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Microdialysis Techniques In Pharmacokinetic and Biomarker Studies. Past, Present and Future Directions. A Review.

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Abstract

Microdialysis (MD) techniques were first applied in the early 1960s. The fields of their application comprised probe implantation into the central nervous system (CNS) at the beginning, and then expanded to almost every organ summarized in this article. After its early experimental applications MD became an important tool in the human pharmacokinetic/pharmacodynamic (PK/PD) studies as well. This monitoring technique is capable for investigation of local unbound concentrations of both endogenous and exogenous compounds in the interstitial fluid. The review shows examples for the role of MD in pharmacodynamic studies and for its use in tissue distribution and drug-transporter interaction studies.

Determination of test substances in the dialysate samples needs sensitive bioanalytical methods. The main analytical techniques coupled with MD are summarized under the subtitle "Target molecules".

New trend in the application of MD is the determination of large molecular entities in the extracellular fluid of target tissues. This approach greatly helps in the discovery of new pathophysiological pathways and identification of new therapeutic intervention strategies for several disorders.

Finally, the article gives an overview on the complementary techniques (positron emission tomography, magnetic resonance spectroscopy and open flow microperfusion), and presents their advantages and limitations versus to *in vivo* MD.

In summary, MD techniques have a wide variety of the fields of application. There are several new approaches using this methodology. The relatively low price and the importance of the information gained on the pharmacologically active form of the test articles at the site of interest guarantees a significant position of this technique in the preclinical and clinical research.

Keywords: Microdialysis; PK/PD studies; Biomarker research; Drug-drug interactions; Bioanalysis

The Beginnings of the Application and Principle of Microdialysis Techniques

The microdialysis principle was first employed in the early 1960s, when push-pull cannulas [1] and dialysis sacs [2] were implanted into animal tissues, especially into rodent brains, to directly study the tissues' biochemistry [3]. While these techniques had a number of experimental drawbacks, such as the number of samples per animal or no/limited time resolution, the invention of continuously perfused dialytrodes in 1972 helped to overcome some of these limitations [4]. Further improvement of the dialytrode concept resulted in the invention of the "hollow fiber", a tubular semi-permeable membrane with a diameter of ~200-300 μm, in 1974 [5]. Today's most prevalent shape, the needle probe, consists of a shaft with a hollow fiber at its tip and can be inserted by means of an introducer and split tubing or a guide cannula into the brain or other tissues.

Microdialysis technique is usable for continuous monitoring of the biochemical milieu surrounding the probe-membrane in the extracellular fluid of the target organ. A physiological perfusion fluid (artificial cerebrospinal fluid or artificial peripheral perfusion fluid) is perfused through the inner tube of the microdialysis probe with concentric design. At the tip of the probe the test substances located in the interstitial fluid of the target tissue can diffuse trough the pores of the semipermeable membrane into the direction of the concentration gradient. The cut off value of the probe membrane is usually 20 kDa, but lower and higher cut off membranes are also available in the market (6 kDa – 100 kDa). The molecules that are able to cross the membrane reach the perfusion fluid and flow away in the outer tube of the probe. At the outlet of the probe there are connecting plastic tubings leading the dialysate of the extracellular fluid into the collection vials. The principle of microdialysis sampling is presented in Figure 1.

Investigation of PK/PD profile of test compounds can be performed both in anesthetized and awake animals by microdialysis. The changes in the neurotransmitter levels in specific brain regions or alterations in biomarker concentrations in health or disease conditions suggested to be studied preferentially in freely moving animals. The constructions of microdialysis setup for anesthetized and awake animals are presented in Figures 2 and 3.

Main Fields of Application of Microdialysis Techniques

Target organs

MD techniques are widely employed for sampling the extracellular fluid (ECF) in living organs. The first target for application of MD was the central nervous system (CNS) of experimental animals. The main goal of these studies was to investigate brain function and changes in levels of endogenous compounds such as neurotransmitters or metabolites [6] . Nevertheless, in CNS studies, reverse MD (retrodialysis) has also been used extensively to investigate the effects of diverse pharmacological and toxicological agents, such as antidepressants,

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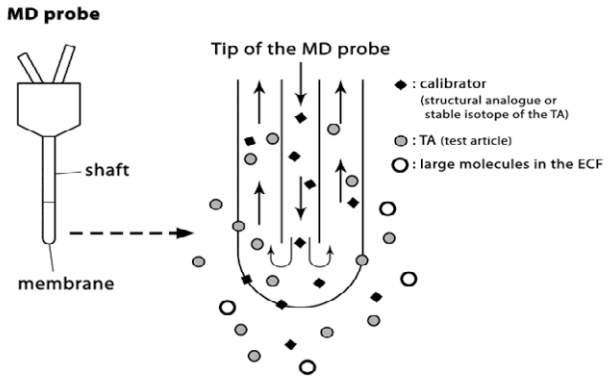

Figure 1: Principle of microdialysis technique: The most important element of the technique is the microdialysis probe (left side). The probe is introduced into the target tissue where the free molecules in the ECF can diffuse through the probe membrane into the perfusion solution (right side). The cut off value of the probe membrane limits the size of the molecules diffusing through the membrane. For quantitative microdialysis calibrator molecules can be perfused through the probe and the loss of their concentrations in the dialysates can be measured. The recovery of the probe for a specific test substance can be calculated.

