Department of Pharmaceutics, College of Pharmaceutical Science, Zhejiang Chinese Medical University, Hangzhou, China

Microdialysis for pharmacokinetic-pharmacodynamic studies

W. Yu, Q. Cheng, J. Feng, F. Li

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Prof. Li Fanzhu, College of Pharmaceutical Science, Zhejiang Chinese Medical University, NO.548, Road Bin Wen, District Binjiang, Hangzhou 310053, P.R. China
lifanzhu@zjtcm.net; Lilongzhu911@sohu.com

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Microdialysis (MD) has become one of the major tools to sample endogenous and exogenous substances in extracellular spaces. It is more suitable for pharmacokinetic-pharmacodynamic (PK-PD) studies than other techniques. This review aims to give an overview of MD for PK-PD (MD/PK-PD) studies, including PK-PD studies, three aspects (principles, recovery, advantages) of MD/PK-PD, and application examples of MD/PK-PD organized by types of drugs and information collected. It can be concluded that MD offers an unique opportunity, to study simultaneously pharmacokinetic (PK) behavior of a drug and its effect on the extracellular levels of endogenous compounds, which may facilitate proof-of-concept demonstrations for target modulation, enhance the rational selection of an optimal drug dose and schedule. In addition, MD/PK-PD can also minimize uncertainties associated with predicting drug safety and efficacy, reduce the high levels of drug attrition during development, accelerate drug approval, and decrease the overall costs of drug development.

1. Introduction

Microdialysis (MD) is a catheter-based sampling technique that is used to measure the concentration of unbound fraction of endogenous and/or exogenous substances in the extracellular fluid (ECF) of tissues (e.g., adipose tissue, brain, heart, lung, tumors) (Muller et al. 1998; Muller 2000, 2002). As we know, it emerged from the neurosciences where it was originally used for measuring concentrations of neurotransmitters (NTs) in rat brain (Ungerstedt and Pycock 1974). From this experimental field, it gradually spread to other research areas, for instance, toxicology (McKim et al. 1993), and drug delivery systems (DDS) which our laboratory were engaged in before (Li et al. 2007), and it has now found increasing applications in pharmacology, particularly in pharmacokinetic-pharmacodynamic (PK-PD) studies of drugs. The method provides strong support for PK-PD modeling procedures and may also optimize dose selection and help to determine appropriate dose regimens. Table 1 shows some of the analytes successfully monitored and where this technique has been applied.

Table 1: Areas of application of MD and some of the analytes successfully monitored

Areas of application	Analytes	Selected references
Neurotransmission	Amino acid NTs	(Brodie et al. 1987)
Behavioral neurochemistry	Monoamines	(Osborne 1995)
	Catecholamines	
PK	Drugs	(Hurd et al. 1988)
Metabolism	Drug metabolites	(Touchet and Bennett 1989)
Pharmacology	Drugs	(Shimura et al. 1995)
	Secondary messengers	
	Peptides (low MW)	
Endocrinology	Hormones	(Shichiri et al. 2002)
Toxicology	Drugs	(Nomoto 1995)
	Drug metabolites	
DDS		(Duvvuri et al. 2005)
PD	Endogenous compounds	(Clinckers et al. 2005)
PK-PD	Drugs/Endogenous compounds	(Hocht et al. 2006)

* MW = molecular weight

This review gives an overview of MD for PK-PD (MD/PK-PD) studies, including PK-PD studies, three aspects (principles, recovery, advantages) of MD/PK-PD, and application examples of MD/PK-PD organized by types of drugs and informations collected. It ends with the potential advances of MD/PK-PD, realizing that it will continue to open new doors in PK-PD studies when combined with sensitive analytical techniques (ATs), such as high performance liquid chromatography (HPLC) (Day et al. 2001; Hows et al. 2004), high performance capillary electrophoresis (HPCE) (Bowser and Kennedy 2001; Huynh et al. 2004), nuclear magnetic resonance (NMR) (Kanamori and Ross 2005; Lucas et al. 2005) and mass spectrometry (MS) (Baseski et al. 2005), etc.

2. Pharmacokinetic-pharmacodynamic studies

PK-PD studies play an important role in drug development and evaluation (Derendorf and Meibohm 1999). In general, PK describes what the body does to the drug, namely the time course of the drug concentrations in plasma or tissue fluid (TF). While PD describes what the drug does to the body, in a more quantitative sense, it studies the relationship between drug concentration and effect. PK-PD modeling is a well-established approach that links the PK and PD of the drug, and describes the time course of pharmacological effects of a given dose, which is helpful to determine appropriate dose regimens (Fig. 1) (Liu et al. 2002a).

