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Application of microdialysis for pharmacokinetic–pharmacodynamic modelling

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Pharmacokinetic–pharmacodynamic (PK–PD) modelling describes the relationship between the pharmacokinetics and pharmacodynamics of a drug allowing the prediction of clinically relevant parameters. PK–PD modelling has several advantages over classical dose–response studies because it allows a better pharmacodynamic characterisation of drugs and screening of dosage–regimen. However, PK–PD studies are limited by the need for simultaneous measurement of drug tissue levels and corresponding pharmacological effects at multiple time points. The microdialysis technique is a unique research tool that allows the simultaneous determination of unbound concentrations of drugs at several tissues and its action on biochemical and clinical markers during several hours and days. Therefore, microdialysis sampling is an attractive methodology for PK–PD studies. The aim of this review is to describe the applicability of the microdialysis technique for PK–PD modelling of therapeutic agents, including the description of PK–PD modelling concepts, an overview of the microdialysis technique and the analysis of PK–PD studies using microdialysis sampling both in the preclinical and clinical setting.

Keywords:drug development, microdialysis, pharmacokinetics, pharmacokinetic–pharmacodynamic modelling, pharmacodynamics

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1. Introduction

The demand for efficacious pharmacological agents is increasing worldwide due to higher lifestyle expectations and changes in demographic profiles [101]. Moreover, the discovery of a large number of orphan receptors will increase the number of new therapeutic agents to be tested in the future [1]. Therefore, a more rigorous selection process is needed at early stages of drug development to select promising compounds. Integration of pharmacokinetic–pharmacodynamic (PK–PD) concepts through PK–PD modelling is a potential tool to enhance the information gain and the efficiency of drug selection during development [2,3].

The discovery of pharmacological properties of drugs includes both preclinical and clinical phases of drug development, which are designed to accrue the necessary information for assessing the therapeutic potential of a pharmacological agent (**Table 1**). Although each phase of drug development has different objectives, the pharmacokinetic and pharmacodynamic behaviour of drugs is evaluated in all phases by means of the assessment of pharmacokinetic parameters and the characterisation of the dose (concentration)–effect relationship (**Table 1**).

Two different approaches exist for characterising the dose (concentration)–effect relationship of a drug; namely, the classical dose–response trial and PK–PD modelling [4]. PK–PD modelling describes the relationship between the pharmacokinetics and pharmacodynamics of a drug allowing the estimation of PK–PD parameters and the prediction of clinical relevant parameters. PK–PD modelling has several advantages over dose–response studies. It allows not only better pharmacodynamic

Table 1. Application of pharmacokinetic–pharmacodynamic modelling during drug development.

Stage of drug development	Objectives	Benefits of pharmacodynamic–pharmacokinetic modelling
Preclinical	Demonstration of pharmacological activity in experimental animal models of disease Toxicology studies to define initial dosing in Phase I	Precise definition of the dose–concentration–pharmacological effects and dose–concentration–toxicity relationship Determination of the appropriate dosing regimen for Phase I studies Identification of biomarkers and animal models for efficacy and toxicity Explore any dissociation between plasma concentration and duration and onset of pharmacological effect Provide information on drug effects that would be difficult to obtain in human subjects
Phase I	Assessment of limit tolerable dose Study of pharmacokinetic and pharmacodynamic behaviour	Understanding the dose-concentration–pharmacological effects and dose–concentration–toxicity relationship in healthy volunteers. Characterization of pharmacokinetics and pharmacodynamics in special population Study of tolerance development Determination of the dosing regimens for Phase II studies.
Phase IIA	Study of efficacy in the intended population	Confirms and explores the relationship between dose–concentration–effect in patients. Examines a variety of therapeutic endpoints to understand the most adequate for further modelling.
Phase IIB	Optimal use in target population	Determination of the dosing regimens for Phase III studies Predicts the probability distribution of further clinical trial outcomes
Phase III	Demonstrate safety and efficacy for clinical use	Assessment of phatmacokinetic and pharmacodynamic changes or relationship in the patients population

characterisation of drugs, but also permits screening and dosage–regimen selection [4]. The potential applications of PK–PD modelling during preclinical and clinical drug development are summarised in **Table 1**.

One disadvantage of PK–PD modelling is the necessity of simultaneous measurement of drug tissue levels and its corresponding pharmacological effect at multiple time points [4]. Blood sampling (which has traditionally been used for this purpose) has the disadvantages that the removal of samples may interfere with PK and PD drug behaviour, especially in preclinical studies with small animals [5]. Furthermore, traditional sampling techniques allow the measurement of plasma concentrations of pharmacological agents rather than drug levels in the target tissue. These limitations could be resolved by the application of new sampling techniques, such as *in vivo* microdialysis.