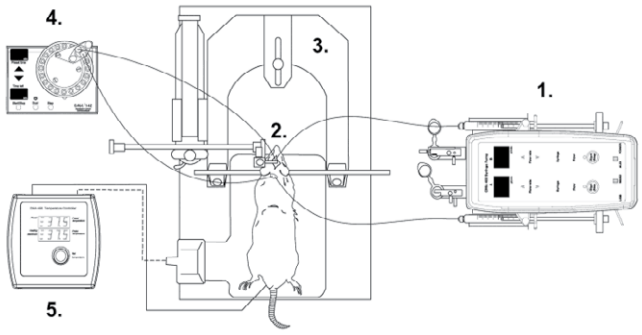

Figure 2: Microdialysis setup for anesthetized animals (1) Microdialysis syringe pump, (2) Microdialysis probes placed into the brain and jugular vein of a rat, (3) Stereotaxic instrument, (4) Fraction collector, (5) Temperature controller. (The figure is a slightly modified adaptation of the picture of CMA product catalogue with permission of the CMA representative).

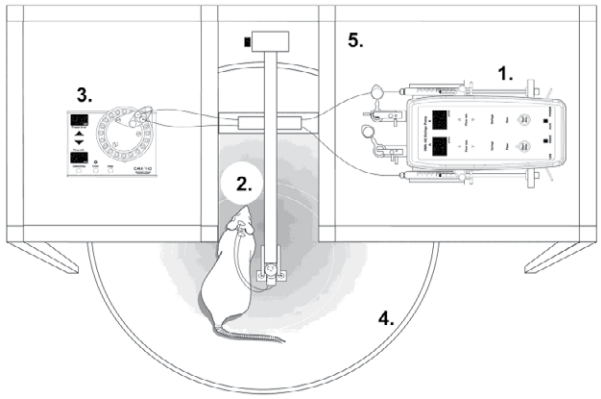

Figure 3: Microdialysis setup for freely moving animals (1) Microdialysis syringe pump, (2) Microdialysis probes placed into the central guide cannula and jugular vein of a rat, (3) Fraction collector, (4) Plastic bowl for awake animals, (5) Instrument table. (The figure is a slightly modified adaptation of the picture of CMA product catalogue with permission of the CMA representative).

antipsychotics, antiparkinson molecules, hallucinogens, drugs of abuse, and experimental drugs, on local effects on neurotransmission at various central nuclei. Thus, the MD approach has contributed largely not only to clarification of the physiological role of the serotonergic, dopaminergic, glutamatergic etc. neuronal systems, but also to the development of therapeutic strategies for the treatment of a number of neuropsychiatric disorders.

Since MD was established for measuring concentrations of substances within living organisms, the range of capable tissues has extended continuously. The first MD in lung tissue was performed in 1991 in rats; it was 10 years before a MD probe was inserted into a human lung. This might be explained by two major reasons: (1) ethical and safety concerns, and (2) alternative measurement techniques for lung [7]. For other organs like skin and muscle the human studies were developed in parallel with the animal experiments.

MD probes can be inserted easily into superficial compartments like skin or subcutaneous adipose tissue without notable risks to healthy subjects, this is not the case in deeper compartments. Thus, MD in internal organs is not permitted in healthy volunteers. As the insertion of MD probes into internal organs is more invasive, the technique has generally been limited to animals [8] in strictly experimental studies.

Four main areas of employing MD in internal organs can be distinguished. 1) Strictly metabolic processes have been studied under different conditions, e.g., metabolites like lactate have been quantified to monitor organ ischemia. 2) Researchers have aimed at elucidating organ-specific physiological or pathophysiological pathways. For instance, cytokine cascades and their interactions have been identified in internal organs in addition to metabolites. 3) A considerable number of pharmacokinetic studies have been conducted in organs with various drugs. 4) MD has been evaluated as a tool permitting local delivery of drugs to specific organs or tumors [8].

For some examples on the application of MD techniques in different organs, see Table 1.

Target disorders

To obtain detailed information on the pathomechanism of different disorders the continuous *in vivo* sampling and monitoring by MD provided an appropriate tool. For summary on disorders investigated in humans or experimental animals by *in vivo* MD, see Table 2.

Target molecules

Neurotransmitter and biomarker studies using microdialysis: MD allows not only the evaluation of target - site distribution of new chemical entities, but also the assessment of their pharmacodynamics effects on physiological variables and biomarkers of disease processes. An important application of this technique is its use in the measurement of drug-induced changes in concentrations of monoamine and amino acid neurotransmitters and acetylcholine, and their respective metabolites [9] in specific brain regions.

The overall objective of antidepressant therapy over the past several decades has been to increase monoamine neurotransmitter concentration at the synapse. Antidepressant agents produce elevated monoamine levels either by inhibiting monoamine metabolizing enzymes or by monoamine transporter blockade and inhibiting neurotransmitter reuptake (serotonin /5-HT/reuptake inhibitors, norepinephrine /NE/ reuptake inhibitors, NE-5-HT reuptake inhibitors, 5- HT-NE-dopamine reuptake inhibitors etc.). MD is capable for the neurochemical characterization and elucidation of the

Organ	Species	References
Brain	human	[68]
brain, blood	mouse	[31]
brain	mouse	[69]
brain (cortex, hippocampus, striatum)	rat	[12]
brain (cortical, subcortical)	rhesus monkey	[70]
Breast	human, mouse	[71]
skin	human	[72]
skin	human, *ex vivo*	[73]
subcutaneous fat, muscle	human	[20]
skin, soft tissue	human	[74]
muscle	human	[75]
bone, adipose tissue	human	[19]
skeletal muscle, adipose tissue	human	[76]
lung	human	[77]
lung	rat	[78]
kidney, lung, liver	rat	[79]
liver	rat	[80]
heart	rabbit	[81]
pancreas	rat	[82]
peritoneum	human	[49]
heart	pig	[83]
pancreas	dog	[84]
synovial fluid	rabbit	[85]
cerebrospinal fluid	rat	[33]

Table 1: Target organs for *in vivo* microdialysis studies.

