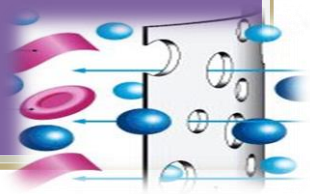
PROBES

A publication of the AAPS Microdialysis Focus Group

July 2017
Volume 6. Issue 2



PROBING FURTHER...

Notes from the Editors

By Hiren Patel and Shabnam Sani

The Microdialysis Focus Group steering committee proudly presents the July 2017 issue of Probes. This issue focuses on the contribution of target site metabolism to CNS drug response. The "Special Feature" section provides a brief, yet seminal overview of the field coupled with the "Pearls of Wisdom" section with profile of Dr. Rachel Tyndale's career. Briefly, Dr. Rachel Tyndale is a leading scientist in this field and a head of Pharmacogenetics at the Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, and the Canada Research Chair in Pharmacogenomics. She is also a Professor in the Department of Pharmacology & Toxicology, and Psychiatry, at the University of Toronto. Dr. Tyndale has supervised over 100 scientists, post-doctoral fellows, and graduate students. She has published over 300 original research manuscripts and book chapters, and has given over 200 invited presentations. She is the recipient of over 50 awards in both basic and clinical pharmacology and neuroscience.

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.

INSIDE THIS ISSUE

Special Feature:

Contribution of Target Site Metabolism to CNS Drug Response

By Robert E. Stratford, Jr., Ph.D.

Therapeutic and/or adverse effects of several CNS drugs are either known or suspected to be linked to the disposition of their metabolites. Furthermore, there is substantial inter-patient variability in these therapeutic and untoward effects. Through the work of Dr. Rachael Tyndale at the University of Toronto, our understanding of how genetic, environmental, and age-related factors that govern the expression and activity of drug metabolizing enzymes expressed in brain contribute to this variability has grown considerably[1,2,3].

The observation more than 20 years ago that clinical response to CNS acting drugs does not always correlate to plasma drug levels[4] has served as a stimulus for investigating possible causes of such disconnects. Measurement of drug exposure in brain relative to plasma exposure has suggested that metabolism in brain tissue may contribute to this discrepancy. For instance, brain levels of the anesthetic propofol, which is metabolized by CYP2B6, correlate better with sleep duration than systemic exposure[5]. This observation is only an example of a host of others that has led to studies evaluating metabolite disposition in the brain[2]. Several P450s are known to be expressed in brain[6]. Of these, two have received a great deal of attention in Dr. Tyndale's lab with respect to their contributions to inter-patient variability in drug and metabolite exposure, and CNS drug response: CYP2B6 and CYP2D6.

CYP2B6 in humans, and its related gene products in the CYP2B family in other species, metabolize nicotine, which is responsible for the pharmacologic effects that support tobacco dependence. Expression of this enzyme is highly polymorphic. One variant, CYP2B6*6, has been shown to have reduced expression in brain[7], and individuals with this variant have been found to have an increased likelihood of becoming smokers[8], and to relapse during quitting attempts versus normal metabolizers[9,10]. Studies conducted in rats have shown that localized inhibition of CYP2B in brain increased nicotine brain levels measured by microdialysis, but did not alter systemic nicotine exposure[11]. These studies also connected the higher nicotine levels in brain to behaviors associated with increased nicotine dependence. Brain levels of CYP2B have also been found to be induced by nicotine in humans and animals[3]. Given that CYP2B6 is responsible for metabolism of other CNS drugs, such as bupropion, ketamine and propofol, as well as endogenous substrates such as serotonin and anandamide[2], variability in drug effects and behaviors between smokers and non-smokers due to variable CYP2B6 expression in the brain would not be surprising. Such differences have been noted for propofol[12].

CYP2D6 expression is also highly polymorphic, and similar to CYP2B6, it participates in the metabolism of both CNS drugs and endogenous substrates[2]. Individuals with lower expression of CYP2D6 in the liver also have lower expression in the brain[13]. The significance of brain CYP2D6 in the action of the analgesic drug codeine has been demonstrated in Dr. Tyndale's lab[14]. Codeine, which has lower potency at mu opioid receptors than morphine[15], is converted to morphine by CYP2D in animals and humans[16], and is more permeable to the blood brain barrier than is morphine[17], thus it can be considered as a prodrug

of morphine. Studies have shown that rats receiving localized brain administration of CYP2D6 inhibitors by intracerebroventricular administration have lower analgesia, and lower morphine concentrations and morphine-to-codeine concentration ratios in brain (but not in plasma) at early time points compared to controls following subcutaneous administration of codeine. As well, analgesia is correlated with brain morphine exposure, but not with plasma morphine concentrations. Interestingly, smokers have higher expression of CYP2D6 in brain than non-smokers, and chronic nicotine treatment has been shown to increase CYP2D6 in the brain[18]. Consistent with this higher central expression, smokers have a higher analgesic response to codeine[19]. Other studies have focused on the role of brain CYP2D6 in behavior and susceptibility to neurodegenerative disease. With respect to behavior, CYP2D6 has been found to produce dopamine from tyramine[20], and to produce serotonin from 5-hydroxytryptamine[21], thus the enzyme can be associated with inter-individual differences in personality traits and susceptibility to psychiatric disorders associated with these important neurotransmitters. Microdialysis was used in both of these studies to demonstrate these localized effects of CYP2D6. CYP2D6 poor metabolizers have an increased risk of developing Parkinson's disease[22] and brain CYP2D6 protein levels are lower in patients with this neurodegenerative disease than in age-matched healthy controls[23]. Localized brain delivery of the precursor of the dopaminergic neurotoxin, MPP+, elicits neurodegenerative effects in rodents similar to the neuroanatomical symptoms observed in Parkinson's disease. MPP+ is metabolized by CYP2D6, and inhibiting the enzyme increases the cytotoxic effect of this neurotoxin[24].