The development of microdialysis for measuring drug concentrations was initiated during the late 1980s [6-8]. This technique provides a method for continuous drug sampling in different tissues without repeated tissue sampling. Its applicability to the study of drug metabolism and pharmacokinetics has been widely demonstrated [5,9]. The possibility of microdialysis sampling without tissue loss makes this technique useful for PK–PD correlation studies [10].

This review describes the microdialysis technique applicability for PK–PD modelling of therapeutic agents, including the description of PK–PD modelling concepts, an overview of the microdialysis technique and an analysis of PK–PD studies using microdialysis sampling both, in the preclinical and clinical setting.

2. Principles of pharmacokinetic–pharmacodynamic modelling

The principle of PK–PD modelling is described in **Figure 1**. PK–PD relationships build a bridge between the time course of drug concentrations in the organism, as assessed by pharmacokinetics, and the intensity of the pharmacological response, as quantified by pharmacodynamics. The link between the PK and PD of a drug is established by the use of mathematical models, allowing the estimation of parameters, such as effective concentration to yield half-maximal response (EC_{50}) and maximal efficacy (E_{max}). PK–PD modelling also provides information about the onset, magnitude and duration of the therapeutic effect [11].

PK–PD modelling requires the simultaneous measurement of drug tissue levels and its corresponding pharmacological effects at multiple time points. Measurements of the active compound should be performed with fully validated analytical methods [12-14]. Although concentrations of the therapeutic agent should be measured at the target site, in most situations this is not possible and plasma sampling is the only alternative [15]. Moreover, an accurate measurement of the intensity of the pharmacological effect of the active compounds is necessary for a PK–PD modelling design.

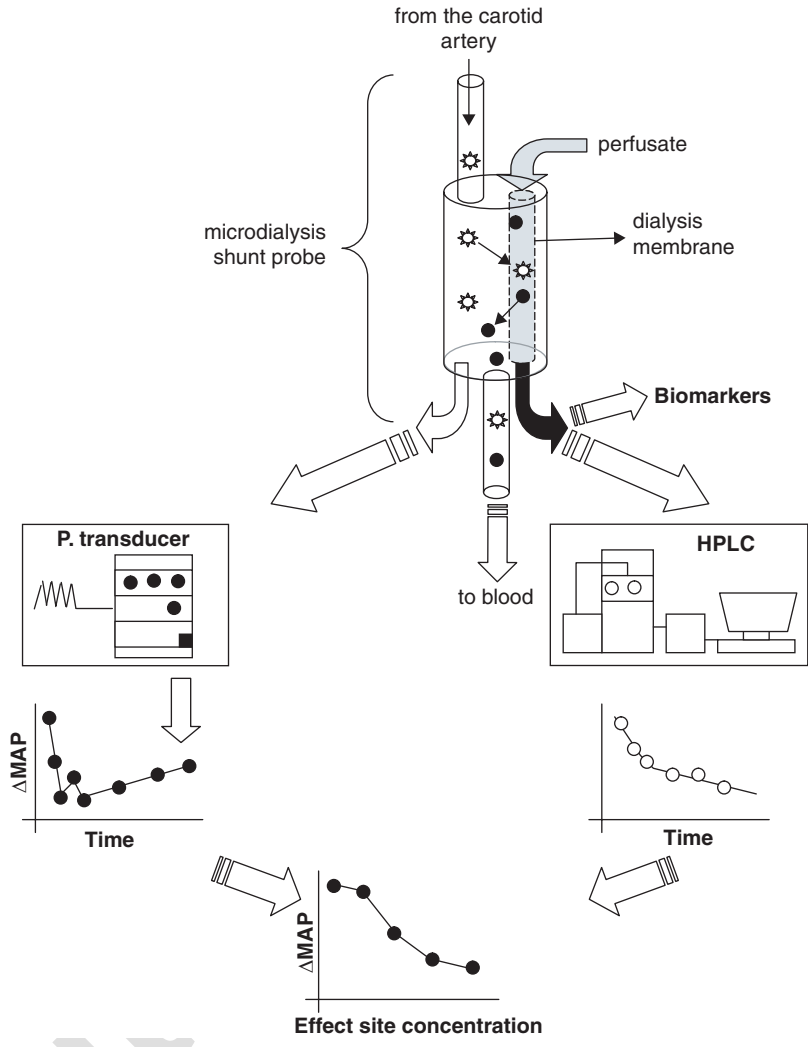

Figure 1. **Representation of the applicability of microdialysis for pharmacokinetic–pharmacodynamic modelling.** A shunt microdialysis probe is inserted in the carotid artery and perfused with a Ringer solution. The media is pumped through the inner cannula, allowing the diffusion of compounds in both directions. Dialysate levels of antihypertensive drugs are determined by liquid chromatography obtaining plasma unbound concentrations as a function of the time. Simultaneously, biochemical markers can also be monitored in the dialysate. In addition, an outlet of the microdialysis probe is connected to a pressure transducer, allowing the determination of the blood pressure effect of an antihypertensive drug as a function of the time. Finally, the relationship between the plasma concentration of the antihypertensive and its effect on blood pressure is determined by means of pharmacokinetic–pharnacodynamic modelling.