Disease (disease model)	Species	Analyte	Reference
Stroke	Human	glutamate, glycerol, lactate, pyruvate	[68]
Parkinson's disease	Human	GABA, glutamate	[86]
Pulmonary tumor surgery followed by infection	Human	cefplrome	[77]
Glial tumor	Human		[87]
Traumatic brain injury	Human	Tau and beta amyloid proteins	[88]
Subarachnoid hemorrhage	Human	lactate	[89]
Subarachnoid hemorrhage	Human	glutamate	[90]
sepsis	Human	cefpirome	[91]
diabetes, leg infection	Human	ertapenem	[17]
diabetes, cardiac surgery	Human	vancomycin	[18]
Alzheimer's disease	Mouse	amyloid beta	[92]
diabetes, leg infection	Human	daptomycine	[19]
Alzheimer's disease	Mouse	amyloid beta	[69]
Alzheimer's disease	Mouse	apolipoprotein E	[93]
sepsis	Human	antimicrobials	[76]
Parkinson's disease	Human	catecholamines, L-dopa	[94]
Parkinson's disease	Rat	GABA, glutamate, glycine	[95]
Stroke	Rat	glutamate	[96]
sepsis	Rat	fluconazole	[78]
cocaine addiction	Mouse	dopamine	[97]
Stroke	Mouse	adenosine	[98]
Peritonitis	Pig	cytokines	[99]
Obesity	Human	ciprofloxacin	[21]
Cutaneous tumor	Human	carboplatin	[23]
Rheumatoid arthritis	Rabbit	sinomenine	[85]

Table 2: Therapeutic fields as targets for application of *in vivo* microdialysis.

pharmacological profile of several classes of antidepressants.

In vivo MD is a powerful tool in the development of therapeutic strategies over depression for addiction, anxiety, attention deficit, hyperactivity disorder and schizophrenia as well as neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, epilepsy and cerebral ischemia [9]

Besides the CNS applications, MD is used to monitor biomarkers also in peripheral tissues. In inflammation processes, metabolic disorders, peripheral ischemic diseases, dermatological disorders and pain many markers can be determined using these techniques (Table 3).

Drug tissue distribution studies using microdialysis techniques: Several characteristics of MD make it a valuable addition to the techniques available for pharmacokinetic studies. The small size of the probe generates minimal perturbation of tissues, organs and systems. Thus, the samples are representative of normal physiology. The dialysis process does not change the fluid volume of the surroundings. This allows continuous sampling with good temporal resolution, including long-term experiments in awake animals. Samples can be collected prior to administration of the dose, so each animal serves as its own control and few animals are required overall [10]. Knowledge on distribution within the brain is important for drugs that directly act on targets in the central nervous system [11] such as anticonvulsants, antidepressants, learning and memory enhancers [12], anesthetics, antibiotics [13], antinociceptive, and anticancer agents. In some cases it is important to avoid brain exposure of drugs, and MD is a tool to provide evidence for the absence or low level of a pharmacologically active compound in the CNS [14].MD techniques are frequently used to determine the unbound anti-infective drug distribution in critically ill, diabetic, ischemic and obese patients. A reduction in the cardiac output caused by heart failure can affect the tissue distribution [15,16] of antibiotics. Inflamed tissues and bones are very common in diabetic patients, especially in the lower extremities. Several studies have used MD to evaluate anti-infective concentrations in tissues of diabetic patients[17-19]. The use of MD sampling is also feasible for the study of target – site pharmacokinetics of new antimicrobial agents in both preclinical and early clinical development.

Jonsson and coworkers employed MD in critical limb ischemia (CLI) patients to study penetration of the antibiotic cloxacillin to the target tissue [20]. Cloxacillin concentrations were measured in serum and in MD samples from skin and muscle of the lower part of the calf and, as reference, subcutaneously at the pectoral level in eight patients suffering from CLI. In CLI patients, the tissue penetration of cloxacillin was comparable to that of healthy controls, despite impaired blood circulation [15,20].

The choice of the correct anti-infective agent at the right dosage regiment plays a crucial role in treatment success, safety, and prevention of resistance [16]. However, dose adjustments according to body weight are not usually performed. Therefore, under dosing in overweight or obese patients may occur [21,22]. Hollenstein et al. compared the concentrations of ciprofloxacin (2.85 mg/kg) in obese and lean patients at the target site, adjusting the dose by weight. The free drug concentrations were measured with MD in both skeletal muscle and adipose tissue. Plasma concentrations did not predict the actual target concentrations quantified with MD. The authors concluded that the penetration process of ciprofloxacin is diminished in obese patients and the doses have to be adjusted [21]

Measurement of target - site concentrations of antineoplastic drugs

in malignancies by MD and relating pharmacodynamic parameters are of great interest for the design of active new chemical entities with cytotoxic effects [23,24]. Tumor drug exposure, a marker linked to clinical outcome, may be reduced dramatically, due to diffusion barriers in solid tumors [25].