The contribution of localized brain metabolism to drug effects is not limited to brain P450s. The recent identification that the analgesic effects of the commonly used drug acetaminophen are in part a consequence of its conjugation to arachidonic acid in the brain by the enzyme fatty acid amide hydrolase (FAAH)[25,26] speak to this. Studies using FAAH knockout mice coupled with systemic delivery of brain permeable versus non-permeable inhibitors of this enzyme[27], or intracerebroventricular administration of capsazepine[28] demonstrated that brain-derived enzyme was responsible for metabolism of acetaminophen to a conjugate that was a potent TRPV1 (transient receptor potential vanniloid 1) agonist.

The above cases hopefully provide a sense of the effects that brain tissue metabolism can have on inter-patient variability in CNS drug response and susceptibility to CNS disorders. As Dr. Tyndale concludes in one of her many review articles on the subject, "Although there have been substantial advances in knowledge of brain CYPs, much remains to be explored…."[2]. In this regard, microdialysis can play an important role in the field of target site metabolism contributions by providing rich PK data of brain relevant concentrations of drug and metabolite to support modeling and simulation of drug and metabolite uptake and efflux from the brain, and as a convenient means of inhibiting brain specific metabolism, through localized delivery, to demonstrate the contributions of target site metabolism to inter-patient variability.

References

1. Ferguson, C. S., et al., Cytochrome P450 enzymes in the brain: emerging evidence of biological significance. Trends Pharmacol Sci., 2011. 32(12): 708-714.
2. Miksys, S., et al., Cytochrome P450-mediated drug metabolism in the brain. J Psychiatry Neurosci., 2013. 38(3): 152-163.

3. Foti, R. S., et al., "Target-Site" Drug Metabolism and Transport. Drug Metab Dispos, 2015. 43(8): 1156-1168.
4. Michels, R., et al., Progress in psychiatry (1). N Engl J Med., 1993. 329(8): 552-560.
5. Khokhar, J. Y., et al., Drug metabolism within the brain changes drug response: selective manipulation of brain CYP2B alters propofol effects. Neuropsychopharmacology, 2011. 36(3): 692-700.
6. Hedlund, E., et al., Cytochrome P450 in the brain; a review. Curr Drug Metab, 2001. 2(3): 245-263.
7. Miksys, S., et al., Smoking, alcoholism and genetic polymorphisms alter CYP2B6 levels in human brain. Neuropharmacology, 2003. 45(1): 122-132.
8. Hoffmann, E., et al., CYP2B6 and CYP2A6 Genotype: Impact on Acquisition of ICD10 Tobacco Dependence in Caucasian Adolescents. Society for Research on Nicotine and Tobacco (2006): Orlando, FL, USA.
9. Lee, A. M., et al., CYP2B6 genotype alters abstinence rates in a bupropion smoking cessation trial. Biol Psychiatry, 2007. 62(6): 635-641.
10. Lerman, C., et al., Translational research in medication development for nicotine dependence. Nat Rev Drug Discov, 2007. 6(9): 746-762.
11 Garcia, K. L., et al., Effect of Brain CYP2B Inhibition on Brain Nicotine Levels and Nicotine Self-Administration. Neuropsychopharmacology, 2015. 40(8): 1910-1918.
12. Lysakowski, C., et al.,The effect of cigarette smoking on the hypnotic efficacy of propofol. Anaesthesia, 2006. 61(9): 826-831.
13. Miksys, S., et al., Regional and cellular expression of CYP2D6 in human brain: higher levels in alcoholics. J Neurochem, 2002. 82(6): 1376-1387.
14. Zhou, K., et al., First demonstration that brain CYP2D-mediated opiate metabolic activation alters analgesia in vivo. Biochem Pharmacol, 2013. 85(12): 1848-1855.
15. Adler, T. K., et al., The metabolic fate of codeine in man. J Pharmacol Exp Ther, 1955. 114(3): 251-262.
16. Pert, C. B. et al., Properties of opiate-receptor binding in rat brain. Proc Natl Acad Sci U S A., 1973. 70(8): 2243-2247.
17. Bouw, M. R., et al., Pharmacokinetic-pharmacodynamic modelling of morphine transport across the blood-brain barrier as a cause of the antinociceptive effect delay in rats--a microdialysis study. Pharm Res, 2000. 17(10): 1220-1227.
18. Mann, A., et al., Induction of the drug metabolizing enzyme CYP2D in monkey brain by chronic nicotine treatment. Neuropharmacology, 2008. 55(7): 1147-1155.
19. McMillan, D. M. et al., Nicotine Increases Codeine Analgesia Through the Induction of Brain CYP2D and Central Activation of Codeine to Morphine. Neuropsychopharmacology, 2015. 40(7): 1804-1812.
20. Bromek, E., et al., Cytochrome P450 mediates dopamine formation in the brain in vivo. J Neurochem, 2011. 118(5): 806-815.
21. Haduch, A., et al., The cytochrome P450 2D-mediated formation of serotonin from 5-methoxytryptamine in the brain in vivo: a microdialysis study. J Neurochem, 2015. 133(1): 83-92.
22. McCann, S. J., et al., The association between polymorphisms in the cytochrome