Measurement of the effect should meet validation parameters, including continuity, sensitivity, objectivity and repeatability [15].

To obtain the greatest precision in estimating PK–PD relationships, the number of measurements of drug tissue levels and its corresponding effect must be as large as possible [15]. However, multiple time-point sampling is often not possible in the clinical setting. To overcome this limitation, population PK–PD modelling is increasingly introduced.

PK–PD relationships have been described using diverse mathematical models depending on the nature of drug administration, the magnitude of the pharmacological effect and the time dependency of the pharmacodynamics of the tested drug [3,16]. Relatively simple PK–PD models are needed to describe PK–PD relationships after multiple doses or long-term infusion, because the system is kinetically at steady state [16]. The most common mathematical equations employed in steady-state conditions are the linear, log linear and E_{max} models. Although the linear and log linear model allow an easy parameter estimation, these models erroneously assume that the effect can increase with concentrations without limits [15].

Therefore, the E_{max} model is the most broadly applied to characterise a myriad of pharmacological effects. This model derives from the classical theory of drug–receptor interaction, relating the effect to drug concentrations, as in Equation 1:

$$E = E_O + \frac{E_{max} * C}{EC_{50} + C} \quad (1)$$

where E_{MAX} is the maximal effect, E_0 the baseline value and EC_{50} the effective concentration yielding half-maximal response [15].

More complex PK–PD models are needed to describe the relationship between PK and PD after single-dose administration or when time dependency in the pharmacodynamics of the drug is present [16].

Plotting drug effects as a function of drug concentrations and connecting data in a chronological order allows the determination of possible delays in the drug response [4]. A hysteresis loop appears in the plotting when the magnitude of an effect corresponds to more than one drug concentration. Therefore, an anticlockwise hysteresis loop could be explained by the disequilibrium between biophase and plasma compartment [17], appearance of active metabolites [18] or indirect mechanism of action [19]. On the other hand, tolerance in the pharmacological effect could be suggested if a clockwise hysteresis loop is observed [20].

In these cases, plasma concentrations cannot be directly linked to drug effect and more complex PK–PD models such as an effect compartment model and a physiological indirect response model, are needed.

The most applied PK–PD model is the effect compartment model that considers a hypothetical effect compartment as an additional compartment of a pharmacokinetic compartment model, representing the drug concentration at the effect site [15]. The time-dependent aspects of the equilibrium between plasma concentration and the effect are characterised by the first-order rate constant, K_{e0}, which represents the irreversible disappearance of the drug from the effect compartment [15]. The time course of drug concentration in the effect compartment is described by Equation 2:

$$(\Delta CE)/(\Delta t) = K_{e0} * (Cp - Ce) \quad (2)$$

where Ce and Cp represent the concentration in the effect compartment and plasma, respectively [15]. This approach has been successfully applied to predict the PK–PD relationship of diverse drugs [17,21-23].

In PK–PD modelling studies the complete pharmacodynamic range of a drug should be covered after a single administration [4]. Often, in clinical pharmacology, it is not possible to determine the maximal effect of a drug because of the appearance of adverse reactions, in which case an alternative PK–PD model designed by Schoemaker *et al.* must be applied [24]. In their model, the authors introduced a new parameter (S_0) equal to E_{max}/EC_{50} in the E_{max} Equation which represents the initial sensitivity to the drug. This model allows an accurate estimation of potency and maximal effect without attaining the maximal pharmacological response during the PK–PD experiment.

Considering that the selection of an inadequate PK–PD model according to the PK–PD study characteristics might lead to an erroneous interpretation, it is extremely important to determine which PK–PD model is going to be applied for the analysis of the data.

When selecting the PK–PD model, the investigator must keep in mind experimental variables, including type of drug administration, type of pharmacological effect measured, the existence of time dependency in the pharmacological effect of a drug and the possibility to reach maximal response in their experimental design.

3. Principles of the microdialysis technique for PK–PD modelling

PK–PD modelling needs the determination of drug tissue concentration and corresponding pharmacological effect data at multiple time points. Different techniques, such as

biopsies, saliva, blood sampling, microdialysis and imaging techniques, are available for the study of PK properties of drugs [25]. However, microdialysis seems to be the most adequate methodology for the study of PK–PD relationships [25].

