by subcutaneous microdialysis. • Penetration of skin barrier by topically applied drugs. • Tissue distribution of local anesthetic drugs. • Blood pharmacokinetics in special populations	• Determination of the effect of antineoplasic drugs on growth tumor factors. • Mechanism of action of nonsteroidal anti-inflammatory drugs. • In vivo selectivity of nonsteroidal anti-inflammatory drugs to COX-2. • Mechanism of local anesthetic nerve block potentiation. • Monitoring of drug effect on central ischemia. • In vivo study of lipolytic and anti-lipolytic drugs. • Effect of drugs on muscle metabolism. • Continuos glucose monitoring in diabetic therapy. • Study of skin adverse drug reactions.

effects on physiological events. This permits parallel determination of the local drug levels and its pharmacological effects and the study of a pharmacokinetic-pharmacodynamic correlation.

Lastly, the introduction of a drug to the microdialysis perfusate allows to study related local effects. This type of experiments could help to elucidate the mechanism of action of different drugs and could serve as a new therapeutic tool.

ABBREVIATIONS

PK	=	Pharmacokinetic
PD	=	Pharmacodynamic
T	=	Time
AUC	=	Area under the curve
MIC	=	Minimum inhibitory concentration
Cmax	=	Maximum concentration
PK-PD	=	Pharmacokinetic-pharmacodynamic
AUC	=	Area under the curve
Cmax	=	Maximum concentration of drug in serum
5-FU	=	5-fluorouracil
IGF-1	=	Insulin-like growth factor-1
CSF	=	Cerebrospinal fluid
NSAIDs	=	Nonsteroidal anti-inflammatory drugs
COX	=	Cyclooxygenase
PG	=	Prostaglandin
Tx	=	Tromboxane

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