By Robert E. Stratford, Jr.

The Microdialysis Focus Group steering committee proudly presents the January 2016 issue of Probes. This issue focuses on the application of microdialysis in the design and development of anti-infective agents. The “Special Feature” section provides a brief, yet seminal overview of the field, and is authored by a leader in this area, Professor Hartmut Derendorf of the University of Florida. Coupled with this overview, the “Pearls of Wisdom” section profiles Dr. Derendorf’s career. Briefly, Professor Derendorf is a Distinguished Professor and the Chair in the Department of Pharmaceutics, and the V. Ravi Chandran Professor in Pharmaceutical Sciences. During his nearly 25 years at the University of Florida, Dr. Derendorf has published well over 400 original research articles, many related to the pharmacokinetics and pharmacodynamics of anti-infective agents. In “Role of Microdialysis in the Research of Young Scientists,” Dr. Nivea Maria Falcao Voelkner, who recently completed her Ph.D. training in Dr. Derendorf’s lab, describes her dissertation work in which she developed a PK/PD model to describe pyrazinamide treatment of cutaneous leishmaniasis, a potentially fatal skin infection. Central to her work was the application of microdialysis to measure concentrations of pyrazinamide in the dermis. Finally, “Recent Microdialysis Publications” provides a compilation of publications in the last six months in which microdialysis was listed as a key word.

As always, whether you are a seasoned veteran, or are relatively new to the microdialysis field, we hope you find this issue valuable to your research.
**Special Feature -**

**Application of Microdialysis in the Development of Novel Anti-infectives**

**Hartmut Derendorf, Ph.D.**

The cost of drug development has exploded in recent years and risen to a level that soon will no longer be affordable to society. One reason for the high cost of drug development is the many unnecessary studies where the results could have been predicted with reasonable certainty. PK/PD modeling is a tool that can be used to collect and integrate all the available information about a drug candidate and its class in order to make rational decisions on studies that will decrease the uncertainty of the compound. In the drug development process, it bridges the complete cycle from discovery to clinical use. The advantage of this approach is to define objective go/no-go decision criteria for the development process rather than relying on subjective empirical decisions. There is no way that today all developing questions can be answered by experimental evidence, and modeling and simulation is a powerful alternative approach. This modeling and simulation approach is of particular need in the field of new anti-infective agents where the rise of resistance has become an international threat to society. However, very few drug companies are currently developing new antibiotics due to the poor prospective of return on investment. However, the cost of anti-infective drug development can be dramatically lowered by applying pharmacometric concepts and selection of some key experiments based on pharmacokinetic/pharmacodynamics concepts. Using microdialysis, it is today possible to measure the local exposure at the infection site in both animals and humans. This PK information is much more useful than traditional serum pharmacokinetics. Microdialysis is one of the most exciting new technologies in biomedical research and drug development and that is still vastly underutilized. Microdialysis enables measurement of unbound local concentrations at almost any site, both in–vitro and in–vivo. The in–vitro use is of great significance when measuring drug concentrations in complex media such as cell cultures where drug binding to biological material and/or glass– and plastic ware may be significant and in the past frequently was ignored. For example, in in–vitro drug–drug interaction studies unbound concentrations measured by microdialysis are much more meaningful for extrapolation to the in–vivo conditions than total concentrations. Microdialysis is much gentler than centrifugation and avoids any breakdown of cellular material that could produce artifacts. In–vivo microdialysis allows direct access to almost any site of interest, both in animals and in humans. In our group, we do a lot of studies in healthy volunteers and learned quickly that the monitored unbound drug concentrations in the extracellular space are frequently quite different than the respective plasma or serum concentrations. This can be of direct therapeutic consequence. If you adjust the dose of an antibiotic based on the serum concentration, it can be very different than if you focus on the unbound extracellular concentration at the infection site, e.g. a diabetic foot. One cool feature of microdialysis is that you can stick your probe anywhere you want to measure the concentration, sort of like a magic wand. An example of this is the monitoring of drug concentrations in the target tissue after topical administration, such as with a dermal patch or ointment. In the preclinical setting, microdialysis allows almost unlimited pharmacokinetic sampling from small animals over long periods of time without any concern for loss of too much blood. Similarly, the use of clinical microdialysis in neonates provides access to frequent sampling without any fluid loss. However, microdialysis has more uses than just for monitoring unbound drug concentrations. It also allows monitoring of endogenous compounds at any site of interest for pharmacodynamic evaluation. In the past,
biochemical biomarkers have been mainly derived from serum. Microdialysis allows access to specific sites of endogenous biochemical reactions so as to monitor events that may not be detectable in serum. Even better, one can also use the microdialysis probe to deliver a biochemical reagent or precursor locally and then measure the local turnover events followed by their modifications by drugs of interest. The potential applications of microdialysis are endless and we have just scratched the surface.