In the past 20 years, the microdialysis technique has become a method of choice in the tissue concentration studies of both endogenous and exogenous substances [26]. In this technique, a probe inserted into a tissue mimics a capillary blood vessel (**Figure 1**). The probe has a dialysis membrane, usually with a 20-kDa molecular weight cutoff, that is permeable to water and small molecules. When the microdialysis probe is implanted, a perfusion fluid enters into 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 [26]. 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 [26]. Thus, microdialysis can be used for both collecting a substance in the dialysate as well as delivering it into the periprobe fluid [27]. Finally, the collected dialysate samples are analysed using highly sensitive techniques, such as liquid chromatography and capillary electrophoresis (**Figure 1**).

As endogenous compounds and xenobiotics diffuse simultaneously through the dialysis membrane, microdialysis sampling not only allows the study of tissue drug concentrations, but also the effects of drugs on endogenous compounds, such as neurotransmitters, metabolites and peptides [28].

The microdialysis technique allows continuous tissue sampling without removing liquid and, therefore, a higher temporal resolution can be achieved than with traditional techniques without interfering with the pharmacokinetic and pharmacodynamic behaviour of the drug. An ethical advantage is that 5- to 10- times fewer animal experiments have to be performed to determine the time profile of a drug [26].

In microdialysis experiments with conventional dialysis membranes, large molecules are precluded to diffuse through the dialysis probe. Therefore, microdialysis samples the bioactive concentration of drugs, because only the unbound fraction diffuses through the dialysis membrane. Traditional techniques for pharmacokinetic sampling, including measurement of drugs in blood samples and biopsies, are limited by the fact that they do not discriminate between free concentration and drug bound to cell components and proteins [26].

Moreover, the microdialysis technique provides protein-free samples that allows the sample analysis to be performed directly online without any pretreatment of samples. Traditional blood sampling requires clean-up procedures prior to analysis, with the possibility of losing analytes during protein precipitation and the need of an internal standard for an accurate drug determination [29].

Nevertheless, if the protein binding of the drug is high, only a very small amount of drug is available for analysis, which thus requires highly sensitive analytical methods.

Microdialysis probes can differ in their shape and material, depending on the tissue to be sampled in animals or humans [30-34]. The different geometry of microdialysis probes enables their use in virtually any tissue and fluid of the body [30], allowing concentrations of therapeutic drugs to be monitored at the target site. In basic research, drug levels in tissues, such as blood, bile, central nervous system (CNS), adipose tissue, skeletal muscle, heart, liver and tumours have been monitored by means of microdialysis [26]. In a clinical setting, microdialysis has also been used in other tissues, such as lung, tendons, bone, peritoneal cavity and infective tissue [34]. However, clinical microdialysis in brain, liver and tumours is not easy to perform because of ethical concerns and the requirement of special settings; for example, during surgery.

In addition, the placement of multiple microdialysis probes in different tissues allows the monitoring of the drug time course in different organs in the same animal, supplying information about the distribution process of xenobiotics [26].

Another important issue is that microdialysis is not performed under equilibrium conditions because the perfusate is constantly being pumped through the probe and, therefore, the concentration of the drug in the dialysate is a fraction of that in the surrounding tissue. To obtain tissue concentrations, the factor by which dialysate concentrations are interrelated needs to be determined. This factor, called relative recovery, can be obtained by *in vivo* or *in vitro* calibration procedures [26]. 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 the reduced capacity of compounds to diffuse through the extracellular space surrounding the membrane when compared with diffusing capacity in an aqueous solution [35]. The microdialysis probe can be calibrated *in vivo* through different methods: the flow-rate or stop-flow method [36], the zeronet-flux [37] and the retrodialysis method [38]. Because the zero-net-flux 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 their application for pharmacokinetic purposes.

In the retrodialysis method, 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 loss 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 [26].

Contrary to pharmacokinetic studies, accurate calibration of the microdialysis probe is not necessary for endogenous compounds because the desirable information is the relative change in its concentration induced by the drug

Table 2. Summary of advantages and limitations of microdialysis sampling technique for pharmacokinetic–pharmacodynamic modelling.

Advantages	Limitations
Determination of the bioactive concentration of the drug in the biophase	Semi-invasiveness technique
Good time and spatial resolution compared with other sampling techniques	Diluting effect of the microdialysis procedure
Protein free samples	Need of highly sensitive analytical methods
No further enzymatic degradation of the drug	*In vivo* calibration of the microdialysis probe during the experiment
Online coupling of analytical determination	Sticking of lipophilic drugs to tubing and probe components
No fluid loss	Low recovery of large molecules
Simultaneous collection of endogenous compounds	
Monitoring of drug concentrations in different tissues by multiprobe microdialysis. Simultaneous determination of clinical markers (blood pressure, heart rate, electroencephalogram, inflamatory response)	

administration. Only the concentration independence and stability of the recovery need to be determined by *in vitro* calibration of the microdialysis probe [26].

