Human Microdialysis

M. Stahl, R. Bouw, A. Jackson and V. Pay, Current Pharmaceutical Biotechnology, 3 (2002) 165-178.

Microdialysis sampling has been employed in human pharmacokinetic studies for about a decade. Human microdialysis studies are being conducted by pharmaceutical companies, and regulatory agencies are recognizing it as a promising technique for determining tissue concentrations in humans.

After a clear and concise description of the microdialysis sampling process, the authors discuss pilot experiments that should be conducted prior to undertaking a large-scale microdialysis study. *In vitro* pilot experiments should establish that analyte recovery is independent of concentration and that diffusion of the compound is equal in both directions across the membrane. A few preliminary *in vivo* experiments are recommended to obtain a target tissue profile without *in vivo* calibration and to determine the appropriate duration of calibration as well as the "washout" time required after calibration.

After reviewing studies of insertion trauma, the authors conclude that one hour is widely accepted as a sufficient interval to allow tissue surrounding the probe site to re-equilibrate. Stahl et al also discuss molecular weight, lipophillicity, and plasma protein binding with respect to microdialysis sampling. While microdialysis sampling is generally thought of as being limited to small water-soluble molecules, high molecular weight and very lipophillic compounds also have been successfully sampled using microdialysis. Microdialysis samples the free drug in the interstitial fluid. If plasma binding for the drug is high, the free concentration available for sampling by the microdialysis probe may be very low. In such cases, feasibility of microdialysis sampling to study the drug depends on the analytical capabilities of the assay method.

In discussing calibration methods, the authors point out the necessity of an *in vivo* calibration method in studies where drug distribution in specific tissues is the goal. Included are descriptions of the no-net flux, dynamic no-net flux and reference techniques. The authors describe microdialysis/PET studies seeing the techniques as complementary, each contributing to elucidation of various receptor, transport and other questions of interest in pharmacology. Microdialysis studies of the antinociceptive effect delay of morphine are presented as a case study illustrating applications of microdialysis in both animals and humans. This is followed by a selection of examples of human *in vivo* microdialysis studies.

Stahl and colleagues present an overview of current regulatory opinion with respect to human clinical application of microdialysis sampling, concluding that regulatory authorities regard microdialysis as a promising means to obtain target/tissue site drug concentrations. The authors present a case for the utility of microdialysis sampling in a number of situations where knowledge of free plasma concentration alone is insufficient to understand the *in vivo* behavior of drugs. They predict that applications of microdialysis sampling will extend to additional categories of compounds as analytical techniques become more sensitive.

A Method to Evaluate the Renin-angiotensin System in Rat Renal Cortex Using Microdialysis Technique Combined with HPLC-fluorescence Detection

T. Kajiro, Y. Nakajima, T. Fukushima and K. Imai, Analytical Chemistry, 74 (2002) 4519-4525.

The renin-angiotensin system (RAS) consists of several peptides (7-10 amino acid residues) and is found in various tissues, including the kidneys. RAS is involved in regulating fluid volume, and thus plays a role in long-term control of blood pressure. Renin cleaves angiotensin resulting in angiontensin I (AngI). Angiotensin converting enzyme (ACE) cleaves AngI to angiotensin II (AngII) which influences several biological processes that change fluid volume and hence, blood pressure. Angiotensin III and angiotensin 1-7 (Ang1-7) also have been characterized.

Kajiro et al used 3-(7-fluro-2,1,3-benxoxadiaxole-4-sulfonamindo) benenesulfonic acid to derivatize the N-terminal amino residue of the peptides for separation and determination using liquid chromatography with fluorescence detection. A size exclusion column performed the initial removal of low molecular weight amino acids. The angiotensin-containing segment of effluent from the size exclusion column was switched to an ODS column. The four analytes of interest were monitored by fluorescence detection ($_{ex}$ = 426 nm, $_{ex}$ = 564 nm).