P-450 2D6 gene and Parkinson's disease: a case-control study and meta-analysis. J Neurol Sci, 1997. 153(1): 50-53.
23. Mann, A., et al., The neuroprotective enzyme CYP2D6 increases in the brain with age and is lower in Parkinson's disease patients. Neurobiol Aging, 2012. 33(9): 2160-2171.
24. Mann, A. et al., Cytochrome P450 2D6 enzyme neuroprotects against 1-methyl-4-phenylpyridinium toxicity in SH-SY5Y neuronal cells. Eur J Neurosci, 2010. 31(7): 1185-1193.
25. Hogestatt, E. D., et al., Conversion of acetaminophen to the bioactive N-acylphenolamine AM404 via fatty acid amide hydrolase-dependent arachidonic acid conjugation in the nervous system. J Biol Chem, 2005. 280(36): 31405-31412.
26. Mallet, C., et al., Endocannabinoid and serotonergic systems are needed for acetaminophen-induced analgesia. Pain, 2008. 139(1): 190-200.
27. Dalmann, R., et al., Supra-spinal FAAH is required for the analgesic action of paracetamol in an inflammatory context. Neuropharmacology, 2015. 91: 63-70.
28. Mallet, C., et al., TRPV1 in brain is involved in acetaminophen-induced antinociception. 2010. PLoS One 5(9).

Pearls of Wisdom:

A Scientist Profile of Dr. Rachel Tyndale

Dr. Rachel Tyndale is a head of Pharmacogenetics at the Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, and the Canada Research Chair in Pharmacogenomics. She is also a Professor in the Department of Pharmacology & Toxicology, and Psychiatry, at the University of Toronto.

Dr. Tyndale's work mostly focuses on the sources of variation between individuals in drug response in the clinical area of addictions and mental health. Addictions adversely affect millions of people; however, the risk for drug dependence and treatment response varies substantially. She seeks to identify and understand risk factors, and underlying mechanisms, in substance abuse and to implement genetic approaches to personalize the treatment. Her work mainly focuses on how genetic variation in drug metabolism and drug targets alters this risk, using extensive biomarker, phenotypic, pharmacokinetic, datasets, imaging, and pharmacogenomic approaches. Research in this area spans preclinical molecular biology through to leading the first prospectively randomized pharmacogenomic trial in addictions. Her laboratory program also has a major interest in understanding how inter-individual variation in drug metabolizing enzymes within the brain alters drug and toxin effects. Her work in this area seeks to understand how variable drug metabolism in the brain alters CNS drug levels and response, identifying novel mechanisms underlying variation in drug response, risk for addiction and therapeutic failure, leading to treatment optimization and new therapeutic targets. This work has produced many examples of drugs and neurotoxins which are activated or inactivated within the brain resulting in differential response and behaviors. This attempt has led her to use microdialysis as an additional tool for understanding the localized drug metabolism within the brain.

Dr. Tyndale serves on numerous scientific advisory councils and external advisory boards including the Canadian Centre on Substance Use and Addiction, Brain Canada, the Peter Boris Centre for Addictions Research, McMaster University, and the International Advisory Committee of Microsomes and Drug Oxidations. She has served on a number of scientific committees including being the chair of steering committee for NIH's PharmacoGenomic Research Network (PGRN.org), Clinical Pharmacogenetic Implementation Consortium Steering Committee, the Riken:Pharmacogenomics Research Network (Japan:NIH) executive committee and the International CYP nomenclature steering committee. In addition, she is on the editorial board for Trends in Pharmacological Sciences and is an associate editor for Clinical Pharmacology and Therapeutics and for Pharmacology & Therapeutics. She is currently serving as a lead writer for the

2018 Surgeon General's report on Tobacco Cessation.

Dr. Tyndale has supervised over 100 scientists, post-doctoral fellows, and graduate students. She has published over 300 original research manuscripts and book chapters, and has given over 200 invited presentations. She is the recipient of over 50 awards in both basic and clinical pharmacology and neuroscience. Since 2016, she has been honored with the Faculty of Medicine Graduate Teaching Award for Sustained Excellence in Graduate Teaching and Mentorship, and she was the Endowed Chair in Addictions and became the Canada Research Chair in Pharmacogenomics. She became an Honorary Fellow for the Society for Research on Nicotine and Tobacco, gave the Peter Boris Lecture on the Science of Addiction (McMaster University) and the 12th annual Gruber Lecturer (An endowed presentation that honors the first Chair of Pharmacology Thomas Jefferson University, Philadelphia); she was featured at the ECNP meeting, in Research2Reality http://research2reality.com/ and the American Society for Clinical Pharmacology and Therapeutics Featured Career in Clinical Pharmacology and Therapeutics. Other significant lifetime awards include Wendy and Stanley Marsh 3 Endowed Lectureship in Pharmacology and Neurochemistry of Substance Abuse/Addiction Disorders; the Society for Research in Nicotine and Tobacco Langley Award, the Canadian College of Neuropsychopharmacology Heinz Lehmann Award, and the Ochsner Award Relating Smoking and Health from the American College of Chest Physicians.