Transporter interaction studies using microdialysis: Several papers reported the localization of drug transporters at the main physiological barriers of the organism (blood-brain barrier, blood-CSF barrier, hepatic-, renal- and intestinal barriers). The role of these proteins is huge in drug-drug interactions, drug absorption, distribution and elimination. First P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) were identified at the apical membrane of brain capillary endothelial cells as the mechanism responsible for the restricted brain penetration of various molecules**.**

P-gp mediated brain distribution of morphine was studied by Groenendaal and coworkers [26] in rats using MD approach and population pharmacokinetic modelling analysis. They found that brain distribution of morphine is determined by three factors: limited passive diffusion; active efflux, reduced by 42% by P-gp inhibition and low capacity active uptake.

Similar interactions with P-gp were reported in mdr1a (-/-) mice by Xie and coworkers using cerebral MD techniques in an earlier study [27].

O'Brian and coworkers reported P-gp mediated BBB transport of imipramine [28] and escitalopram [29]. MD based pharmacokinetic studies demonstrated that administration of the P-gp inhibitor cyclosporin A or verapamil resulted in increased brain levels of escitalopram and imipramine without altering plasma escitalopram levels in the rat.

P-gp mediated drug-drug interactions were studied not only in the brain but also in the periphery by Ma and coworkers using triple probe MD in rats [30]. The authors tested brain, blood and bile concentrations of spinosin in the presence and absence of cyclosporine A, as selective P-gp transporter inhibitor.

Our group reported a dual- and triple-probe mirodialysis study using PSC-833 (valsprodar), as specific P-gp inhibitor and quinidine as P-gp substrate in anesthetized and awake mice [31] and rats [32], respectively. PSC-833 significantly increased the brain exposure of quinidine in both species irrespective of the presence or absence of anesthesia. The local perfusion of the inhibitor by retrodialysis also enhanced the brain concentration of quinidine in the perfused hemisphere compared to the contralateral, control side.

Recently, Chen and coworkers reported the transporter-mediated brain exposure of cefadroxil, a cephalosporin antibiotic [33]. Cefadroxil is a substrate for several membrane transporters including peptide transporter 2 (PEPT2), organic anion transporters (OATs), multidrug resistance-associated proteins (MRPs), and organic anion transporting polypeptides (OATPs). These transporters are expressed at the blood–brain barrier (BBB), blood-cerebrospinal fluid barrier (BCSFB), and/or brain cells. The distribution of cefadroxil in brain was compared in the absence and presence of probenecid, an inhibitor of OATs, MRPs and OATPs using triple probe MD technique (brain parenchyma, cerebral ventricle and blood probes). The ratio of unbound cefadroxil AUC in brain ECF to blood (Kp,uu,ECF) was ~2.5-fold greater during probenecid treatment. These findings demonstrate that drug-drug interactions via relevant transporters may affect the distribution of cephalosporins in the brain ECF.

Further example for the non-P-gp mediated transport studies was reported by Westerhout and coworkers who studied methotrexate distribution in the presence and absence of probenecid by MD technique [34].

Analytical considerations for microdialysis: There are many different ways to analyze MD samples for the test molecules. Samples can be analyzed off line or directly online using sensors. For offline analysis, a specified volume of sample (usually 1–20 ml) is collected in vials or tubes for later analysis. The temporal resolution that can be obtained in these experiments is usually determined by the MD flow rate, analyte recovery, and the sensitivity of the analytical method. At the typical MD flow rate of 1ml/min, most offline experiments have temporal resolutions from 5 to 10min. An exception to this is if the analyte concentration is very high. Then smaller volume samples can be collected and diluted prior to analysis. However, in general, sample volumes of less than 1ml become very difficult to manipulate offline due to problems with surface tension and evaporation [35].

Online sample analysis offers several potential advantages over offline analysis. In an online system, the sample collection, manipulation, injection, and analysis steps are all integrated in a planar device in a continuous, streamlined fashion. Therefore, problems related to handling submicroliter volumes of sample (sample loss, mislabeling, evaporation, and surface tension) as well as sample degradation that can occur with sample exposure to air (e.g., ascorbic acid and catecholamines) can be avoided [36-38]. Such systems are usually capable of manipulating and analyzing submicroliter sample volumes, which allow high temporal resolution analysis to be performed and near real-time data, which provide immediate feedback on the biological process under investigation.

Most applications require a separation of analyte in dialysate samples prior to detection. The most commonly employed separation methods are liquid chromatography (LC) and electrophoresis (in either the capillary (CE) or microchip format). A variety of detection methods have also been used, including ultraviolet, fluorescence, electrochemical, and mass spectrometric detection [35,39].

LC is the most commonly used analytical method for the separation of analytes present in MD samples. The detection method employed for LC analysis is dependent on the analyte of interest [35]. Ultraviolet detection is popular for detection of drugs for pharmacokinetic studies. Electrochemical detection is employed for the detection of catecholamines and other redox - active compounds of biological interest, such as thiols and aromatic amines. Fluorescence detection has also been popular, but usually requires derivatization of analytes prior to analysis. Finally, mass spectrometry (MS) is becoming increasingly popular for off line analysis of MD samples following liquid chromatographic separations [35].

CE is particularly attractive for the analysis of MD samples because it has very low sample volume requirements (nanoliters to picoliters) and can perform extremely fast separations. A variety of detectors can be used, including ultraviolet, laser – induced fluorescence (LIF), electrochemical (amperometric and conductivity), and MS [35].

Over the past decade, microchip electrophoresis has evolved to become an attractive analytical platform for the online analysis of MD samples. Microchip devices have at least two major advantages: very fast separations on - chip for monitoring fast biochemical processes and miniaturization of the system for on - animal sensing.