Microdialysis is still a technique in its infancy and there are a number of challenges that we all need to work on in the years to come. One issue is probe calibration which is still very time consuming and sometimes scares away potential users. However, further miniaturization of the probes and flow rates may provide increased recoveries close to 100% so that probe calibration may become mute. This further miniaturization also requires even more sensitive assays than those available today. The ideal scenario will be compound-specific probe-detector combinations that will allow sampling and quantification in one continuous process online. Other challenges include non-specific binding to probe material that sometimes can be a nuisance. More probe materials need to be made available to choose from. This will happen as soon more scientists will demand these probes and microdialysis becomes mainstream. Finally, it is always important to stress that *in-vivo* microdialysis measures unbound *extracellular* concentrations. Intelligent ways to also monitor unbound *intracellular* concentrations are desperately needed. Imaging techniques can only detect total drug concentrations but not unbound drug. The use of intracellular biochemical probes in combination with extracellular microdialysis in the vicinity may be one approach that seems promising.

Furthermore, pharmacodynamic activities can be much better captured by analyzing time–kill curves rather than simple minimum inhibitory concentrations (MICs). Sophisticated pharmacometric models have been developed to characterize the resulting concentration–time–effect relationships and convert them into dosing recommendations that ultimately will need to be tested clinically. However, the use of microdialysis has facilitated streamlining of drug development of new anti-infective agents and has added an exciting new tool that hopefully will help to bring new badly needed antibiotics to our infected patients quickly.
Pearls of Wisdom:

A Profile of the Career of Dr. Hartmut Derendorf

Hartmut Derendorf is Distinguished Professor, V. Ravi Chandran Professor of Pharmaceutical Sciences and Chairman of the Department of Pharmaceutics at the University of Florida College of Pharmacy in Gainesville. He received his B.S. (1976) and Ph.D. (1979, summa cum laude) in Pharmacy from the University of Münster, Germany and then joined the University of Florida, first as a Postdoctoral Fellow (1981/82) and later (1983) as a faculty member.

He has been teaching Biopharmaceutics, Pharmacokinetics and Clinical Pharmacokinetics. He was awarded numerous teaching awards such as the UF Teaching Improvement Award, HHMI Distinguished Mentorship Award, UF Research Foundation Professorship, CVS Pharmacy Endowed Professorship, International Educator of the Year Award and UF Doctoral Advisor/Mentoring Award. He has supervised over 45 Ph.D. students.

Prof. Derendorf has published over 400 scientific publications and given over 750 presentations at national or international meetings. He has published six textbooks in English and German. He is Editor or Associate Editor of the Journal of Clinical Pharmacology, European Journal of Pharmaceutical Sciences and Editor of the International Journal of Clinical Pharmacology & Therapeutics, International Journal of Antiinfective Agents and Die Pharmazie, and serves on the Editorial Board of eight other Journals. His research interests include the pharmacokinetics and pharmacodynamics of corticosteroids, analgesics, antibiotics as well as drug interactions.