Dr. Hiren Patel in conversation with Dr. Rachel Tyndale

Could you please provide your personal experience with microdialysis? How did you get inclination towards the technique and a little history behind how you started working on microdialysis?

The main research interests in our laboratory center on inter-individual differences in drug response are focusing on the genetic and environmental differences in drug metabolism. Our clinical areas of interest include research in addictions and mental health, and specifically relevant to the use of microdialysis is our examination of drug and toxin metabolism within the brain. We want to investigate why people differ in their response to centrally acting drugs and neurotoxins, and to understand how metabolism within the brain contributes to this difference. Most drug metabolism takes place in the liver, and genetic differences in drug metabolizing enzymes account for much of the variation between individuals. However, these drug metabolizing enzymes also function in the brain, where they are regulated differently from their hepatic counterparts. We have shown that metabolism within the brain affects drug response meaningfully and independently from hepatic metabolism. To demonstrate this, we induced or inhibited brain drug-metabolizing enzymes and observed changes in central drug levels and behavioral and biochemical responses; liver enzymes remained unaltered and there was no change in plasma drug levels. To explore these observations further, we examined how altering the activity of these enzymes alters local brain extracellular, unbound drug and metabolite levels, and then determined how these altered activities correlate with the drug response. In the past, we have measured drug levels in whole post-mortem brain or a brain region of interest. However, the brain is a very

heterogeneous organ, with tissues that differentially sequester and bind drugs and metabolites, using microdialysis to assess unbound drug and metabolite levels in the brains of living, responding animals improves our ability to correlate brain drug profiles, neurotransmitter release, and altered drug response in real time.
We initially implemented microdialysis to measure nicotine metabolism and resulting nicotine levels in rat brain. Specifically, we demonstrated that inducing the nicotine drug metabolizing enzyme in the brain, but not in the liver, lowered brain nicotine levels by increasing its metabolism centrally; plasma levels were unaltered. We then selectively inhibited the enzyme in the brain through localized delivery of a mechanism-based inhibitor, which reversed the impact of enzyme induction on brain nicotine levels.

Please share your thoughts on application of microdialysis in the research conducted by you.

Microdialysis is underutilized in neuroscience research, particularly for the measurement of local unbound drug (or toxin) and metabolite, and in the simultaneous measurement of endogenous compounds. For example, in combination with animal models of opioid analgesia and manipulations of brain drug metabolism, we use microdialysis to directly assess changes in brain drug and metabolite levels, to measure subsequent changes in neurotransmitter levels, and to associate these with the opioid analgesic properties. We are currently expanding these studies to include models of drug dependence behaviors. In addition to our focus on brain drug metabolism, microdialysis within the brain can be used to answer a myriad of questions regarding the impact of drug and toxin levels on endogenous compounds and behavioral and biochemical outcomes.

According to you, what is the potential of microdialysis and the challenges associated with microdialysis and metabolism.

Microdialysis is a powerful tool for the continuous measurement over the time of unbound drug, metabolite, and endogenous compounds in the extracellular space in the brain of a living animal. The associated technology has advanced considerably with the development of improved materials and reduction in probe size. Microdialysis has the potential to be very useful for neuropharmacology and pharmacokinetic research, enabling the continued search for drug targets, particularly for diseases of the central nervous system, and elucidating the role of transporters at the blood brain barrier and within the brain. There are plenty of areas within the field of neuroscience that would benefit from using microdialysis in conjunction with other techniques, such as behavioral testing and endogenous compound assessment.

Please provide your opinion about the approach of the young scientists in generating interest in microdialysis as a potential technique and explore more about the technique.

The field is still relatively new and the technique is greatly underutilized in neuroscience, for drug level assessment, and to a lesser extent for neurotransmitters assessment. Microdialysis provides many opportunities for young scientists to develop new, innovative approaches to address diverse topics. With that being said, it is a tool; the primary goal is to develop good research questions in which microdialysis is a technique to facilitate its inquiry. Many existing research questions and approaches could benefit from incorporating microdialysis; it is important to encourage young researchers to be versatile when selecting

appropriate techniques to address their scientific questions.

The future of microdialysis from your point of view.

The role of extrahepatic drug metabolism in drug response and abuse liability is a new and growing area of research. I envision that microdialysis will play a greater role in my own work in this area, as well as in the work of others. In my lab, we strive to understand the complex interplay between changes in drug and metabolite levels over the time in the brain, the neurotransmitter release that this elicits, and the corresponding behavioral and biochemical consequences. It allows delivery of drugs and toxins locally, which can aid in mechanistic understanding. It also enables us to refine our animal work compared to other approaches, which often involve sacrificing many animals at many time points to estimate brain levels of drug, metabolite, and endogenous compounds over time. Microdialysis has an important role in detecting unbound drug and metabolite levels in plasma and in tissues of interest, contributing importantly to physiologically-based pharmacokinetic modelling. Further improvements in the detection and quantification of drugs and metabolites in microdialysate with the advancing technologies of HPLC, LCMS and GC ensure that the applications of microdialysis continue to expand beyond what is currently possible; the potential is immense.