Besides the separation- based analytical approaches there are non-

Type of pathology	Molecule(s)	References
Ischemia/reperfusion injury	glutamate, aspartate, glycine GABA lactate pyruvate glucose adenosine myoglobin (cardiac ischemia)	[81, 83, 96, 98, 100, 101]
Subarachnoid hemorrhage	glutamate glutamine histidine glycine lactate IL-6	[89,90, 102,103]
Traumatic brain injury	tau, amyloid beta glucose	[88, 104]
Parkinson's disease	dopamine 3-methoxytyramine 3,4-dihydroxyphenylacetic acid serotonin HVA GABA glutamate cathecholamines	[86, 94, 95, 105]
Alzheimer's disease	acetylcholine apolipoporotein E beta-amyloid protein amyloid beta oligomers	[69, 92, 93]
Epilepsy	glutamate GABA adenosine acetylcholine	[106, 107,108]
Psychiatric disorders (schizophrenia)	serotonin dopamine GABA	[109, 110]
Inflammation	interleukin-1 interleukin-8 interleukin-10 interleukin-6 TNF-alpha VEGF NGF	[99, 111, 112]
Enterocholitis	glucose lactate pyruvate glycerol	[49]
Diabetes	glucose lactate pyruvate glycerol urea	[113, 114]
Pain	substance-P beta endorphin pyruvate lactate cortisol glutamate serotonin norepinephrine dopamine leukotriene B4 PGE2	[115-117]
Smoking	dopamine	[118-120]
Drug abuse	dopamine glutamate	[121-123]

Table 3: Target molecules of microdialysis in neurotransmitter and biomarker studies

separation based techniques for bioanalysis of microdialysate samples, as well.

Biosensors are analytical devices with a biological recognition element that produce an electrical signal in response to a biological change. An ideal biosensor should be able to perform continuous and reliable monitoring of analyte from complex body fluids over a significant period of time. There are many types of biosensors. Most of these are enzyme - based and employ either electrochemical or optical detection [35]. Online MD sampling coupled to biosensors has been reported for analytes such as ascorbate [40], glucose, lactate and glutamate [41].

Another non-separation-based analytical approach is immunoassay. Immunoassays have been employed following MD sampling for measuring peptides and some drug substances in MD samples. Neuropeptides are present at nanomolar - to - picomolar concentrations in the extracellular fluid of the brain. In addition, the recovery of peptides across the dialysis membrane is generally much lower than that of smaller -molecular - weight neurotransmitters, generating extremely dilute samples of analyte [35].

MS detection has the advantages of conclusive analyte identification based on molecular weight as well as the sensitivity to detect low concentrations of analyte(s) often present in MD samples. More frequently, MS is coupled to a separation method (LC-MS; LC-MS/MS). Matrix – assisted laser desorption ionization (MALDI) has been shown to be an attractive choice for off - line analysis of peptides in MD samples.

Analyte recovery is a very important parameter in MD sampling and bioanalysis. At typical flow rates used in MD sampling (1 to 5 μL/min), there is not enough time for complete equilibration between the perfusate and the surrounding environment to occur, and therefore the concentration of analyte in the perfusate reflects only a percentage of the total amount present in the extracellular space or sample. The recovery is defined as the ratio of the dialysate concentration to the actual tissue concentration [35]. For compounds that are present at very low concentrations in the extracellular fluid, one way to reduce the dependence of the assay on the sensitivity of the analytical method is to increase recovery. This can be accomplished by decreasing the flow rate [42] or by adding a substance (beta-cyclodextrine, bovine serum albumin etc.) to the perfusate that has a strong affinity for the compound of interest [14,43].

Drug delivery by microdialysis

The MD probes inserted into the target tissue can be used in sampling mode and also in delivery mode (retrodialysis or reverse dialysis). If they are used in delivery mode, then specific compounds can diffuse from the perfusion fluid into the interstitial fluid of the target tissue into the direction of the concentration gradient and can express their influence locally without any systemic side effect.

To avoid or diminish the tissue injury caused by implantation of MD probe which might trigger ischemia, gliosis and cell death at the sampling site, Nesbitt and coworkers delivered dexamethasone, a glucocorticoid anti-inflammatory agent, and XJB-5-131, a mitochondrially targeted reactive oxygen species scavenger, using retrodialysis [44] techniques. Dexamethasone and XJB-5-131 each diminished the loss of evoked dopamine activity, diminished ischemia, diminished the loss of neuronal nuclei, diminished the appearance of extravasated macrophages, and diminished the loss of dopamine axons and terminals next to the probes.

Another example for the application of reverse MD was described by Ludwig and co-workers, who studied the local effect of naloxone, an opioid antagonist against the systemic effect of morphine in supraoptic nucleus oxytocin neurons [45]. They found that the effect of opioid agonists primarily occurs within the supraoptic nucleus itself, since the antagonist was effective when given directly into the supraoptic nucleus by retrodialysis.

Vazquez-De-Rose reported that retrodialysis of nociceptin into the nucleus accumbens shell blocks cocaine-induced increases in extracellular dopamine and locomotor activity [46]. Extracellular dopamine and locomotor activity can be dissociated within the nucleus accumbens and may reflect motor output differences in shell versus core regions of the nucleus accumbens.

Our group applied retrodialysis in a drug-drug interaction study [32]. The specific P-gp inhibitor PSC-833 was administered by retrodialysis via the MD probe into one cerebral hemisphere and quinidine was given systemically. The enhancement in the brain concentration of quinidine in the inhibitor-treated hemisphere provided evidence on the role of efflux transporters localized at the BBB in the brain exposure of this compound.