Prof. Derendorf has served as President of ACCP (American College of Clinical Pharmacology) in 2006/08 and President of ISAP (International Society of Antiinfective Pharmacology) in 2004/06. He won the McKeen-Cattell Award for the best publication in J. Clin. Pharmacology (1994) and the Faculty Award of the University of Utrecht (2005). In 2003, he was awarded the Nathaniel T. Kwit Distinguished Service Award of ACCP and the Research Achievement Award in Clinical Science of the American Association of Pharmaceutical Sciences (AAPS). He is a Fellow of AAPS and ACCP as well as a former review panel member of the NASA Human Research Program. In 2010, he was awarded the Volwiler Award of the American Association of Colleges of Pharmacy (AACP) as well as the ACCP Distinguished Investigator Award, the highest research awards of both organizations. In 2013, he was awarded the First Leadership Award of the International Society of Pharmacometrics.

In an interview with Dr. Derendorf conducted in 2014, Dr. Ravi Juluru, who was a member of the microdialysis focus group at the time, asked Dr. Derendorf the following question. Dr. Derendorf’s response follows.

What is your advice for students and young scientists?

As you can see from the few thoughts in the previous questions, there are still many unanswered questions in this field. I can certainly recommend microdialysis and its related aspects as a great choice for a research area. This is still a new field and there are not too many groups in the world specializing in this technique. However, more generally, the most important advice for students and young scientists is to always challenge the current thinking and question...
established paradigms. For example, for many years millions of animals were sacrificed to take tissue biopsies since it was believed that these total tissue concentrations could be related to efficacy or safety and thereby to dose optimization. Today we know that it is the unbound concentration that usually correlates with drug activity and, hence, total tissue concentrations are of only very limited use. The concept of a ‘tissue concentration’ is flawed since tissue is not homogenous, has many sub-compartments and potential binding sites. So a ‘tissue concentration’ is a hybrid number that usually is very different than the relevant unbound drug concentration at the target site and not very helpful. Still, ‘tissue-partition-coefficients’ are reported as if tissue would be an oil drop. I use this as an illustration of something that is called Eng’s Law: The easier it is, the harder it is to change. So my advice is to speak up and question when something does not seem right, and follow a critical rational approach rather than just believing what the opinion leaders pontificate.
ROLE OF MICRODIALYSIS IN THE RESEARCH OF YOUNG SCIENTISTS – A Doctoral Candidate’s Profile