What are the challenges of applying microdialysis in your lab setting? (e.g. cost, time, availability of equipments and experienced personnel, acceptability, etc.)

A microdialysis probe can be placed into the brain ventricles or into a brain region of interest, each of which presents common and unique challenges. There are obvious technical issues such as probe calibration, adsorption to the probe, breakdown of compounds during sampling, sampling in a relatively closed space, altered kinetics due to flow rates, tissue damage, etc. One of the challenges we face is the technical aspect of animal surgery to implant guide cannulae into the brain, and maintenance of cannulae patency and integrity for sufficient time to conduct microdialysis in conjunction with animal behavioral experiments. Another major challenge is to develop analytical assays that are sufficiently sensitive to quantify compounds of interest, e.g. drugs and neurotransmitters in the dialysate, while keeping the drug treatment doses in a relevant range. This may be even more relevant for low dose toxicity studies. These various types of challenges can be overcome, but they often take time and can be costly, which can be a deterrent.

Notes from Dr. Hiren Patel: The interview with Dr. Tyndale ended cordially and the steering committee thanked Dr. Tyndale for sharing with us her “Pearls of Wisdom”.

TECHNICAL OVERVIEW:

Microdialysis Further: Application in the Detection of Drug Metabolism

By Benjamin A. Kuzma

The technique of microdialysis allows the researchers to understand the availability of active moiety at the target site by continuous monitoring of the drug/neurotransmitter/metabolite of interest. This is particularly important within the brain and the CNS, which are very delicate areas. The miniature nature of microdialysis probes can monitor biochemical changes within the brain – particularly in terms of drug metabolism and relevant metabolites.

In the absence of microdialysis technique, an animal represents a single time point or experiment[1,2] but with the implementation of the technique, a continuous Pharmacokinetic (PK) brain profile can be obtained which therefore reduces the variability that is seen within animal data. Brain microdialysis was used in a rat model to understand how increased CYP expression can reduce the effect of a general anesthetic (by 2.5-fold), propofol, especially if nicotine was given for 7 days prior to the administration of the propofol. The researchers had induced/inhibited the CYP2B expression, which in turn, demonstrated that the duration of sleep was correlated to the brain concentrations of propofol and not those of plasma[1].

Another group of researchers investigated the CYP2D mediated metabolism of tyramine in the synthesis of dopamine from an alternative pathway[3]. Microdialysis was used in this study as a second model to understand the synthesis of dopamine. The other model was sacrificing rats at certain time points to analyze the formation of dopamine, based upon the administration of drugs. Reserpine and a-Methyl-p-tyrosine were administered to deplete dopamine stored in vesicles and to block dopamine formation from tyrosine, respectively. Retrodialysis was used to administer the drugs – tyramine and quinine. The researchers found that the administration of tyramine and quinine together had extracellular striatum dopamine levels in the brain that were significantly lower than the group that had tyramine administered alone. The comparison of techniques showed that the microdialysis technique can be used to demonstrate the functioning of the CYP2D-mediated synthesis of dopamine *in-vivo*. This study suggests more selective inhibitors and inducers for brain CYP2D isoforms to understand the pharmacological effects on drugs metabolized and possible treatment of neurodegenerative and psychiatric diseases.

In a comparable study, the regeneration of serotonin was increased with the administration of quinine. This study was conducted in a similar fashion to the one mentioned above (two models – one being microdialysis)[4]. These findings indicate that there is an alternative pathway for serotonin synthesis from 5-methoxytryptamine, which can be considered in the pharmacological activity of drugs as a potential target for therapeutic activity.

There are many drug-drug interactions that may occur in metabolism or delayed metabolism of the drug of interest, and while this occurs mainly in the liver, it can also occur in the brain. Wang et al reported a drug-drug interaction in-situ with administration of tramadol, nicotine, and propranolol simultaneously. They found that the administration of propranolol with tramadol inhibited the metabolism of tramadol in the brain due to the decreased level of O-desmethyltramadol (major metabolite of tramadol)[2]. They also found

an increase in the level of O-desmethyltramadol after pre-treatment with nicotine, indicating that there was increased metabolism of tramadol in the brain, while the nicotine metabolism did not change in the blood – indicating that expression of hepatic CYP2D remains unchanged.

Variation of the enzymatic level within the brain may alter the pharmacological and toxicological response. The researchers at University of Toronto, gave either C8-xanthate(CX8) or artificial cerebral spinal fluid (ACSF) intracerebrally to understand the effect of inhibition of cerebral nicotine levels as well as the nicotine levels in the plasma. Microdialysis was used to measure the brain concentration of nicotine over the time and found the administration of CX8 increased the level of nicotine circulating within the brain[5]. Along with the increased nicotine levels in the brain, the nicotine mediated behaviors such as "asking for more nicotine" through a behavioral study. The rats that had a higher concentration of nicotine were more active in pushing a lever to indicate the rats wanted a dose of nicotine. It was determined that the CX8 inhibited the CYP2B and brain levels of CYP2B could be altered without changing liver expression of CYP2B. Similarly, brain drug-drug interactions can be as important as hepatic drug-drug interactions as drugs or environmental factors, such as smoking can alter the drug response producing a clinically relevant alteration.