A minimal lesion of the tissue surrounding the probe is produced by implantation of the microdialysis probe, causing tissue responses such as a compromise of the blood–brain barrier [39,40] and acute inflammation in different tissues [41-43]. However, several studies have demonstrated that baseline conditions are reached following a period of ~ 60 min after probe implantation. Once basal concentrations of endogenous compounds have become 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) can be calculated as a percentage of the basal mean [26].

In conclusion, the vast applicability of microdialysis for PK–PD modelling is supported by the fact that this technique allows the simultaneous determination of drug concentrations in one or more tissues and its effect on biochemical and clinical markers in the same animal with high temporal resolution. **Table 2** summarises the principal advantages and drawbacks of the microdialysis technique for PK–PD studies.

4. Application of microdialysis for pharmacokinetic–pharmacodynamic modelling

Microdialysis sampling has been used for PK–PD studies of therapeutic agents in both preclinical and clinical studies. Some important examples are discussed in Section 4.1. **Table 3** shows an overview of PK–PD studies by means of the microdialysis technique.

4.1 Antihypertensive drugs

With regard to the blood pressure effect of antihypertensive drugs, a poor concentration–response relationship has been found. The suggestion that there is no relationship between plasma levels of antihypertensive drugs and its effect on blood pressure reflects an inadequacy or failure in the approaches designed to detect such correlation. A number of factors have hampered the possible identification of a correlation, including failure to study individual patients, inability to collect sufficient pharmacodynamic data, failure to identify and account for temporal delay in the onset of the pharmacological effect, the use of restricted concentration ranges and the use of dose rather than concentration [44,45].

Using a 'shunt' intra-arterial microdialysis probe (**Figure 1**), a good relationship was found between metoprolol concentration in the effect compartment and its hypotensive and chronotropic effect [10,46,47]. Moreover, the maximal response was significantly greater in hypertensive animals, such as spontaneously hypertensive rats and animals with aortic coarctation, with regards to its respective control animals. Therefore, this data suggests that the proposed lack of relationship between plasma levels of β-blockers and its antihypertensive effect is probably a consequence of an inadequate experimental design and data analysis.

4.2 Anti-infective drugs

Traditionally, pharmacokinetic assessment of antimicrobial agents was based on measuring of 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, measurement of unbound drug concentrations in the interstitial fluid of the target tissue should be considered as a gold standard for improvement of antimicrobial therapy [48,49].

Several techniques, such as skin blisters, saliva sampling, microdialysis and imaging techniques, have been used to monitor free drug concentrations in interstitial fluid in human studies [49]. However, skin blisters and saliva have been shown to be poor surrogates for interstitial fluid and imaging techniques cannot discern different compartment. Microdialysis sampling does not deal with these limitations and seems to be the most adequate technique for the study of tissue concentrations of antimicrobial agents [49].

The applicability of microdialysis for the study of pharmacokinetic properties of antimicrobial drugs has been previously reviewed [50]. Microdialysis has been used to measure various antimicrobial agents, including aminoglycosides, penicillins, cephalosporines, fosfomycin, fluoroquinolones, metronidazole and antiviral agents in healthy volunteers and patients with sepsis [34,50,51]. These studies have served to develop *in vivo* PK– *in vitro* PD models in the target site using the 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 (C_{max}) to the MIC (C_{max}/MIC), and the area under the inhibitory curve or the area under the curve (AUC)/MIC ratio [51].

A three-step approach has been used for the *in vivo* PK–*in vitro* PD modelling by means of microdialysis. First, interstitial fluid concentrations of the antibacterial drug at the target site are measured by means of microdialysis. Second, time versus drug concentration profile measured *in vivo* is simulated in an *in vitro* setting on bacterial cultures. In a third step, unbound antibiotic concentrations are linked to bacterial kill rates by means of PK–PD models [52].

Delacher *et al.* [52] have demonstrated a significant correlation between the maximal bactericidal effect and several pharmacokinetic surrogate parameters, including AUC/MIC, C_{max}/MIC and T > MIC. The authors concluded that the therapeutic success or failure in antibacterial therapy depend on the target site concentrations of the antimicrobial agent. Moreover, *in vivo* PK–*in vitro* PD modelling provides valuable guidance for drug and dose selection of antibacterial drugs [52].

In vivo PK–in vitro PD modelling of antimicrobial drugs was also studied in critically ill patients by means of microdialysis. Zeitlinger *et al.* [53] have applied an *in vivo* PK–*in vitro* PD method to simulate bacterial killing in plasma and the interstitium of skeletal muscle tissue after intravenous administration of cefpirome and fosfomycin alone and in combination to patients with sepsis. The *in vitro* simulation of *in vivo* plasma and tissue pharmacokinetics of cefpirome and fosfomycin has shown that both antimicrobial agents kill *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains after single-dose administration, observing a synergic antimicrobial effect by the combined use. Therefore, this data confirms antimicrobial strategies of simultaneous administration of cefpirome and fosfomycin in patients with severe soft tissue infection [53].