2.1. *Pharmacokinetic-pharmacodynamic features*

It is well known that PK-PD modeling has several advantages which can basically be summarized in four major points (Derendorf and Meibohm 1999; Latz et al. 2006). The first characterizes the link between measured drug concentration and the response system, direct link versus indirect link. The second considers how the response system relates effect site concentration to the observed outcome, direct versus indirect response. The third regards what clinically or experimentally assessed information is used to establish the link between concentration and effect, hard link versus soft link. And the fourth considers the time dependency of PD model parameters, distinguishing between time-variant versus time-invariant.
However, one disadvantage of PK-PD modeling is the need for simultaneous measurement of drug tissue levels and corresponding pharmacological effects at multiple time points (Toutain 2002). In this regard, traditional blood sampling is not ideal because removal of the samples themselves interferes with PK and PD behavior of the drug (Elmquist and Sawchuk 1997). The development of MD provided researchers with a special tool to study PK-PD (Chenel et al. 2004; Ezzine and Varin 2005). Since this technique not only allows the sampling of extracellular levels of drugs but also endogenous compounds such as NTs, metabolites, glucose, lactate and low MW peptides.

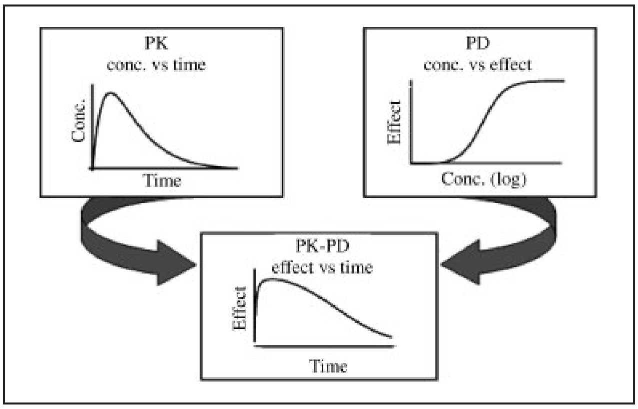

Fig. 1: PK-PD modelling as a combination of the classic pharmacological disciplines PK and PD

2.2. *Pharmacokinetic-pharmacodynamic modeling*

In PK-PD modeling study, the most used model for a direct (no time delay) and reversible concentration-effect relationship is the Sigmoid maximum effect (E_{max}) model (de Lange et al. 2005):

$$E = E_0 + \frac{E_{max} C^h}{EC_{50}^h + C^h} \tag{1}$$

in which E is the response observed for a given concentration at time t, C; E_0 is the baseline response; E_{max} is the maximal effect of the drug; EC_{50} is the plasma concentration of the drug that produces 50% of E_{max} and h is the hill coefficient, which determines the steepness of the concentration-effect relationship.
The sigmoidal E_{max} equation used to fit a plasma concentration-effect profile provides estimates of EC_{50} and E_{max} values that result from the combined ability of the drug to bind to its receptor (the affinity of the agonist) and the ability of the drug to cause an effect after binding to the receptor (the efficacy of the agonist). Actually, it may estimate identical EC_{50} and E_{max} values for a drug with high affinity and low efficacy and a drug with low affinity and high efficacy. It, therefore, lacks the power to predict drug responses under different physiologic or pathologic conditions, where both affinity and efficacy may be affected.
In order to predict the intrinsic activity and potency of a drug for a particular pharmacological effect or response, a model is required that explicitly distinguishes between drug-specific and system-specific properties. To that end, derived from the receptor occupation theory, a modified operational model seems to be very useful (Black and Leff 1983; Black et al. 1985):

$$E = E_0 + \frac{E_m \cdot \tau^h \cdot C^h}{(K_A + C)^h + \tau^h \cdot C^h} \tag{2}$$