In a similar study, CYP2B was also investigated to understand the increase in the metabolic activity in smokers. The researchers used brain microdialysis to understand if the induction of the CYP2B enzyme decreased the nicotine levels. They also found that chronic administration of nicotine led to lower brain levels, as measured by brain microdialysis compared to the group that had an acute treatment of nicotine[6] (induction can decrease nicotine levels in the brain). This would lead to an increase of nicotine intake to reach similar nicotine levels as a person with an acute treatment of nicotine.

References

1. Khokhar, J.Y., et al., Drug metabolism within the brain changes drug response: selective manipulation of brain CYP2B alters propofol effects. Neuropsychopharmacology. 2011 Feb;36(3):692-700.
2. Wang, Q., et al., Regulation of cerebral CYP2D alters tramadol metabolism in the brain: interactions of tramadol with propranolol and nicotine. Xenobiotica. 2015 Apr;45(4):335-44.
3. Bromek, E., et al., Cytochrome P450 mediates dopamine formation in the brain in vivo. J Neurochem. 2011 Sep;118(5):806-15.
4. Haduch, A., et al., The cytochrome P450 2D-mediated formation of serotonin from 5-methoxytryptamine in the brain in vivo: a microdialysis study. J Neurochem. 2015 Apr;133(1):83-92.
5. Garcia, K.L., et al., Effect of Brain CYP2B Inhibition on Brain Nicotine Levels and Nicotine Self-Administration. Neuropsychopharmacology. 2015 Jul;40(8):1910-8.
6. Garcia, K.L., et al., Brain CYP2B induction can decrease nicotine levels in the brain. Addict Biol. 2016 May 27.

Microdialysis based literature published from january 2017 to june 2017

1. Alves I.A., et al., Influence of Experimental Cryptococcal Meningitis in Wistar Rats on Voriconazole Brain Penetration Assessed by Microdialysis. Antimicrob Agents Chemother. 2017 Jun 27;61(7)
2. Bancirova M., Paramecium caudatum as a source of nitric oxide: Chemiluminescent detection based on Bluestar(®) Forensic reagent connected with microdialysis. Luminescence. 2017 Jun 5. [Epub ahead of print]
3. Bank J.H.H., et al., Gene expression analysis and microdialysis suggest hypothalamic triiodothyronine (T3) gates daily torpor in Djungarian hamsters (Phodopus sungorus). J Comp Physiol B. 2017 Jul; 187(5-6):857-868.
4. Bazzu G., et al., Microdialysis as a New Technique for Extracting Phenolic Compounds from Extra Virgin Olive Oil. J Agric Food Chem. 2017 Mar 1;65(8):1829-1835.
5. Benade V., et al., Mechanistic evaluation of tapentadol in reducing the pain perception using in-vivo brain and spinal cord microdialysis in rats. Eur J Pharmacol. 2017 Aug 15;809:224-230.
6. Bernardi P.M., et al., Application of a LC-MS/MS method for evaluating lung penetration of tobramycin in rats by microdialysis. J Pharm Biomed Anal. 2017 Feb 5;134:340-345.
7. Calderón N., et al., A ketogenic diet modifies glutamate, gamma-aminobutyric acid and agmatine levels in the hippocampus of rats: A microdialysis study. Neurosci Lett. 2017 Mar 6;642:158-162.
8. Carpenter K.L., et al., Advanced monitoring in traumatic brain injury: microdialysis. Curr Opin Crit Care. 2017 Apr; 23(2): 103-109.
9. Chen X., et al., Influence of peptide transporter 2 (PEPT2) on the distribution of cefadroxil in mouse brain: A microdialysis study. Biochem Pharmacol. 2017 May 1;131:89-97.
10. Das C., et al., Multiplexed and fully automated detection of metabolic biomarkers using microdialysis probe. Sens Actuators B Chem. 2017 Jan;238:633-640.
11. Deitchman A.N., et al., Utility of Microdialysis in Infectious Disease Drug Development and Dose Optimization. AAPS J. 2017 Mar;19(2):334-342.
12. Fong S.Y., et al., A novel microdialysis-dissolution/permeation system for testing oral dosage forms: A proof-of-concept study. Eur J Pharm Sci. 2017 Jan 1;96:154-163.
13. Griffin A.E., et al., Development of microdialysis methodology for interstitial insulin measurement in rodents. J Pharmacol Toxicol Methods. 2017 Apr 6;86:67-75.
14. Gulati G.K., et al., Programmable carbon nanotube membrane-based transdermal nicotine delivery with microdialysis validation assay. Nanomedicine. 2017 Jan;13(1):1-9.
15. Gustafsson S., et al., Combined PET and microdialysis for in vivo estimation of drug blood-brain barrier transport and brain

unbound concentrations. Neuroimage. 2017 Apr 30;155:177-186.