In another study, penetration of cefpirome into soft tissues was evaluated by means of microdialysis [54]. To assess the antibacterial effect of cefpirome at the target site, the measured pharmacokinetic profiles were simulated *in vitro* with select strains of *S. aureus* and *P. aeruginosa*. Although tissue penetration of cefpirome was significantly impaired in septic patients compared with healthy subjects, effective bacterial growth inhibition was observed in all *in vitro* simulations because of the prolonged half-life of cefpirome in tissue. Therefore, the authors concluded that cefpirome is an appropriate agent for the treatment of soft tissue infections in septic patients. However, due to the high interindividual variability of the pharmacokinetics of cefpirome in tissue, dosing intervals no longer than 8 h should be preferred to ensure that susceptible bacterial strains are killed in each patient [54].

However, it needs to stressed that all PK–PD studies of antimicrobial drugs by means of microdialysis have used a combined *in vivo* PK–*in vitro* PD simulation without applying mathematical PK–PD models in their analysis. Recently, Liu *et al.* [55] demonstrated that a PK–PD model based on unbound antibiotic concentrations at the site of infection, and a sigmoid E_{max} relationship, effectively described the antimicrobial efficacy of both cefpodoxime and cefixime. This approach offers a more detailed information than the MIC does about the time course of antibacterial efficacy of antibiotics [55].

4.3 Antineoplasic drugs

So far, microdialysis has been extensively used for the study of tumoural concentrations of antineoplasic drugs in both animals and humans [34,56,57]. Tumour drug exposure, a marker linked to clinical outcomes, may be dramatically reduced due to diffusion barriers in solid tumours [56]. Therefore, plasma anticancer drug profiles are frequently inappropriate for predicting outcome in oncology. Thus, microdialysis appeared as a valuable minimally invasive tool that allows *in vivo* investigations [57].

More recently, microdialysis has been used to assess the pharmacodynamics of chemotherapeutic agents [57]. Castejon *et al.* [58] have determined plasma concentrations of serotonin and 5-hydroxyindoleacetic acid during cisplatin treatment by means of microdialysis. Microdialysis has also been used for the monitoring of extracellular levels of growth factors, such as the vascular endothelial growth factor during treatment with tamoxifen in a mouse model of human breast cancer [59].

Therefore, PK–PD modelling studies applying microdialysis allow the integration of the pharmacological response with tumour PK profiles of the corresponding drug helping to define PK–PD relationship, which is essential for rational design of drug administration regimens in cancer patients.

Table 3. Examples of recently published PK–PD studies by means of microdialysis sampling.

Therapeutic use	Drug	Experimental subject	PK input	PD input	PK-PD model	Ref.
Antimicrobial agents	Cefpirome	patients with sepsis	Subcutaneous adipose tissue concentrations	I*n vitro* susceptibility test to *Staphylococcus aureus* and *Pseudomonas aeruginosa*	*In vivo* PK–*in vitro* PD	[54]
	Cefpirome and fosfomycin	patients with sepsis	Interstitial muscle concentrations	*In vitro* susceptibility test to *S aureus* and *P aeruginosa*	*In vivo* PK–*in vitro* PD	[53]
	Ciprofloxacin	healthy volunteers	Subcutaneous adipose tissue concentrations	*In vitro* susceptibility test to *Pseudomonas aeruginosa*	*In vivo* PK–*in vitro* PD	[53,67]
	Fosfomycin	healthy volunteers	Interstitial muscle concentrations	*In vitro* susceptibility test to *Staphylococcus aureus*	*In vivo* PK–*in vitro* PD	[68]
	Levofloxacin	Patients with sepsis	Interstitial muscle concentrations	I*n vitro* susceptibility test to *Staphylococcus aureus*	*In vivo* PK–*in vitro* PD	[69]
	Norfloxacin	Rat	Blood and hippocampal concentrations	Quantitative EEG recording	Effect compartment	[65]
Antineoplasic drugs	5-fluorouracil, methotrexate	Patients with breast cancer	Interstitial tumor concentrations	*In vitro* tumour response	*In vivo* PK– i*n vitro* PD	[60]
Cardiovascular drugs	Metoprolol	Rat	Arterial blood concentrations	Hypotensive and chronotropic effect	Effect compartment	[10,46,47]
Neuromuscular blockers	Rocuronium	Dog	Interstitial muscle concentrations	Neuromuscular function	Effect compartment	[66]
Psychomimetic drugs	Morphine-6-g lucuronide	Rat	Brain and blood concentrations	Antinoceptive effect	Effect compartment	[62]
	Psychomimeti c drugs	Rat	Blood, striatal and prefrontal cortex concentrations	Dopamine concentrations in striatum and prefrontal cortex	No PK–PD model applied	[63]
Others	L-arginine	Rat	Blood and hippocampal concentrations	Hippocampal nitric oxide concentration	Comprehensive PK–PD model	[70]

EEG: Electroencephalogram; PD: Pharmacodynamic: PK; Pharmacodynamic.