By Nivea Maria Falcao Voelkner

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Infections by Leishmania parasites which cause cutaneous Leishmaniasis (CL) encompass clinical symptoms, including skin lesions to serious disfigurement and fatal systemic infection. Pyrazinamide (PZA) is an antibiotic usually effective in treating infections caused by Mycobacterium tuberculosis. Studies not only suggested PZA sensitivity of Leishmania during parasite stages, but also have indicated collateral immunostimulation (Mendez, Traslavina, Hinchman, Huang, Verde, Cynamon, et al. 2009). In the context of drug delivery, there has been an intensified interest on the determination of unbound drug concentrations of antibiotics at the site of infection. Studies have reported variability between antibiotic tissue concentrations and corresponding plasma levels (Nwaka, Ramirez, Brun, Maes, Douglas & Ridley, 2009). Although previous microdialysis studies suggested PZA as an antibiotic rapidly and readily distributed into the bile and brain (Ellard, 1987), there is limited quantitative information describing the in vivo assessment of PZA pharmacokinetic (PK) profile in dermal tissue as the sampling site. It appears acceptable to study the skin penetration of PZA following intravenous application since the development of new therapies, new drug formulation and dose optimization for CL disease is required. Currently, we know very little about the PK and PD of any of the drugs used for Leishmaniasis and nothing about drug action and drug concentration at the site of infection. For a drug to be effective it should ideally reach a concentration at the site of infection (PK) which can kill the parasite (PD) without being toxic to other parts of the body. Regarding the dosing regimens used in the actual treatment it has not been optimized based upon this PK/PD understanding. The PK/PD modeling approach could lead to improved efficacy, lower toxicity, and less chance of resistance; the same applies to the design of drug combinations. Nowadays, the availability of techniques for quantification of free drug concentrations in dermis are still limited. Considering microdialysis as a minimally-invasive sampling technique, in vivo dermal microdialysis has become a very useful tool in conventional PK studies which has been successfully applied to assess drug delivery of water-soluble molecules in the skin (Chooluck, Singh, Sathirakul & Derendorf, 2012). Indeed, the low lipophilicity (log P= −1.31 / CLogP = −0.67632) (Brunner, Derendorf & Muller, 2005) and the low plasma protein binding (10%) (Dolezal, Zitko, Osicka, Kunes, Vejsova, Buchta, Dohnal, Jampilek & Kralova (2010) of PZA, made this antibiotic an ideal candidate for microdialysis sampling in my doctoral study. The purpose of my dissertation was to correlate PK parameters obtained through dermal microdialysis, and pharmacodynamics (PD)
parameters assessed by time–kill curve experiments to model and simulate outcomes of various dosing regimens of PZA for CL. In initial studies, a HPLC method was developed and validated for determination of free concentrations in microdialysates and in plasma samples. We applied in vitro dialysis and retrodialysis methods to evaluate the influence of perfusion flow rate on recovery and subsequently, to estimate the influence of PZA concentrations in a fixed flow rate. Recoveries determined by dialysis and retrodialysis suggested nonspecific binding of PZA to the inlet tubing of the linear microdialysis probe, indicating this to be dependent on the perfusion flow rate. An increase in flow rate demonstrated a decreased relative recovery and relative loss percentage in PZA concentration. Three PZA concentrations were evaluated through dialysis and retrodialysis in a fixed flow rate. However, different drug concentrations did not affect the binding to the probe or affect relative recovery or loss. Recoveries were independent of concentration but inversely dependent on perfusion flow rate in the in vitro microdialysis experiments. After validating the microdialysis conditions in in vitro experiments, the methods was also further tested in vivo in a Wistar rat model.

In our study, for characterization of the PK of PZA, we performed dermal microdialysis at the target site of infection by Leishmania spp. In each animal, procedures such stabilization, calibration by retrodialysis and washout of the probe were performed before the application of the microdialysis technique. Following intravenous (i.v.) bolus administration of PZA in individual healthy Wistar rats, drug levels were measured using CMA 30 linear microdialysis probes and compared to plasma concentrations at different time points. Microdialysis technique is based on simple diffusion of free analyte through the semipermeable membrane, which allows for the measurement of unbound concentrations continuously at the site of drug action over time. Contrasting with plasma samples, microdialysates are released to be quantified excluding extra extraction procedures. The technique also enables assessment of full local PK profile of dermal drug penetration from each sampling site. Pharmacokinetic parameters of PZA in plasma and dermal tissue as $C_{\text{max}}$, AUC, clearance, $t_{1/2}$, volume of distribution and free drug levels were also determined through this technique. The results show that biologically active PZA levels in the skin were not significantly different compared to unbound plasma concentrations. The results also indicate a linear PK for PZA with high concentrations dispersed in the dermis per concentration administered for different doses (Figure 1). In addition, to correlate PK to PD parameters, time–kill curves were performed using macrophages infected with amastigotes–like parasites from L. (L.) amazonensis (Figure 2). A population PK model proposed by Wilkins and coworkers (Wilkins, Langdon, Mclleron, Pillai, Smith & Simonsson, 2006) was used for all posterior simulations in our analyses. Subsequently, human PK data were correlated with PD data based in our study (Figure 3).

In conclusion, dermal microdialysis was considered a valuable and useful technique, which enables continuous monitoring of drug levels, and the assessment of full local PK profile of drug penetration in the dermis. The characterization of the PZA PK/PD relationship in our study revealed a reasonable dosing strategy for PZA in the treatment for CL, and also led to a predictive model that may be useful in drug development and in determining dose regimens in humans.
References:


Figure 1. Mean unbound concentration–time profiles for pyrazinamide levels in dermal tissue and plasma after i.v. bolus administration of 50 mg/kg (N = 6) and 25 mg/kg (N = 4) in Wistar rats.
Figure 2. Number of parasites within macrophages after PZA treatment.