This equation is used to analyze agonist concentration-effect curves in terms of the concentration of the drug (agonist) at time t, C; the baseline response E_0; the maximal tissue response (E_m); the slope of the transducer function (h); the agonist-receptor dissociation equilibrium constant (K_A); and the efficacy parameter (τ). The efficacy parameter:

$$\tau = \frac{R_0}{K_E} \tag{3}$$

is expressed in terms of the total number of available receptors R_0 and the concentration of the number of receptors occupied at the half-maximal effect K_E. While receptor affinity and intrinsic efficacy, the "drug-specific" properties, can be estimated *in vitro*, with the maximal response of the drug:

$$E_{max} = \frac{E_m \cdot \tau^h}{\tau^h + 1} \tag{4}$$

and the concentration at half-maximal response of the agonist:

$$EC_{50} = \frac{K_A}{(2 + \tau^h)^{1/h} - 1} \tag{5}$$

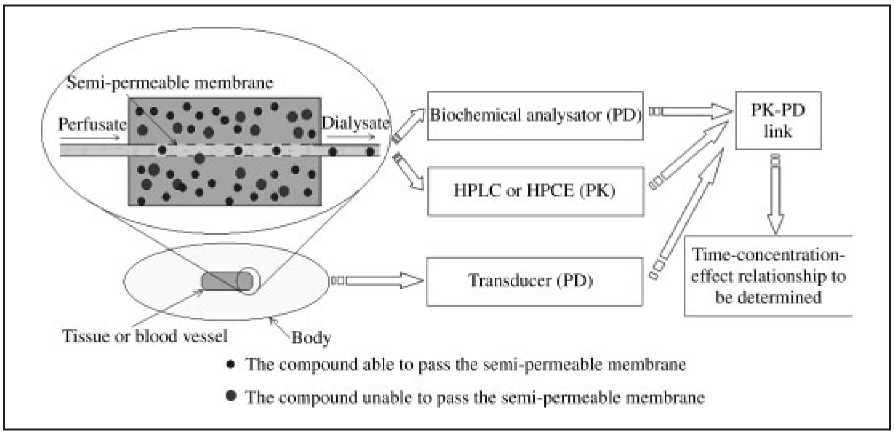

Fig. 2: The basic principles of MD/PK-PD

The modified operational model has not only been successfully applied in numerous *in vitro* studies, but can also be used for PK-PD analysis of *in vivo* drug effects (Van der Graaf and Danhof 1997). For instance, affinity and efficacy values of agonists at cardiac adenosine A_1 receptors have been estimated based on *in vivo* data and appeared to be highly consistent with estimates from *in vitro* radioligand binding studies (Van der Graaf et al. 1999).

To estimate the parameters in a modified operational model, simultaneous analysis of different PK-PD relationships which may be obtained from one agonist under control conditions and the number of receptors available for binding must be performed. This can be achieved by a compound that irreversibly binds to the receptor to such an extent that the agonist is no longer able to produce its maximal effect (Furchgott 1966; Christ 1990). Alternatively, simultaneous analysis of these relationships that result from a series of drugs with varying degrees of agonism for the specific receptor can be used (Van der Graaf and Danhof 1997).

3. Microdialysis for pharmacokinetic-pharmacodynamic studies

3.1. Principles of MD/PK–PD

MD is based on the principle that solute diffusion between two compartments separated by a semi-permeable membrane results from the concentration gradient across the membrane. Applied to an *in vivo* situation, these two compartments represent tissue ECF and artificial physiological perfusion fluid inside MD probe which consists of a small semi-permeable hollow fiber membrane, connected to an inlet and outlet tubing with a small diameter. Once implanted into a selected tissue, the probe is continuously perfused with a physiological solution at a low flow rate (Chu and Gallo 2000; de Lange et al. 2000). The perfusate is an aqueous solution that mimics the composition of the surrounding medium, therefore it prevents the excessive migration of molecules into or out of the periprobe fluid due to osmotic differences. The direction of the diffusion process is dependent on the concentration gradient, while the perfusate passes the membrane, molecules up to a certain molar mass diffuse into or out of the perfusate. The dialysate that exists in the probe can be collected (Plock and Kloft 2005). Finally, dialysate levels of drugs are determined by highly sensitive techniques, such as HPLC and HPCE, etc, obtaining unbound drug concentrations as a function of the time. Simultaneously, biochemical markers can also be monitored by biochemical analysators in the dialysate. In addition, some transducers, such as pressure transducer and respiratory-flow transducer etc, can be connected to the specified part of body, allowing the determination of drug effects as a function of time. Thus, the relationship of time-concentration-effect of drugs can be determined by means of PK-PD modeling. Figure 2 shows the basic principle of MD/PK-PD (de Lange et al. 2000).