16. Hifumi T., et al., Association of brain metabolites with blood lactate and glucose levels with respect to neurological outcomes after out-of-hospital cardiac arrest: A preliminary microdialysis study. Resuscitation. 2017 Jan;110:26-31.
17. Hu Y., et al., The Impact of Liposomal Formulations on the Release and Brain Delivery of Methotrexate: An In Vivo Microdialysis Study. J Pharm Sci. 2017 Mar 18.
18. Huang H.C., et al., Components of Goutengsan in Rat Plasma by Microdialysis Sampling and Its Protection on Aβ1-42-Induced PC12 Cells Injury. Evid Based Complement Alternat Med. 2017;2017:7593027.
19. Ilias I., et al., Microdialysis-Assessed Adipose Tissue Metabolism in Critically Ill Patients. Recent Pat Endocr Metab Immune Drug Discov. 2017 Jun 8. [Epub ahead of print]
20. Jadhav S.B., et al., Tissue Distribution of a Therapeutic Monoclonal Antibody Determined by Large Pore Microdialysis. J Pharm Sci. 2017 Apr 13. [Epub ahead of print]
21. Joshi A, et al., Pharmacokinetic applications of cutaneous microdialysis: Continuous+intermittent vs continuous-only sampling. J Pharmacol Toxicol Methods. 2017 Jan - Feb;83:16-20.
22. Kala A, et al., Development and validation of LC-MS/MS methods for the measurement of ribociclib, a CDK4/6 inhibitor, in mouse plasma and Ringer's solution and its application to a cerebral microdialysis study. J Chromatogr B Analyt Technol Biomed Life Sci. 2017 Jul 1;1057:110-117.
23. Kho, C.M., et al., A Review on Microdialysis Calibration Methods: the Theory and Current Related Efforts. Mol Neurobiol. 2017 Jul;54(5):3506-3527.
24. Kodama T., et al., Oral Administration of Methylphenidate (Ritalin) Affects Dopamine Release Differentially Between the Prefrontal Cortex and Striatum: A Microdialysis Study in the Monkey. J Neurosci. 2017 Mar 1;37(9):2387-2394.
25. Kong W.M., et al., Evaluation of pharmacokinetics and blood-brain barrier permeability of mitragynine using in vivo microdialysis technique. J Pharm Biomed Anal. 2017 May 13;143:43-47.
26. Lenkin P.I., et al., Continuous Monitoring of Lactate Using Intravascular Microdialysis in High-Risk Cardiac Surgery: A Prospective Observational Study. J Cardiothorac Vasc Anesth. 2017 Feb;31(1):37-44.
27. Liu M., et al., Intracerebral microdialysis coupled to LC-MS/MS for the determination tramadol and its major pharmacologically active metabolite O-desmethyltramadol in rat brain microdialysates. Drug Test Anal. 2017 Jan 4. [Epub ahead of print]
28. Martínez-Valverde T., et al., Characterization of the Ionic Profile of the Extracellular Space of the Injured and Ischemic Brain: A Microdialysis Study. J Neurotrauma. 2017 Jan 1;34(1):74-85.
29. Ngo K.T., et al., Monitoring Dopamine Responses to Potassium Ion and Nomifensine by in Vivo Microdialysis with Online Liquid

Chromatography at One-Minute Resolution. ACS Chem Neurosci. 2017 Feb 15;8(2):329-338.

30. Papadopoulos D., et al., Regional cerebral blood flow and cellular environment in subarachnoid hemorrhage: A thermal doppler flowmetry and microdialysis study. Neurol Neurochir Pol. 2017 Jan - Feb;51(1):66-71.
31. Patet C., et al., Bedside cerebral microdialysis monitoring of delayed cerebral hypoperfusion in comatose patients with poor grade aneurysmal subarachnoid haemorrhage. J Neurol Neurosurg Psychiatry. 2017 Apr;88(4):332-338.
32. Pinczolits A., et al., Standard-sampling microdialysis and spreading depolarizations in patients with malignant hemispheric stroke. J Cereb Blood Flow Metab. 2017 May;37(5):1896-1905.
33. Quist S.R., et al., Preclinical pilot study monitoring topical drug penetration and dermal bioavailability of a peptidase inhibitor from different galenic formulations into pig dermis, using cutaneous microdialysis. Clin Exp Dermatol. 2017 May 29. [Epub ahead of print]
34. Rogers M.L., et al., Simultaneous monitoring of potassium, glucose and lactate during spreading depolarization in the injured human brain - Proof of principle of a novel real-time neurochemical analysis system, continuous online microdialysis. J Cereb Blood Flow Metab. 2017 May;37(5):1883-1895.
35. Sanna F., et al., Dopamine, Noradrenaline and Differences in Sexual Behavior between Roman High and Low Avoidance Male Rats: A Microdialysis Study in the Medial Prefrontal Cortex. Front Behav Neurosci. 2017 Jun 7;11:108.
36. Slimano F., et al., First plasma and tissue pharmacokinetic study of the YSNSG cyclopeptide, a new integrin antagonist, using microdialysis. Eur J Pharm Sci. 2017 Jul 15;105:178-187.
37. Su C.K., et al., Sequential enzymatic derivatization coupled with online microdialysis sampling for simultaneous profiling of mouse tumor extracellular hydrogen peroxide, lactate, and glucose. Anal Chim Acta. 2017 Mar 1;956:24-31.
38. Tabatabaei P., et al., Radiotherapy induces an immediate inflammatory reaction in malignant glioma: a clinical microdialysis study. J Neurooncol. 2017 Jan;131(1):83-92.
39. Tasca G., et al., Muscle Microdialysis to Investigate Inflammatory Biomarkers in Facioscapulohumeral Muscular Dystrophy. Mol Neurobiol. 2017 Apr 29. [Epub ahead of print]
40. Thelin E.P., et al., Microdialysis Monitoring in Clinical Traumatic Brain Injury and Its Role in Neuroprotective Drug Development. AAPS J. 2017 Mar;19(2):367-376.
41. Turkina M.V., et al., Evaluation of dynamic changes in interstitial fluid proteome following microdialysis probe insertion trauma in trapezius muscle of healthy women. Sci Rep. 2017 Mar 7;7:43512.
42. Varner E.L., et al., Enhancing Continuous Online Microdialysis Using Dexamethasone: Measurement of Dynamic Neurometabolic