Figure 3. Pyrazinamide dose optimization prediction for human based on human PK. Skin concentration profile (PK) and parasitic response–time profile (PD) prediction of antileishmanial activity of PZA under three dosing regimens.
The anti-infective agents are drugs that can either eradicate an infectious agent such as pathogen or inhibit it from spreading within the body. The common PK–PD approach to characterize efficacy of an anti-infective drug, relies upon the plasma concentration and the minimum inhibitory concentration (MIC) measurement as the PK and PD endpoints respectively. Most of the infections occur in the peripheral tissues, far away from plasma. Hence, the therapeutic efficacy of anti-infective agents should be evaluated based on their concentrations at the target site rather than plasma concentrations. PK studies are an essential component of anti-infective drug development and are required to provide information on the tissue drug distribution. The global regulatory agencies recommend to report the measurements of the distribution of antibiotics to both unaffected and infected target sites. Clinically, estimation of PK parameters based on the free drug concentration in target tissue is a rational approach, since unbound fraction of the drug exerts pharmacological action. The onset, magnitude, and duration of the therapeutic effect depend on not only the availability of the drug in the target site but also on its disposition at the site of interest. Consequently, direct target site concentration measurements can be more relevant in predicting therapeutic response. Several techniques including tissue biopsies, saliva and skin blister fluid sampling, imaging and microdialysis, have been developed to monitor target site concentrations of anti-infective agents in animals and humans.

1. Traditional technique
   a. Indirect method
   This method involves use of compartment and physiology based mathematical algorithms to obtain indirect information about peripheral tissues from plasma-derived data. This approach relies on several assumptions that may not hold true in most clinical situations.

b. Direct method
   Several experimental methods were developed to overcome the limitations of the indirect method. These methods involve quantification of antibiotic in different surrogates for interstitial fluid (ISF), such as in-vitro models, skin blister sampling (SBS), fibrin clots, tissue chambers, wound exudates, surface fluids, implanted fibrin clots and peripheral lymph. These approaches provide only limited information on the tissue penetration due to the lack of pathophysiologic counterpart in humans. The major limitations of traditional techniques are: (i) only one–point measurement is provided and (ii) the free drug concentrations in ISF cannot be discriminated.

2. Conventional technique
   a. Imaging techniques
   Imaging methods consists of novel radiopharmaceutical techniques based on the conceptual extension of autoradiography. The two–dimensional (2D) technique includes planar gamma scintigraphy (PGS) whereas the three–dimensional (3D) technique involves single photon emission computed tomography (SPECT), positron emission tomography (PET) and magnetic resonance spectroscopy (MRS). These techniques enable the visualization of the entire pattern of distribution in given organs and they also provide a means to quantify the inter– and intra–subject variability associated with the in–vivo distribution process. The major limitations are (i) only drugs that lend themselves to radiolabeling may be studied, (ii) the signal is not necessarily a measure of intact drug concentration and (iii) they do not provide information about specific tissue compartment such as the ISF. The regulatory agencies are therefore very
cautious in using data from imaging studies for regulatory purpose. Currently, the use of these techniques is also limited to large research centers with good funding opportunities.

b. Microdialysis

Microdialysis is a unique technique that enables continuous monitoring of local unbound drug concentration and metabolites in the ISF of various tissues in animals and humans. It is well suited for the pharmacokinetic studies and has been widely applied to assess drug distribution to target tissues. The working principle of this technique is passive diffusion driven by concentration gradient of the analyte across semipermeable membrane.