3.2. Recovery of MD/PK–PD

One of the critical and most difficult questions in MD/PK–PD is how to estimate the true concentration of an analyte in the interstitial fluid (IF) of the tissue from that measured in the dialysate. In essence, to what extent is the compound of interest recovered in the dialysate, or lost if using MD as a delivery method. The dynamic nature of MD, due to the continuous perfusion of dialysate and removal of the analyte, normally results in the dialysate concentration being less than that in the ECF. The relation between these two concentrations is defined to be recovery which can be assessed by both *in vitro* and *in vivo* methods (Larsson 1991; Yokel et al. 1992; Van Belle et al. 1993). The main influencing factors of *in vitro* and *in vivo* recoveries are summarized in Table 2 (Plock and Kloft 2005; Zhou and Gallo 2005). From this table, it can be concluded that *in vivo* method is more reliable and allows better estimation of actual extracellular concentrations of a given analyte as compared with those from the *in vitro* methods.

3.3. Advantages of MD/PK–PD

There are several techniques for PK-PD studies, such as position emission tomography (PET) and magnetic resonance spectroscopy (MRS) (Malizia et al. 1996; Dietz

Table 2: Influencing factors of in vivo and in vitro recoveries

Main influencing factors	In Vivo recovery	In Vitro recovery
Temperature	•	•
PH	•	•
Probe geometry	•	•
Characteristics of the membrane	•	•
Concentration gradient over the membrane	•	•
Composition and flow rate of the perfusate	•	•
Physicochemical properties of the analyte	•	•
Tortuosity of the interstitial space	•	
Volume of the interstitial compartment	•	
Transport capacity over the cell membrane or certain physiological barriers	•	
Release, uptake, and clearance processes	•	

Table 3: Comparison with other techniques for PK-PD studies

Technique	MD	PET	MRS
Analytes	Any compounds (endogenous + exogenous)	Positron emitting isotope containing compounds (^{11}C, ^{13}N, ^{15}O, ^{18}F)	Compounds containing MRS-active nuclei (^{13}C, ^{1}H, ^{19}F, ^{31}P, ^{15}N)
Detection method	LC-MS/MS, UV, ect.	Radioactive decay (radiolabeling)	Nuclear magnetic resonance
Measured compartment	ECF of tissue concentration (unbound)	Total tissue concentration (bound + unbound)	Total tissue concentration (unbound)
Multiple sites	Yes	Yes	Yes
Technical complexity	Low	High	High
Metabolite monitoring	Yes	No	Yes
Temporal resolution	High	High	Poor
Spatial resolution	High (Focal sampling)	Moderate (1–5 mm)	Poor (> 10 cm)
Sensitivity	High ($10^{-9} - 10^{-3}$ mol* l – 1)	High (10^{-12} mol* l – 1)	Poor ($10^{-5} - 10^{-3}$ mol* l – 1)
Selectivity	High	High	High
On-line	Yes	Yes	Yes
Cost	Low	High	High

et al. 2001; Gomeni et al. 2001). Compared with these techniques (Table 3) (de Lange et al. 1997; Seddon and Workman 2003; Brunner and Langer 2006; Workman et al. 2006), MD is more practical and much cheaper. For these reasons, it is accessible for each laboratory as an "in-house" technique and plays a special role for PK-PD studies.

4. Application of microdialysis for pharmacokinetic-pharmacodynamic

MD has been used for PK-PD studies of therapeutic agents in both preclinical and clinical studies. Table 4 gives an overview of PK-PD studies by means of MD.

4.1. Central nervous system drugs

The particular benefit of MD/PK-PD of central nervous system drugs (CNSDs) lies in the fact that it enables the determination of free-drug concentrations as a function of time in plasma and in ECF of brain, thereby providing important data to determine blood-brain barrier (BBB) transport characteristics of drugs. Furthermore, the concentrations of (potential) extracellular biomarkers of drug effects or disease can be monitored with this technique (de Lange et al. 2005). It could be anticipated that MD/PK-PD will provide key knowledge for prediction and herewith optimization of dose regimens of CNSDs (Danhof et al. 1993).