A potential strategy to increase the efficacy of topotecan to treat CNS malignancies is modulation of the activity of ATP-binding cassette (ABC) transporters at the blood-brain and blood-cerebrospinal fluid barriers to enhance topotecan CNS penetration. Zhuang and co-workers focused on topotecan penetration into the brain ECF and ventricular cerebrospinal fluid (CSF) in a mouse model and the effect of modulation of ABC transporters at the blood-brain and blood-cerebrospinal fluid barriers by a tyrosine kinase inhibitor (gefitinib) [47]. Topotecan brain ECF penetration was lower compared with ventricular CSF penetration. Gefitinib increased topotecan brain ECF penetration but decreased the ventricular CSF penetration. These results are consistent with the findings that expression of Bcrp1 and P-gp at the apical side of the choroid plexus facilitates an influx transport mechanism across the blood-cerebrospinal fluid barrier, resulting in high topotecan CSF penetration.

Diagnostic applications of microdialysis

Low and coworkers reported the application of MD as a diagnostic tool in patients with severe head injury. The measurement of brain neurochemistry is based on the principle that secondary insults such as hypoxia result in alterations in cellular metabolism of neuronal tissues; this in turn results in a cascade of metabolic changes that result in further cellular damage. Glutamate is an excitatory amino acid that is an early marker of cerebral ischemia. Glucose levels fall as a consequence of hyperglycolysis during a hypermetabolic state, and this in turn results in elevation of lactate levels and lactate: pyruvate ratios. Since these biochemical variables obtained from MD assays and partial pressure of oxygen in brain tissue ($PbtO_2$) act as early indicators of the tissue's ischemic response to injury, they potentially have a critical role in influencing patient outcomes and assisting in the prediction of outcomes following traumatic brain injury [48].

Peritoneal MD is a safe procedure and an applicable method in surveillance of the metabolic and inflammatory changes in the peritoneal cavity after surgery for necrotizing enterocolitis (NEC). Pedersen and coworkers reported a MD study in infants on the determination of the concentration of glucose, lactate, pyruvate, and glycerol in the peritoneal microdialysates. The results of peritoneal MD in patients with complications were significantly different from those with an uncomplicated course (lactate/pyruvate ratio and glucose concentration) suggesting the predictive value of MD monitoring of the biochemical changes [49] in this pathology.

Similar ischemia markers were followed by Pynnönen and coworkers in small intestine of pancreaticoduodenectomy patients. The metabolic changes were measured by intraperitoneal and intraluminal MD probes. The results support the hypothesis that intraluminal application of MD and metabolic parameters from the small intestinal lumen indicate onset of ischemia earlier than intraperitoneal MD with higher sensitivity and specificity [50].

Advantages and Limitations of Microdialysis Techniques

The technique of MD has a number of benefits and drawbacks reported by several authors which are summarized in Table 4 [11,51,52].

The three most important benefits are as follows: (1) The method measures the concentration of drug or endogenous molecule at the site of action. (2) Unbound, pharmacologically active concentrations can be determined by MD. (3) The number of the animals examined can be kept to a low level contrary to traditional pharmacokinetic studies (simply blood collections) where every time point needs different animals. Moreover, MD not only offers the possibility of sampling from certain tissues but also of delivering drugs directly to the site of action (retrodialysis) [32,53,54] contrary to complementary techniques (see the next section).

Benefits
1. It measures the concentrations (drug or endogenous molecules) at the site of action
2. It measures the unbound, pharmacologically active concentrations of the drugs
3. The samples are protein free and this usually allows the direct determination of the drugs from the collected samples by bioanalytical techniques
4. Microdialysis can be used to obtain concentration-time profiles over a consecutive number of days, because there is no fluid loss during sampling
5. The experiments can be performed not only in anesthetized but also in freely moving animals
6. The number of animals can be kept to a low level contrary to traditional PK studies where every time point needs different animals
7. Microdialysis not only offers the possibility of sampling from certain tissues but also of delivering drugs directly to the site of action (retrodialysis)
8. In the case of delivery mode, the drug effects can be studied locally without the risk of systemic adverse events or confounding drug effects
9. More than one compound can be measured simultaneously
10. More than one tissue (or brain region) can be studied simultaneously
11. High resolution concentration-time profiles can be obtained from distinct brain regions
Limitations
1. The implantation of the microdialysis probe into the brain or other target tissue could cause tissue trauma or damage (semi-invasive technique)
2. The probe insertion into the brain parenchyma can increase the BBB permeability for 1-2 days
3. The size of the molecules to be determined is limited by the cut off value of the probe membrane
4. Several molecules show non-specific binding to the microdialysis setup
5. Calibration is necessary as the probe recovery does not reach 100 %
6. This techniques measure a mean concentration over a time interval
7. Highly sensitive analytical method is necessary, capable of dealing with small sample volumes
8. Microdialysis techniques are highly sophisticated low throughput methods
9. Special surgical skills are needed to set up the experiments

Table 4: Strengths and weaknesses of microdialysis techniques.

Comparison of Microdialysis and Complementary Technologies: Positron Emission Tomography (PET), Magnetic Resonance Spectroscopy (MRS) and Open Flow Microperfusion (OFM)

MD is a powerful experimental tool to assess unbound concentrations of drugs in the interstitial space fluid of different tissues and organs, both in preclinical species and in human subjects. In the next part of this review, the non-invasive nuclear imaging methods positron emission tomography (PET), magnetic resonance imaging (MRI)/ magnetic resonance spectroscopy (MRS) and open flow microperfusion (OFM) will be discussed as complementary techniques to study drug distribution and pharmacokinetics in the living body.