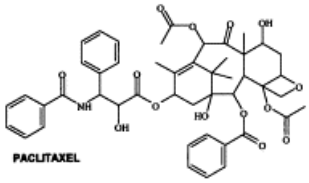
Linear microdialysis probes were implanted in the renal cortex of male Sprague-Dawley rats which remained anesthetized throughout the experiments. Phosphate buffered Ringers' (PB-R) solution was perfused at 0.5 µL/min. Endogenous concentrations of the analytes were not detected in the kidney dialysis samples. When the perfusate contained AngI, locally generated AngII and Ang1-7 were detected. Changing back to PB-R resulted in disappearance of the metabolite peaks. Local generation of Ang1-7 in kidney tissue was dependent over the entire range on the AngI concentrations used in the perfusate. In contrast, generation of AngII was found to be saturable. Co-perfusion of phosphoramidon (a neprilysin inhibitor) with AngI resulted in no Ang1-7 production suggestion that neprilysin is the enzyme responsible for conversion of AngI to Ang1-7 in kidney tissue. Unexpectedly, co-perfusion of captopril (an ACE inhibitor) with AngI did not decrease production of AngII. Thus, enzymes other than ACE may be responsible for conversion of AngI to AngII in the kidneys.

The authors conclude that their liquid chromatography-fluorescent detection method combined with microdialysis is suitable for studying local tissue angiotensin-processing pathways. They anticipate applying the method in freely-moving rats to evaluate further the reinin-angiotensin system in kidney tissues and its role in blood pressure control.

Anticancer Agent

Measurement of Paclitaxel in Biological Matrices: High-Throughput Liquid Chromatographic-Tandem Mass Spectrometric Quantification of Paclitaxel and Metabolites in Human and Dog Plasma

M.S. Alexander, M.M. Kiser, T. Culley, J.R. Kern, J.W. Dolan, J.D. McChesney, J. Zygmunt and S.J. Bannister, J. Chromatogr. B 785 (2003) 253-261.



A validated method for determination of paclitaxel, an anticancer agent for breast and ovarian cancer, was developed by BASi Northwest Laboratory. The goal was to develop a high-throughput assay under GLP guidelines. Plasma samples were extracted with methyl-*tert.*-butyl ether, and the ether fraction was dried down and reconstituted for injection. Separation occurred on a 50 x 2.1 mm C_{18} column, using a water:acetonitrile: acetic acid mobile phase. Detection was by positive ion electrospray MS. The method was linear between 0.117 and 117 nM. QC samples were within 3% of their nominal values, and precision averaged 6% RSD. Because of the cleanliness of the extract, about 2000 samples could be injected before the MS source needed cleaning.

Copper Electrodes for Sugars and Alcohols

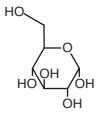
Amperometric Determination of Ethanol in Beverages at Copper Electrodes in Alkaline Medium

T.R.L.C. Paixao, D. Corbo and M. Bertotti, Analytica Chimica Acta 472 (2002) 123-131.

Although Pulsed Amperometric Detection (PAD) is the best known technique for sugars and alcohols, it requires sophisticated equipment. An easier alternative is to determine these compounds at constant potential with a copper (actually copper oxide) electrode. These authors developed a flow-injection apparatus to determine the ethanol content of alcoholic beverages at a constant potential of 650 mV (vs. Ag/AgCl) in a flowing stream of 1 M NaOH. Beverages also contain sugars, which were prevented from reaching the electrode by a PTFE membrane. (Methanol, however, easily crossed this membrane.) The results were linear for injected standards between 2-10% ethanol, and the measured ethanol concentrations of various commercial beverages showed excellent agreement with results from gas chromatography.