To date, MD/PK-PD studies of CNSDs like caffeine (Heppert and Davies 1999), methylphenidate (Weikop et al. 2004), 7-nitroindazole (7-NI) (Bush and Pollack 2002), oxycodone (Bostrom et al. 2006) and other drugs (Feng et al. 2001) were extensively reported. Bush et al. (2002) assessed the PK and PD of 7-NI, a selective inhibitor of neuronal nitric oxide synthase (NOS) using MD/PK-PD. This model allows design of dose regimens that can produce designated changes of NO content in brain, facilitating use of 7-NI to probe the pharmacological implications of NO in the CNS. In a similar study, MD/PK-PD was used by Bouw et al. (2000) to study the processes involved in the delay of anti-nociceptive effect of morphine in rats. It was demonstrated that morphine was actively effluxed at the BBB accounting for 85% of the observed effect delay, indicating possible involvement of rate limiting mechanisms at the receptor level or distributional phenomena for the remaining effect delay. The studies above are essential for development of CNSDs that would be used for the treatment of neurological and psychological diseases.

In other studies, MD/PK-PD in conjunction with automated blood sampling (ABS) in conscious, freely moving rodents offers an attractive approach for CNSDs studies within the same animal (Gunaratna et al. 2004; Bundgaard et al. 2006), which provided multiple PK-PD informations in individual animals, hence minimizing inter-animal variation using a reduced number of animals. Bundgaard et al. (2006) examined the feasibility of this approach for simultaneous PK-PD studies by monitoring plasma and brain ECF concentrations of escitalopram along with SSRI-associated pharmacological activity, monitored as changes in brain 5-hydroxytryptamine (5-HT) levels and plasma corticosterone levels. The authors concluded that combining MD/PK-PD and ABS did not cause any detectable physiological changes with respect to basal levels of plasma corticosterone or brain 5-HT levels, and that the PK of escitalopram could be characterized simultaneously in plasma and the hippocampus of conscious, freely moving rats.

Recently, a technique of MD/PK-PD using three simultaneously implanted probes in the anaesthetized animals was developed, which enables monitoring of PK profiles of a tested drug both in blood (1st probe) and brain (2nd

Table 4: Examples of recently published PK-PD studies by means of MD

Therapeutic use	Representative of drugs	PK-PD model	Selected references
Central nervous system drugs (CNSDs)	Morphine	Effect compartment	(Bouw et al. 2000)
Automonic nervous system drugs (ANSDs)	Metoprolol (MET)	Effect compartment	(Hocht et al. 2004; Hocht et al. 2006)
Antimicrobial drugs (AMDs)	Ciprofloxacin	In vivo PK-in vitro PD	(Joukhadar et al. 2005)
Antineoplastic drugs (ANDs)	5-fluorouracil (5-FU), methotrexate	In vivo PK-in vitro PD	(Muller et al. 2000)
Others	L-arginine	Comprehensive PK-PD model	(Heinzen et al. 2003)

probe) compartments and the PD response of NTs (3rd probe) released into, or accumulating within brain ECF (Weikop et al. 2004). The feasibility of triple-probe MD/PK-PD has been illustrated by monitoring the rate of extracellular accumulation of a drug candidate and dopamine (DA) levels *in vivo* and comparing the resulting PK-PD profiles with those obtained for cocaine and methylphenidate (Huff and Davies 2002). These measures may serve as initial neurochemical indicators of potential psychomimetic or reinforcing properties of the substances tested. However, the triple-probe MD requires large and efficient analytical capacity and the use of sensitive and specific analytical methods for determination of trace levels of NTs, as well as of the exogenous compounds.