Changes during Spreading Depolarization. ACS Chem Neurosci. 2017 May 18. [Epub ahead of print]

43. Vidal-Jorge M., et al., Does Normobaric Hyperoxia Cause Oxidative Stress in the Injured Brain? A Microdialysis Study Using 8-Iso-Prostaglandin F2α as a Biomarker. J Neurotrauma. 2017 Apr 26. [Epub ahead of print]
44. Weisenberger M.M., et al., In Vivo Monitoring of Amino Acid Biomarkers from Inguinal Adipose Tissue Using Online Microdialysis-Capillary Electrophoresis. Anal Chem. 2017 Jan 3;89(1):1009-1014.
45. Wittau M., et al., Population Pharmacokinetics and Target Attainment of Ertapenem in Plasma and Tissue Assessed via Microdialysis in Morbidly Obese Patients after Laparoscopic Visceral Surgery. Antimicrob Agents Chemother. 2016 Dec 27;61(1).
46. Yamada K., In Vivo Microdialysis of Brain Interstitial Fluid for the Determination of Extracellular Tau Levels. Methods Mol Biol. 2017;1523:285-296.
47. Yamamoto Y., et al., Microdialysis: the Key to Physiologically Based Model Prediction of Human CNS Target Site Concentrations. AAPS J. 2017 Jul;19(4):891-909.
48. Yang B., et al., Lung microdialysis study of florfenicol in pigs after single intramuscular administration. J Vet Pharmacol Ther. 2017 Jan 19. [Epub ahead of print]
49. Zhang X., et al., Online microdialysis-ultra performance liquid chromatography-mass spectrometry method for comparative pharmacokinetic investigation on iridoids from Gardenia jasminoides Ellis in rats with different progressions of type 2 diabetic complications. J Pharm Biomed Anal. 2017 Jun 5;140:146-154.
50. Zhou Y., et al., A label-free strategy for measuring the affinity between monoclonal antibody and hapten using microdialysis sampling combined with chemiluminescent detection. Biosens Bioelectron. 2017 Jan 15;87:404-409.

EXTRA EXTRA

Upcoming Microdialysis related program accepted for 2017 AAPS Annual Meeting, San Diego, California

Novel Oncology Modalities: Opportunities & Challenges in Biomarkers and Biopsies

Aim of the program

Some of the challenges in developing novel anticancer therapies are deficiencies in early diagnosis of cancer type and lack of biomarkers or alternative approaches for early prediction of efficacy as well as lack of assessment of tumor tissue concentrations of a drug for better determination of exposure-response relationships. This symposium aims to educate and stimulate thinking on these issues through diverse presentations representing different practical vantage points from the development of non-invasive methods such as liquid biopsies for early cancer diagnosis to the assessment of drug exposure in tumors (microdialysis) and the critical role of biomarkers in cancer therapy, and how each perspective can provide information that can support early diagnosis and optimal dose selection in proof-of-concept clinical trials. Each presenter will provide an overview of the particular modality followed by examples of its use to support early diagnosis and translational modeling for the selection of optimal dose of anticancer drugs.

The following modalities will be represented: 1) microdialysis for the measurement and prediction of free drug concentrations in tumor extracellular fluid; 2) The role of Quantitative Systems Pharmacology (QSP) in identification and development of immuno-oncology biomarkers 3) An overview of the role and application of liquid biopsy techniques in oncology treatment

Speaker 1:
Dr. James Gallo (USA) – Translational microdialysis to predict tumor disposition or
Suggested Presentation Title: *In vivo* microdialysis to support translational PK/PD modelling of anti-cancer drugs

Speaker 2:
Dr. Brian Schmidt (USA), BMS – The role of Quantitative Systems Pharmacology in identification and development of immuno-oncology biomarkers

Speaker 3:
Dr. Steve Shak (USA), Genomic Health (USA) – State of the Science: Liquid Biopsies in Oncology

We're on the Web!

Visit us at:

LinkedIn, AAPS Microdialysis Focus Group

CONTRIBUTORS TO THIS ISSUE

Editors – Drs. Hiren Patel and Shabnam Sani
Special Feature – Dr. Robert E. Stratford, Jr.
"Pearls of Wisdom" – Dr. Hiren Patel in conversation with Dr. Rachel Tyndale
Technical Overview – Benjamin A. Kuzma
Microdialysis Publications – Benjamin A. Kuzma and Dr. Sai Hanuman Sagar Boddu
Questions or Comments? Address correspondence to Dr. Shabnam Sani, ssani@wne.edu