Determination of Sugars in Chinese Traditional Drugs by CE with Amperometric Detection

Q. Hu, T. Zhou, G. Hu and Y. Fang, J. Pharmaceut. Biomed. Anal. 30 (2002) 1047-1053.

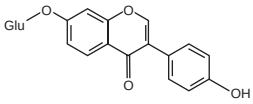


According to these authors, the efficacy of certain traditional Chinese herbs is correlated to their sugar content. (Note that this is a statistical correlation and no cause/effect relationship is implied.) Quantifying the sugar content might then be an aid in quantifying the efficacy of the herbs. A capillary electrophoresis separation was developed, with electrochemical detection of the eluted sugars at a copper electrode. The dried herbs were extracted in methanol and diluted as necessary. Samples were separated on a 45 cm x 25 µm (i.d.) fused silica capillary, using a 50 mM NaOH buffer. A 140 µm diameter copper disk electrode was constructed by gluing a copper wire inside a glass pipet and polishing it flat. The electrode was positioned at the end of the capillary and 650 mV (vs. Ag/AgCl) was applied with a BASi Petite Ampere amperometric detector. Good separations of glucose, sucrose, and fructose were obtained. Results were linear from 5-500 µmol/L and precision averaged 2-3% RSD.

Isoflavone from Soy

Liquid Chromatography Coupled with Multi-Channel Electrochemical Detection for the Determination of Daidzin in Rat Blood Sampled by an Automated Blood Sampling System

F. Tian, Y. Zhu, H. Long, M. Cregor, F. Xie, C.B. Kissinger and P.T. Kissinger, J. Chromatogr. B 772 (2002) 173-177.



Daidzin is an isoflavone abundant in soybeans and some Chinese herbs. It is being investigated for its antioxidant, phytoestrogenic, and anticarcinogenic properties. Reports suggest it also reduces voluntary consumption of ethanol. An assay was developed for daidzin determination in rat plasma, using the BASi Culex® automated blood sampler. Rat plasma collected automatically from freely-moving rats was extracted with ethyl acetate, which was then dried down, reconstituted, and injected onto a microbore column (C_{18}, 100 x 1 mm). A BASi epsilon™ detector was employed, with a quad-glassy carbon working electrode set to four independent potentials (750, 800, 850, 950 mV vs. Ag/AgCl, to facilitate accurate peak identification via peak ratios). Results were linear from 25-1000 ng/mL in rat plasma, and recovery was 75%. The lower limit of quantitation was 15 ng/mL.

Monitoring Molecules in Neuroscience

10th International Conference on *In Vivo* Methods
Karolinska Institutet,
Department of Neuroscience,
Stockholm, Sweden
was held June 24-27, 2003
www.neuro.ki.se/invivo2003
For more information, including abstracts, contact: **Jan Kehr**, *Chairman*, Dept. of Neuro, *jan.kehr@neuro.ki.se,* tel: 46 8 728 7084

During the last 20 years, The Conference on *In Vivo* Methods has become one of the most distinguished international meetings for both academic and industrial researchers active in experimental neuropharmacology and neuropathology. This is further illustrated by the themes of the sessions implementing *in vivo* monitoring technologies, in both the areas of minimal invasive techniques (microdialysis, biosensors) and neuroimaging (fMRI, PET).

Another important mission of this series of meetings is to engage and encourage young scientists and PhD students to direct their future careers toward integrative neuroscience, as represented by combined functional and molecular techniques.

Topics

Affective Disorders: Depression, Anxiety, ADHD
Psychiatric Disorders: Schizophrenia
Drug Abuse
Neurodegenerative Diseases: Alzheimer's, Parkinson's diseases
Neurological Disorders: Stroke, Trauma, Epilepsy, Pain
Clinical Microdialysis (CNS)
Functional Neuroanatomy, Neuroimaging
Neurochemical Correlates to Behaviour
Transgenic Animals: Role of *In Vivo* Techniques in Phenotype Characterization
Voltammetry and Biosensors In Vivo *and* In Vitro
Advances in In Vivo *Monitoring/Sampling Devices/Methods*
Enabling Analytical Technologies
Quantitative Microdialysis, Tissue Damage
Pharmacokinetics/Pharmacodynamics, Blood-Brain Barrier

MicaGenix: A New BASi Partner through PiCRA

MicaGenix is an automated contract toxicology laboratory providing customized GLP testing in genetic toxicology, histopathology and regulatory review. In addition to conventional genetic toxicology testing, namely Ames, Chromosomal Aberration and mouse micronucleus assays for global regulatory submission, the company offers a variety of *in vitro* genetic and spigenetic screening tests, including Comet Assay, Dicentric assay, apoptosis, DNA synthesis and LLNA, for detection of lead candidates for pharmaceutical development.