4.2. Automonic nervous system drugs

In the case of automonic nervous system drugs (ANSDs), measurement of drug concentration in IF would provide an invaluable insight into their concentration-effect relationship. In this respect, MD provides a useful tool for quantitative measurement of ANSDs at their site of action. Ezzine et al. (2005) compared rocuronium effect (C_e) and peripheral (C_2) compartment concentrations predicted by PK-PD modeling with those measured in plasma (C_p) and in the IF of muscle tissue ($C_{ISF,u}$) by MD in anaesthetized dogs. The unbound concentration of rocuronium measured in the muscle IF under steady-state conditions confirmed that MD/PK-PD gives reliable estimates of effect site concentrations.
MD/PK-PD was employed by Raje et al. (2005) to characterize the concentration-effect relationship between the benztropine (BZT) analogues and brain DA levels. It is an important step in the evaluation of these compounds as potential cocaine abuse pharmacotherapies. The authors considered that the slow onset and long duration of BZT analogue-induced DA elevation may avoid the reinforcing effects and craving of cocaine. Furthermore, MD/PK-PD will be useful in other analogues and aid in the assessment of the therapeutic efficacy of the BZT analogues as substitute medications for cocaine abuse.
Other studies have found a correlation between MET unbound concentrations and its chronotropic effect using MD/PK-PD (Hocht et al. 2004, 2006). Hocht et al. (2006) examined this correlation in spontaneously hypertensive (SH) rats and Wistar Kyoto (WKY) animals by means of MD. MET dialysate concentrations and its chronotropic effect were determined during 3 h after the administration of 3 and 10 $mg \cdot kg^{-1}$ of the drug, and PK-PD modeling was used to analyse experimental data. The results suggested a good correlation between plasma MET concentrations and its chronotropic effect in all experimental groups.
MD/PK-PD also offers the possibility to deliver a sufficient amount of the ANSDs transdermally, as well as to optimize dose titration by controlling current intensity. Nugroho et al. (2006) used MD to simultaneously determine PK and dopaminergic effect of dopamine agonist 5-hydroxy-2-(dipropylamino) tetralin (5-OH-DPAT) *in vivo* following transdermal iontophoresis in rats based on drug concentration in plasma (C_p) and dopamine levels in striatum (C_{DA}). This method successfully predicted profiles of C_p and C_{DA}, herewith achieved a considerable dopaminergic effect, indicating the feasibility to reach therapeutically effective concentrations of 5-OH-DPAT upon transdermal iontophoresis.

4.3. Antimicrobial drugs

The traditional approach to link antimicrobial concentrations to its effects is to relate a static parameter, minimum inhibitory concentration (MIC), to the concentration in serum. This approach is usually applied by using cumulative PK-PD variables, such as ratios of area under the plasma concentration time curve to MIC (AUC/MIC), time above the MIC (T > MIC), or ratios of maximum concentration to MIC (C_{max}/MIC) (Derendorf et al. 2000). However, these approaches do not take into account the complex interactions among an administered drug, a host, and an infective agent. Since in practice, PD effect *in vivo* is the result of a dynamic exposure of the infective agent to the unbound drug fraction at the relevant target site rather than a static interaction of two variables. Thus, some authors proposed that PK be linked to PD in a more dynamic way by using several techniques (Nolting et al. 1996; Craig 1998; Delacher et al. 2000).
All of these techniques not only may provide information on PK but also may lead themselves to studies of antimicrobial PD. This is particularly true for MD, as it monitors free antimicrobial concentrations in the fluid which directly surrounds the infective agent, the antimicrobial effect linked to the time-drug concentration profile obtained by MD may be simulated easily in an in vitro setting with bacterial cultures. Some publications described a MD based *in vivo* PK-*in vitro* PD model which may be employed to predict drug effects at a relevant target site (Nolting et al. 1996; Brunner et al. 1999; Delacher et al. 2000). By employing such a combined *in vivo* PK-*in vitro* PD approach (Fig. 3), it supports dose optimization and replaces current concepts for establishing dosing guidelines of selected tissue infections (Delacher et al. 2000). While Chenel et al. (2004) investigated the contribution of

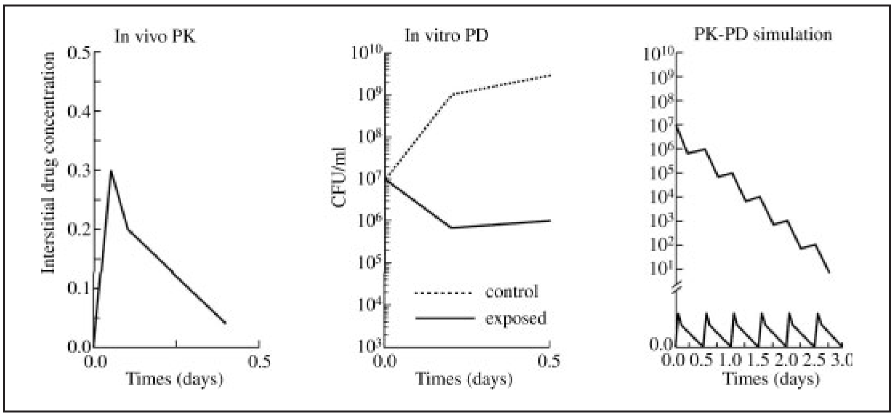

Fig. 3:
The general concept of the *in vivo* PK-*in vitro* PD approach applied in MD studies for antimicrobial drugs. In a first step tissue PK are measured *in vivo* by MD at the target site following single dose administration. Subsequently the time profile obtained *in vivo* is simulated *in vitro* on select bacterial cultures. Thereafter the information generated by the two initial steps is integrated in a combined PK-PD model which simulates an optimal scenario for the eradication of the causative pathogen (Delacher et al. 2000)