To the best of the authors knowledge, only one PK–PD study for anticancer drugs has applied microdialysis sampling. Müller *et al.* [60] have determined the unbound, interstitial drug pharmacokinetics of 5-fluorouracil and methotrexate in solid tumour lesions of patients by means of *in vivo* microdialysis. The authors then made a pharmacodynamic simulation of the time versus drug concentration profile in an *in vitro* setting by exposing breast cancer cells to interstitial tumour concentration of the antineoplasic drugs. They concluded that their *in vivo* PK–*in vitro* PD model might provide a rational approach for describing and predicting pharmacodynamics of cytotoxic drugs at the target site [60].

Some factors limit the use of microdialysis in cancer research. Microdialysis in cancer patients must be conducted in strict compliance with regulatory demands and needs to be based on appropriate ethical conditions. Furthermore, puncture of solid tumours by microdialysis catheter implantation may induce metastasis [61]. However, a recent study estimated that the incidence of metastasis caused by puncture was in the range of 0.003 – 0.005%, and there is no evidence that puncture of tumour lesions affected the course or prognosis of

the underlying disease [61]. Another limitation 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, some antineoplasic drugs, such as 5-fluorouracil, require intracellular enzymatic conversion to exert its cytotoxic activity. In addition, other aspects such as tumour 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 use of microdialysis for studies of antineoplasic drug distribution [34].

4.4 Pharmacokinetic–Pharmacodynamic modelling of effects on the CNS

Microdialysis has been extensively used for neurochemical studies in laboratory animals, especially in the rat [26]. Recently, PK–PD studies evaluating effects of different therapeutic agents at the CNS have been made (**Table 3**).

PK–PD modelling by means of microdialysis allows the study of the mechanism responsible for the time delay of central actions of drugs. In an elegant study, Bouw *et al.* [62] have determined simultaneously blood and brain concentrations of morphine-6-glucuronide and its antinociceptive effect by means of microdialysis sampling. By applying a PK–PD model with an effect compartment, the authors found a greater delay in the onset of the effect when antinociceptation was related to plasma morphine-6-glucuronide concentrations with regard to brain levels. Therefore, it was concluded that half of the effect delay could be explained by transport across the blood–brain barrier, suggesting that the remaining delay is a result of drug distribution in the brain parenchyma [62].

PK–PD modelling was also used to describe the effect of psychomimetic drugs on dopaminergic activity at different nuclei of the CNS [63,64]. The effect of benzatropine analogues on dopamine concentration in the nucleus accumbens after its intravenous administration was evaluated [64]. The authors fitted plasma concentration of the analogues and its effect on extracellular dopamine levels to two different PK–PD models, such as an effect compartment model and a model with indirect physiological response. The authors demonstrated that the indirect model is more suitable for PK–PD modelling of benzatropine analogues than the linked PK–PD model. These results are in accordance with the mechanism of action of the analogues because these drugs bind to the dopamine transporter inhibiting the dopamine re-uptake and consequently elevate dopamine extracellular levels [64].

Microdialysis was used to describe the relationship between norfloxacin concentrations in the CNS and its adverse reactions, such as convulsive effect [65]. Brain extracellular concentrations of norfloxacin by means of microdialysis and a quantitative electroencephalogram (EEG) were simultaneously determined. Blood samples were also collected to determine norfloxacin plasma levels. Although norfloxacin brain concentrations peaked early after its intravenous administration, the effect on the EEG measurement was delayed. By applying a PK–PD model with an effect compartment, the authors demonstrated that the delayed EEG effect of norfloxacin is not due to blood–brain barrier transport [65].

4.5 Neuromuscular blockers

Microdialysis in muscle tissues was used for the study of neuromuscular blockers using PK–PD models. Ezzine and Varin [66] have determined simultaneously interstitial muscle concentrations of rocuronium and the neuromuscular function using the train-of-four stimulation until full recovery. A PK–PD model with an effect compartment successfully predicted concentrations of rocuronium at the effect site, demonstrating an accumulation of rocuronium in muscle tissue, probably by non specific protein binding [66].