MicaGenix was founded by Joseph W. Parton, CEO, who had a 35-year career with Eli Lilly and Company, serving as a principal investigator in their Genetic Toxicology Department. During his years at Lilly, Joe assisted in developing the Loats Associated automated micronucleus scoring system, validated the assay, authored the standard operating procedure for GLP compliance and published the manuscript, establishing a footprint for future automation. As a researcher, he was also responsible for conducting all regulatory-required micronucleus and unscheduled DNA synthesis assays.

K. S. Rao (DVM, PhD, DABT) is MicaGenix Director of Toxicology. Dr. Rao has been a board-certified toxicologist since 1980, and for 30 years he has been active in general toxicology, reproductive toxicology, genetic toxicology, mycotoxicosis, safety evaluation/risk assessment of various toxins/chemicals, and regulatory affairs.

Dr. Yong Xu (MD, MPH) heads the company's Mutagenesis Department and has been instrumental in standardizing the Comet Assay. Dr. Xu has conducted extensive researches on mechanisms of tobacco-induced lung cancer and has worked on free radical oxygen-induced changes in chemical carcinogenesis. He is a member of the Society of Toxicology, American College of Forensic Examiners and Association of Mutation. He has extensive teaching experience in toxicology, carcinogenesis and advanced topics in pharmacology and toxicology.

The company is located in Greenfield, Indiana and more information is available on their website, *www.micagenix.com*.

New BASi Scientific Director for Preclinical Services

Gerry M Henningsen, D.V.M., Ph.D., DABT, DABVT, recently joined BASi as Scientific Director for the preclinical services laboratory at Evansville. Dr. Henningsen's broad science education includes a B.S. degree with honors from the U.S. Air Force Academy's School of Aerospace Medicine (1973), a D.V.M. from Iowa State University (1978), and a Ph.D. in Pharmacology/Toxicology from Washington State University (1985). He holds current board certifications as a Diplomate of the American Board of Toxicology and the American Board of Veterinary Toxicology. Dr. Henningsen also trained in immunotoxicology, public health (board-eligible in preventive medicine), pathology (board-eligible), and laboratory animal medicine (board-eligible).

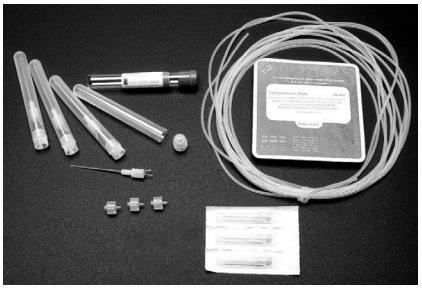
Dr. Henningsen began his science career at the Air Force Academy as Director of Falconry and Base Veterinarian, responsible for public health and zoonotic disease. Following his doctoral training, he was Chief of Aerospace Toxicology at Wright-Patterson AFB where he managed contract research studies on occupational toxicants and began an immunotoxicology program. Dr. Henningsen joined the US Public Health Service in 1987 and served as a Veterinary Research Pharmacologist and then as Chief of Immunochemistry, for the National Institute of Occupational Safety and Health until1992. At NIOSH, he conducted lab research with animal models and field studies of occupationally exposed human subjects in the areas of immunotoxicology, carcinogenesis, biomarkers, aging, and arthritis. Dr. Henningsen was Senior Toxicologist

and Risk Assessor for human health and wildlife at EPA Region 8, in Denver from 1992 to 2001.