norfloxacin BBB transport to its delayed electroencephalogram (EEG) effect in rats by means of MD/PK-PD. The experimental data were successfully expounded by PK-PD modeling with spline function to describe the relationship between effect and concentration at the effect site. Comparison of PK-PD parameters estimated from plasma and ECF concentrations showed that most of the delayed norfloxacin EEG effect is not due to BBB transport, but also that PD parameters derived from plasma data must be carefully interpreted when drug distribution at the effect site is restricted.

MD/PK-PD also can be used to compare tissue penetration of AMDs. Liu et al. (2002b) used this approach to investigate the tissue penetration of cefpodoxime and cefixime. In this study, free concentrations of cefpodoxime in muscle were similar to those in lung and therefore provided a surrogate measure of cefpodoxime concentrations at the pulmonary target site. The total plasma concentrations of each antibiotic were similar and higher than free ones in muscle. The tissue penetration of cefpodoxime was, however, greater than that of cefixime, as shown by two-fold higher peak free muscle concentrations after dosing with cefpodoxime than with cefixime. These findings indicate that, taking into account MD/PK-PD considerations, cefpodoxime is likely to be more efficacious than cefixime, due to its greater tissue penetration. Another example of MD/PK-PD of AMDs is given by Joukhadar et al. (2005) having investigated the effect of microcirculatory blood flow on the ability of ciprofloxacin to penetrate soft tissues. This study showed that the improvement of microcirculatory blood flow due to the warming of the extremity was paralleled by an increased ability of ciprofloxacin to penetrate soft tissue and subsequent PK-PD simulations based on tissue PK data indicated that this increase in tissue penetration was linked to an improved antimicrobial effect at the target site.

4.4. Antineoplastic drugs

Before introduction of MD, the tumor drug concentrations were determined through biopsies, a technique which implies serial sacrifice studies where each animal only contributes one time sample. Another drawback of this method is the impossibility to measure only the unbound concentration of the drug in the tumor. MD represents a powerful technique since it allows the time course determination of the unbound concentrations of the antineoplastic drugs (ANDs) in the tumor in the same animal and in a specific effect compartment. This facilitates a clear pharmacological interpretation and readily supports PK-PD studies of ANDs.

So, in recent years MD/PK-PD of ANDs was designed based on the *in vivo* measurement of interstitial drug PK in breast cancer patients and a PD simulation of the time versus concentration profile in an *in vitro* setting (Muller et al. 2000). Briefly, breast cancer cells (MCF-7) were exposed in vitro to the time versus interstitial tumor concentration profiles of 5-fluoruracil (5-FU) and methotrexate (MTX) from primary breast cancer lesions in patients (Muller et al. 1997, 1998). This led to a maximal reduction in the viable cell count of 69% on day 4 and of 71% on day 7 for 5-FU and MTX. The observed effect was dependent on the initial cell count and was characterized by a high interindividual variability. There was a significant correlation between the maximum antitumor effect and the intratumoral AUC for 5-FU but not for MTX. Data from this approach support the concept that tumor penetration of 5-FU would have a response limiting effect, while the response to MTX may be determined by events beyond IF kinetics.

By applying MD/PK-PD it was shown that success and failure in cytotoxic therapy with 5-FU may be explained by PK variability in interstitial concentrations (ICs) (Muller et al. 2000). It thus emerges that the measurement of ICs in solid tumors by MD may explain drug resistance in select groups of patients and may help optimize dosing and administration schedules. Thereby the selection of novel cytotoxic compounds with favorable tumor penetration characteristics might be facilitated in the future.