5. Conclusions

Regulatory authorities have emphasised the importance of integrating PK and PD information in drug development in order to improve the efficiency of drug selection during development. PK–PD modelling has several advantages compared with dose–response trials, allowing a better pharmacodynamic characterisation and screening of dosage–regimen selection of therapeutic agents. However, only a few PK–PD studies are described in both preclinical and clinical settings. The need for simultaneous measurement of drug tissue levels and corresponding pharmacological effects at multiple time points probably limits the feasibility of PK–PD studies.

The microdialysis technique is a unique research tool that allows the simultaneous determination of unbound concentrations of drugs at several tissues and its pharmacological effect on biochemical and clinical markers.

Therefore, microdialysis overcomes major experimental limitations of PK–PD modelling and might become a reference technique for PK–PD studies. However, the applicability of microdialysis for PK–PD modelling during drug development would be restricted to the preclinical and early clinical phases because of its low throughput, its invasive nature and the need for technical expertise and additional laboratory equipment.

6. Expert opinion

The existence of a large amount of published data underscores the importance of microdialysis sampling technique in pharmacological studies. An up-to-date (May 2006), search of PUBMED for microdialysis displayed 10,500 articles. Surprisingly, only 37 publications are found if the search terms 'microdialysis and pharmacokinetic–pharmacodynamic' are used.

Use of microdialysis is without any doubt of advantage for PK–PD modelling studies, especially in basic research. As discussed in this review, the possibility of the simultaneous

determination of drug concentrations in different tissues, and its effect on biochemical and clinical markers with a good time resolution makes the microdialysis technique a powerful tool for PK–PD modelling. Moreover, microdialysis is a relatively cheap methodology.

Therefore, the probable reason for the existence of few PK–PD studies using microdialysis is the scarce knowledge of PK–PD relationships and, consequently, PK–PD modelling. Another recent search of PUBMED using the search strategy ' pharmacokinetic–pharmacodynamic modelling' only displayed 324 articles. Complex mathematical equations explain the relationship of PK–PD in different PK–PD models, making the understanding of PK–PD modelling difficult for the pharmacologist. In addition, PK–PD studies need computer modelling with special software packages. Although computational programs for PK–PD modelling are available, this type of software has several drawbacks, such as user unfriendliness, severe computational limitations and single task orientation [16]. The inclusion of PK–PD concepts in undergraduate and postgraduate programmes would increase the understanding of PK–PD relationships. In addition, in the last few years several excellent reviews have covered different concepts of PK–PD modelling [3,4,15,16,71-74].

In the authors' opinion, a greater number of papers regarding PK–PD modelling of therapeutic agents by means of microdialysis will be published in the next 5 – 10 years. Recent advances in microdialysis process and calibration understanding, as well as the recognition of microdialysis techniques as an attractive methodology by the regulatory authorities, support the authors' opinion. Therefore, a 100-kDa molecular weight cutoff microdialysis catheter has been introduced recently to allow detection of large molecules such as cytokines [75].

In addition, the development of more sophisticated software for PK–PD modelling would contribute to further advances in this research area.

Regulatory authorities encouraged the study of tissue distribution of therapeutic agents such as antimicrobials and the relationship between unbound drug concentrations at the site of action and its pharmacological effect [102]. Moreover, the FDA advisory committee considered that microdialysis is an attractive approach for clinical studies on tissue distribution of drugs [103].

It is expected that the number of clinical studies using the microdialysis technique for the sampling of drugs will increase in the next few years. To achieve this objective, it is important to improve microdialysis sampling of lipophilic drugs and highly protein-bound drugs. For highly protein-bound drugs, the low recovery could be solved by use of new microdialysis membranes with a high molecular weight cutoff [76]. On the other hand, the addition of solubilisers to the perfusate could improve the recovery of lipophilic drugs [77]. Development of highly sensitive analytical methods may also provide significant progress in the use of microdialysis for PK–PD studies.

The feasibility of chronic microdialysis sampling, by perfusing a microdialysis probe implanted for a period of days or weeks, remains to be elucidated in the next few years. The importance of tissue response to chronic probe implantation must be determined. In addition, the effects of tissue responses such as gliosis on microdialysis catheter recovery need to be evaluated.

The applicability of microdialysis sampling in bioequivalence studies also 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.

Finally, microdialysis sampling could become a reference technique for PK–PD modelling of the effect of therapeutic agents on biochemical biomarkers, and validation of biochemical biomarkers for PK–PD modelling is under development [78]. It is important to mention that, to be of value for PK–PD modelling, a biomarker needs the quantification through a robust and reproducible analytical method and the PK–PD model must be analytical and predictive [79-82].

In conclusion, although only a few PK–PD modelling studies have been carried out so far using the microdialysis sampling technique, this technique offers several advantages and, therefore, probably will become, in the next few years, a reference technique for PK–PD modelling.

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