Positron emission tomography (PET) is a non-invasive nuclear imaging technique which can be used to assess the tissue distribution and pharmacokinetics of drugs labeled with short-lived positron-emitting radionuclides, such as carbon-11 (^{11}C, half-life: 20.4 min) or fluorine-18 (^{18}F, half-life: 109.8 min) [55].

Whereas PET was initially developed for an application in humans, dedicated high-resolution and high-sensitivity PET scanners have been developed which allow for conducting PET experiments in small laboratory animals [56]. Such experiments are of particular interest since diverse animal models of human disease (e.g. transgenic mice, tumor-xenograft mice etc.) are commonly used in drug research. The PET technology therefore takes a key position at the interface between preclinical and clinical—that is translational—research. For the purpose of drug development, imaging with PET bears significant potential in translational medicine as it allows the same methodology to be employed in animal experiments and human studies.

There are many advantages and limitations of PET versus MD in clinical application. (1) For detection of test compound in PET studies radiolabeling is necessary. For MD different analytical techniques can be used for determination of the test article in the dialysate samples (liquid chromatography-tandem mass spectrometry /LC-MS-MS/, high pressure liquid chromatography /HPLC/ with fluorescent or electrochemical detection etc.) and do not need radioactive labeling. (2) The probe implantation into the target organ is a minimally invasive process (depending on the target tissue) contrary to PET which is a non-invasive technology. (3)The route of administration of the test article is intravenous in PET studies, but can be oral, subcutaneous, topical, inhalation or any other mode at MD. (4) PET imaging determines the total drug levels (unbound and protein bound and also metabolites) while MD measures only the unbound drug levels. (5) Temporal and spatial resolutions of PET are higher than that of MD. (6) Sensitivity of PET is about 10^{-12} mol/L while that of MD 10^{-9} -10^{-3} mol/L. (7) The costs of PET are much higher than that of MD [55].

MRI uses radio - frequency pulses and magnetic fields to obtain signals from changes in nuclear magnetic moments. A technique based on the same principle as MRI, but providing a greater degree of molecular characterization, is MRS, in which spectroscopic profiles of the chemical constituents within a sample are obtained. MRS measurements can be performed serially, thus making possible PK analysis with a temporal resolution on the order of minutes. Of importance, MRS is capable of resolving different chemical species, including metabolites, owing to different chemical shifts of the resonance signals [57].

MRS has proven to be particularly feasible for fluorinated drugs, since ^{19}F is one of the lead isotopes for nuclear MRS, and several studies have been published describing brain PK of fluorinated psychiatric medications [57], tumor uptake of anticancer chemotherapeutics, and biodistribution and target tissue PK of fluorinated antibiotics [57] . Recently, the use of ^{19}F MRS was validated to quantify the experimental antihistamine tecastemizole in heart and liver.

MRS has similar advantages to PET versus MD. However, MRS has worse spatial resolution than PET. On the other hand MRS can resolve metabolites and bound or unbound drugs, due to chemical shift differences, while PET cannot provide chemical resolution.

Several imaging techniques are currently available for assessing drug distribution and tissue pharmacokinetics in humans. Each of these techniques has proven its ability to provide new information on drug distribution for compounds, already marketed and potentially, each technique can provide valuable information during drug research and development. The choice of technique or the complementary combination should be based on the compound of interest, the region of the body, where distribution and tissue concentrations should be monitored, and the availability of technical and financial resources [57,58].

Open flow microperfusion (OFM) is an alternative *in vivo* sampling technique that builds on the strengths of continuous, minimally invasive interstitial sampling methods such as MD. But instead of a membrane, OFM uses probes with macroscopic openings to exchange substances in a liquid pathway. Membrane-based sampling systems, like MD, encounter problems when sampling high molecular weight or highly lipophilic substances in the interstitial fluid. OFM overcomes these problems by replacing the membrane with a steel mesh featuring macroscopic openings in combination with a peristaltic OFM pump in push/pull mode to achieve stable recovery of OFM samples [59]. Current applications in adipose subcutaneous tissue (aOFM) and dermal tissue (dOFM) range from preclinical studies to clinical trials [60], and cover a wide range of substances from small ions to lipophilic topical drugs or to large antibodies. The latest development in OFM has been designed for use in cerebral tissue (cOFM).

The key features of OFM and MD show their advantages and limitations and different applications. (1) OFM is a membrane free method, which means that there is no cut off value in the molecular weight of the analytes contrary to MD where the cut off value is 100 kDa. (2) At OFM technique the samples are unfiltered containing small and large molecules and that's why a sample preparation before bioanalysis is necessary. In case of MD technique the samples are filtered and clean (practically protein free), containing small molecules and these samples do not require preparation before bioanalysis. (3) At OFM there is no to low adsorption to the tubings due to direct coupling of sampling vial to OFM probe. While at MD there can be a problem of adsorption of the molecules to the outlet tubings of the MD setup.

Future OFM applications will include preclinical trials with improved anesthesia to prolong experiment time from 8 to 12–14 hours. The use of the OFM system in awake animals will allow a trial time of several weeks, and enables a combination of OFM with behavioral experiments. Furthermore, OFM systems will be tested for use in knock-out mice to study a wide variety of diseases and wound healing.