As we know, metronomic dosed chemotherapy as opposed to conventional dosed chemotherapy is considered an alternate strategy to target angiogenesis and limit host toxicity. Although promising, there has not been any attempt to define optimal metronomic dose regimens by integrating PK-PD studies before. Thus, Zhou et al. (2001) compared the PK and PD of temozolomide (TMZ) following metronomic and conventional dose regimens by means of MD/PK-PD. The results suggested that the metronomic dose regimen may be superior to the conventional dose regimen by preventing tumors from progressing towards a proangiogenic state. At the same time, several PK-PD factors contributing to the antitumor activity of the metronomic dosed TMZ therapy have been identified, and form a foundation for further investigations of low-dose TMZ regimens.

4.5. Other drugs

Apart from the fields of application mentioned above, MD/PK-PD has also been used in other drugs. Heinzen et al. (2003) assessed PK of L-arginine, a NO precursor, and related the disposition of this amino acid to PD endpoint of neuronal NO production by MD. The results were fit with a comprehensive PK-PD modeling to obtain parameters governing the systemic disposition of L-arginine, the uptake of L-arginine into the brain, and subsequent NO production. This supported the hypothesis that administration of exogenous L-arginine to rats resulted in systematic and predictable elevations in hippocampal NO production, and concluded that MD/PK-PD was capable of describing accurately the observed data and represented a valuable tool in the design of L-arginine dose regimens to target specific, sustained elevations in brain tissue NO.

As MD monitors unbound drug concentration and the animal response are not altered by fluid loss, it is possible to study the relationship between the bioactive drug fraction and the cardiovascular response. This way, MD/PK-PD study of methyldopa was made by Hocht et al. (2001) in anesthetized sham operated (SO) and aortic coarctated (ACo) rats. Analysis of the arterial blood dialysates showed a lower half-life of methyldopa in ACo rats than in SO rats. Also a low accumulation and a fast decay of striatal methyldopa levels were seen in ACo rats. However, peak levels of drug were greater in the hypothalamic dialysates of ACo rats than in SO animals samples. It can be concluded that the aortic coarctation modifies the PK and cardiovascular effect of methyldopa in the rat, and the action of this drug on dopaminergic neurotransmission is also altered in the ACo animals.

Rabenstein et al. (1996) proposed a novel "MiniShunt" extracorporeal MD sampling circuit designed to sample core blood in anaesthetised dogs. Discrete microdialysate and plasma samples collected during glucose and lactate monitoring were analysed with a YSI analyzer. In this manner, MD/PK-PD seems to be promising in the studies of anti-diabetic agents.

4. Conclusions and perspectives

In summary, MD/PK-PD studies have been accepted universally and put into practice with greater frequency. By means of MD, the PK behavior of a drug and its effect on the extracellular levels of endogenous compounds can be studied simultaneously, which may facilitate proof-of-concept demonstrations for target modulation, enhance the rational selection of an optimal drug dose and schedule, aid decision-making, such as whether to continue or close a drug development project. In addition, MD/PK-PD can also minimize uncertainties associated with predicting drug safety and efficacy, reduce the high levels of drug attrition during development, accelerate drug approval, and decrease the overall costs of drug development.

But it should be considered that the use of MD/PK-PD is still in the developmental stage. Many technical challenges remain to be resolved. For example, there is still the problem of design of probe, and the possibility to prolong the experimental duration without inducing an inflammatory response of the tissue to the membrane. Thus, modified MD was proposed by some researchers (Kaptein et al. 1998; Schaupp et al. 1999; Hocht et al. 2001). As an application example of this new technique, a vascular "shunt" MD probe has been designed by some laboratories with one inlet and two outlets, the inlet and one outlet are inserted into the left carotid artery and the remaining outlet is connected to a pressure transducer, which allows the continuous and simultaneous determination of unbound plasma levels of drugs and their corresponding effects in the same animal and with a minimal damage, making the probe more suitable for PK-PD studies (Opezzo et al. 2000; Hocht et al. 2001)

Although challenges remain, it is obvious that MD is particular of value in the field of PK-PD studies. Furthermore, rapid developments of analytical techniques allow combination with MD, such as MD-HPLC-MS (Hows et al. 2004; Lindon 2003), MD-HPLC-NMR (Kanamori et al. 2003; Lucas et al. 2005), MD-HPLC-NMR-MS (Lindon et al. 2000; Lommen et al. 2000; Lindon 2003), MD-CE (Bowser and Kennedy 2001; Huynh et al. 2004), etc., which could provide near real-time data in PK-PD studies and give a new perspective in drug discovery and development.

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