In 2001 Dr. Henningsen formed his own consulting company, H & H Scientific Services, in partnership with Dr. David Hobson. Their company provided professional science support to a broad base of clientele, as well as pharmaceutical product development and regulatory advice. Dr. Henningsen is an active member of the AVMA, SOT, ACT, SETAC and related societies.

New Products

Vacuum Needle Accessory for Ultrafiltration



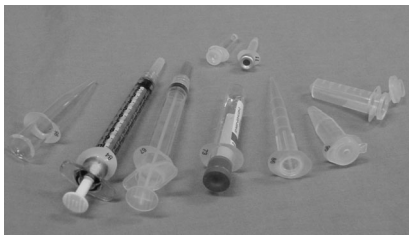
BASi HoneyComb Refrigerated Fraction Collector users can now adapt their instruments for automated collection of ultrafiltrates by using a new vacuum needle accessory. Previously, ultrafiltrates were either collected by (a) exchanging individual vacutainers by hand or (b) using a peristaltic pump to automate collection. The new approach utilizes house vacuum or a portable laboratory vacuum pump and collects smaller volume samples into cold, sealed vials held in the fraction collector. Since the HoneyComb accommodates two needles, it is possible to collect from two separate ultrafiltration probes (from the same animal, or two individually). Use of a vacuum trap lets one vacuum source, or pump, be used for multiple probes. This approach is suitable for both *in vitro* and *in vivo* ultrafiltration using BASi ultrafiltration probes (MF-7023, MF-7025, MF-7026, MF-7027 and MF-7028).

A startup kit (MF-7040) provides four vacuum needles, tubing connectors for ultrafiltration probe tubing, and components for a trap and connection to an external vacuum pump. The needles may be ordered separately (MF-7016) and are disposable to eliminate any possibility of carryover or cross-contamination between studies. Both the needles and the ultrafiltration probes were intended for use with physiological saline or other aqueous solutions. Once the connection to the vacuum source is made, a replacement needle can be substituted easily. The five meters of connecting tubing in the kit makes it possible to move a vacuum pump away from the immediate vicinity of the animal during *in vivo* studies.

As with all current BASi products, no manual is shipped with the kit but an illustrated user's guide is available online at the URL specified on the product sticker.

For more information and photographs, please visit the ***Products*** link at *www.bioanalytical.com* and review the ***Ultrafiltration Sampling*** list under **In Vivo *Sampling, Behavior and Metabolism***.

Chads for Eppendorf Vials



A new size of BASi Chads is now available for standard Eppendorf® tubes and other devices with an outer diameter ranging from 9.5 to 10.8 mm. Small vacutainers, syringes, pipette tips and 1.5 or 2.0 mL Eppendorf tubes were all successfully labeled using this style of Chad. A Chad-rack (CX-1902) supports each sheet of Chads as the item being labeled is punched through the hole in each numbered position. As with other BASi Chads, no adhesives are used and Chads are not affected by freezing (-80°C), common organic solvents or water. The colored, numbered and flexible Chads remain legible throughout common laboratory operations.

Two colors are available: blue (CX-1900B) or white (CX-1900W). This style is available as a set of five sheets providing a total of 480 prenumbered Chads. Sheet 1 covers numbers 1 to 96, sheet 2 covers numbers 97 to 192, sheet 3 covers numbers 193 to 288, sheet 4 covers numbers 289 to 384, and sheet 5 covers numbers 385 to 480. Two sets (1 set = 5 sheets) are provided in each replacement pack of Chads.

Please visit the information on our website at *www.culex.net/chad.html* for more information and ordering details.

New Rodent Cage for Culex Blood Samplers, Rodent Workstations and Microdialysis Applications

The round-bottomed bowl used for caging rodents during microdialysis applications is now available with a removable access panel to facilitate dosing, insertion of intracerebral probes and connection of IV catheters. This cage (MD-1420) is intended for use with bedding materials. An access hole and stainless steel spring have been added for (user's choice) internal or external mounting of an optional water tube (CX-5000). An optional cage lid (MD-1515) is available to discourage escapes.