In contrast to the imaging techniques, microdialysis can be used readily and routinely for many preclinical and clinical studies in almost any research center whereas the new cost– and labor–intensive imaging techniques are not readily applicable in clinical routine settings for PK studies and are only available for a small number of compounds. In–vivo microdialysis offers the opportunity to study the distribution of a large variety of chemical entities in many different clinical settings. Under appropriate ethical conditions, in–vivo microdialysis is feasible for virtually every human tissue.

The particular advantage of microdialysis in studies of anti–infective agents, however, relates to the fact that it allows the online measurement of the unbound, i.e., pharmacologically active, drug fraction in the ISF.

The integration of the microdialysis data in evaluating PK–PD of anti–infective agents comprises of three steps. (i) in–vivo quantification of drug in ISF and estimation of PK at target site, (ii) subsequent PD simulation of concentration–time profile in an in–vivo setting and (iii) data analysis using an integrated PK–PD model to link unbound antibiotic concentration to bacterial killing rates by using $E_{\text{max}}$ model. The resulting data could provide strong support for PK–PD modeling procedures and can assist in dose optimization.

In summary, microdialysis is the method of choice to determine the concentration of anti—infective drugs. Indeed, bacterial infections occur predominantly in the extracellular fluid (ECF) and only the unbound drug that distribute to these sites have the possibility to interact with the micro–organisms and be therapeutically effective. Therefore, microdialysis concentrations are stronger predictor of therapeutic effect then other sampling methods such as blisters or biopsy.

For further information on this topic, the reader is referred to the following references and other scholarly publications available on PubMed.

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Compiled by Amit Joshi, Doctoral Candidate and Shabnam Sani, Chair of Microdialysis Focus Group
notes from the 2015 annual meeting of the Microdialysis Focus Group

The meeting was held on Tuesday, Oct 27 at 4:00 pm in the Orange County Convention Center, Orlando, FL. Dr. Robert Stratford, past chair of the Microdialysis Focus Group (MDFG), welcomed all attendees to the meeting and reviewed the accomplishments and activities of the focus group over the past year. Highlights were: 1) an informative listings of the posters and programming pertaining to Microdialysis at 2015 Annual meeting was provided 2) 6 programming ideas were submitted by focus group within last year, out of which, 5 were accepted for annual and NBC meetings 3) focus group accomplished a very successful programming year with having two symposium, a roundtable, and a sunrise session in 2015 annual meeting as follows:

1. Systems Approach to Biomarker inquiries in Drug Discovery and Development: Can We, Should We, and Integrate Microdialysis with Imaging Modalities?
2. Microdialysis vs Imaging: Us vs Them, or United We Stand?
3. Bio-Equivalence Standards for Topicals (B E S T): Evidence for Integrating Multiple Quality (Q3) and Performance Tests to Evaluate the Best Generic Products
4. “Nuts and Bolts” of bioavailability trial design for local delivery and what do you do with the data?
4) publication of two issues of Probes. The high level of graduate student participation in the steering committee, particularly in contributing to Probes articles, was also acknowledged with appreciation. Following the annual activities report, Dr. Stratford led a discussion related to programming proposals for the 2016 annual meeting. Several ideas were presented and ultimately led to our focus group submitting four proposals and collaborating on a workshop for 2016 annual meeting. Collaborations with several focus groups, within and outside of the PPDM section, were part of these proposals. MDFG was also included in a proposal submitted by the bioanalytical focus group entitled “challenges in the development of peptide antibiotics: PK/PD and bioanalytical considerations”.

Dr. Stratford also requested for the continued support and efforts from the focus group and encouraged further programing submission aligning to the sections goals. Dr. Stratford concluded his services as the chair of the focus group after two years and expressed his sincere thanks to all the steering committee members. On behalf of MDFG, Shabnam Sani, the incoming chair of the focus group, thanked Dr. Stratford for his exemplary leadership and all the outgoing members who had completed two years of their services. Members who have completed two years were Ami Joshi, Jaydeep Mehta, and Charchil Vejani. She also encouraged participation and active involvement of the committee members.
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