Over the above mentioned complementary technologies someone can ask that what the strength of MD contrary to traditional blood collection for PK studies is. Traditional pharmacokinetic studies and bioavailability assessment are critical in early phases of drug research and development. A potential drawback of the current approach of

relating measurement of plasma concentration is inadequate prediction of tissue drug concentrations leading to poor therapeutic intervention and/or drug toxicity. Two examples for poor therapeutic efficacy and toxicity are the observation of sub-therapeutic dose-response for anti-infective drugs and the toxic side effects of anticancer drugs due to poor drug penetration to the site of action [3]. MD has been used to measure the pharmacologically active, in vivo tissue concentrations of endogenous compounds and to assess drug concentrations closest to the site of action in various human tissues in both healthy volunteers and patients. Consequently, MD is gaining recognition as a tool in drug research and development to select appropriate compounds and to optimize dosing regimens [3].

The main properties of MD techniques, PET, OFM and traditional pharmacokinetic blood sampling are compared in Table **5**.

Future Directions

One of the new fields of application of MD techniques is the comparison of protein profiles of extracellular fluids in healthy and diseased subjects. Dayon and coworkers studied [61] the proteins upregulated in stroke in a clinical study. The changes in protein levels associated with ischemic damages were analyzed in microdialysates from the infarct core (IC), the penumbra (P), and the unaffected contralateral (CT) brain regions of patients suffering an ischemic stroke. A shotgun proteomic approach based on isobaric tagging and mass spectrometry was used.

Maurer and coworkers suggested the usefulness of cerebral microdialysate proteomics by comparing microdialysate samples of patients with subarachnoid hemorrhage (SAH) [62]. A major complication of SAH is vasospasm, a narrowing of cerebral blood vessels followed by a regional impairment of blood flow. In a subset of patients, this complication may result in secondary ischemia and is associated with high mortality and unfavorable outcome in those who survive. Maurer and coworkers identified differential proteomic changes in the cerebral microdialysate of SAH patients who later developed cerebral vasospasm compared to SAH patients unaffected by the complication. The current proteomic approach including sample preparation, protein separation, and protein identification by MS (MALDI-TOF) does not provide timely results. Therefore, the proteomic approach may deliver important post-hoc results, but it may not be feasible as a fast diagnostic tool for patients on intensive care units [63].

Another pathology where MD combined with proteomics/metabolomics approach was applied is wound healing. Wound healing of soft tissue and bone defects is a complex process in which cellular differentiation and adaption are regulated by internal and external factors, among them are many different proteins. Kalkhof and coworkers described an approach to sample metabolites by MD and to extract proteins simultaneously by adsorption. With this approach it is possible (i) to collect, enrich, and purify proteins for a comprehensive proteome analysis; (ii) to detect more than 600 proteins in different defects including more than 100 secreted proteins [64]. Many proteins have previously been demonstrated to have diagnostic or predictive power for the wound healing state.

Among the future application trends of MD it is important to mention the topical dermal MD. The crucial step during topical drug therapy is the ability of the free active drug to penetrate the skin in sufficiently high amounts to produce its clinical effect. Regulatory authorities have recognized dermal MD as a potential tool for bioequivalence evaluation of topical dermatological dosage forms during generic drug development [65,66].

MD data are likely to become an important part of new drug submissions, and thus may potentially contribute to the Food and Drug Administration (FDA) Critical Path Initiative to facilitate innovation in drug development [3].

Discussion and Conclusion

MD techniques are presented in this review as constituents of the toolbox of drug research, diagnosis and therapy with preclinical and clinical approaches. Multiple application possibilities may assure the survival, development and refinement of the method. Besides taking further the traditional applications, the investigation of new targets (organs, tissues, disorders) meet the focus of *in vivo* MD in the last decades. The field of drug-drug interactions and proteomic approaches of the disorders are also new areas for the use of MD. Selection of a method for testing tissue distribution or organ specific biochemical changes requires careful consideration of its benefits and shortcomings and a comparison of the assay properties with the complementary technologies. This article makes a comparison between non-invasive imaging, and membrane free open flow microperfusion techniques and MD. MD is the first choice for assessment of neurochemical actions of centrally acting new chemical entities. In addition, as MD is able to simultaneously sample target-site concentrations of new molecules and endogenous biochemical markers with high temporal

Properties	PET	OFM	Traditional PK from blood	Microdialysis
measured drug concentration	total (free, protein bound drug and metabolites) concentrations	total (free and protein bound drug) concentrations	total (free and protein bound drug) concentrations	Only free (active) drug concentrations
invasiveness	non-invasive	minimally-invasive	non-invasive	minimally-invasive
necessity of radiolabeling	labelled drugs needed	no need of labeling	no need of labeling	no need of labeling
drug administration	intravenous	any route	any route	any route
size of the molecules measured	no cut off value	no cut off value	no cut off value	membrane dependent cut off value (<100 kDa)
sample preparation for bioanalysis	no need (no sample)	needed (unfiltered samples)	needed (whole blood)	no need (filtered samples)
costs	high	low	low	low
surgical skills	no need	needed	no need	needed
organ specificity	yes	yes	no, systemic blood	yes
number of subjects (animals or human) used	low	low	high	low
time frame	hours	hours to days	only a very limited number of sampling pro animal	hours to days

Table 5: Comparison of the features of microdialysis techniques with the main complementary technologies and traditional pharmacokinetic blood sampling for the measurement of drug levels in drug research and development studies.

resolution, this technique makes possible the design of mechanism-based PK/PD models of lead compounds. MD can reduce the cost of early drug development because the number of the animals needed is low and the information harvested from one animal is quite wide-ranging. Acceptance of MD data as a part of preclinical and clinical pharmacology packages of drug development by regulatory agencies is actually increasing. It is concluded that relatively low costs and good benefits/limitations ratio makes MD a tool of wide perspective in drug research/development, diagnosis and therapy.

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