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The Beat Newsletter

College of Medicine

The Beat

University of South Alabama
College of Medicine

April 1995

SITE-SPECIFIC DRUG DELIVERY

The microporous infusion catheter is a device that allows drugs to be delivered directly to a blocked artery.

Cardiovascular disease remains the leading cause of death in developed countries. A large proportion of cardiovascular mortality is due to coronary artery disease or blockage in the coronary arteries. In the past 10 years, the treatment of coronary artery disease has advanced markedly with a major advance being the development of coronary balloon angioplasty a little over a decade ago.

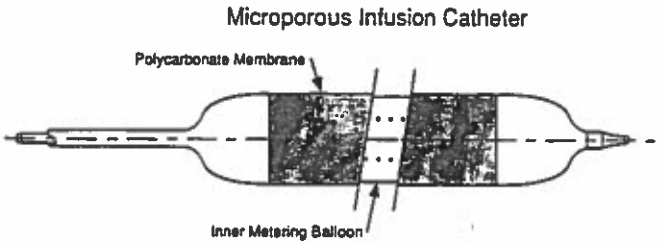
Balloon angioplasty has been joined by other catheter base treatment modalities for coronary disease including directional atherectomy, extraction atherectomy, laser angioplasty, rotational atherectomy and stent implantation. Over 400,000 of these procedures were performed in the United States last year. Unfortunately, although all of these treatment methods have high initial success, they all suffer from restenosis, which is a recurrence of the treated blockage, usually within six months. Restenosis remains the Achilles heel of catheter based therapy for coronary artery disease.

New approaches to the treatment of restenosis have been the major thrust of the research taking place in the laboratory of **Charles R. Lambert, M.D., Ph.D.** in the Division of Cardiology at USA. Dr. Lambert is Director of the Cardiac Catheterization Laboratories and holds the Abraham Mitchell Eminent Scholar's Chair in Cardiology. Millions of dollars have been spent looking for a drug or combination of drugs to prevent restenosis but without success. This led Dr. Lambert and coworkers at the University of Florida College of Medicine to develop the concept of local site specific drug delivery, approximately 5 years ago.

This concept proposed development of a special catheter designed to deliver very high concentrations of a pharmacologic agent to the affected region of the coronary arterial wall immediately following angioplasty. It was hoped this treatment would depress the proliferative processes responsible for restenosis, by achieving very high local concentrations of drugs that could not be reached with systemic administration.

This led to years of research culminating in development of the microporous infusion catheter (see figure). This device allows simultaneous low pressure angioplasty along with delivery of drugs to the site. The device can also be used to deliver drugs locally, independent of angioplasty.

Following his move to USA, Dr. Lambert continued his development of the catheter and has studied it in various animal and in vitro testing systems. A special computer controlled pump has also been designed and constructed to power the catheter and to allow precise control of pressure and volume. Recently parallel research using the catheter has begun at the Cleveland Clinic, Philadelphia Heart Institute, Emory University and the University of Michigan. A multinational trial is about to begin using the microporous infusion catheter to deliver genetic material into coronary arteries of patients.



The microporous infusion catheter.

At present, a clinical trial to be conducted as a physician initiated IDE is being applied for through the FDA. This will be the first U.S. clinical trial of the microporous infusion catheter and should begin in the first quarter of this year and will be conducted at the University of South Alabama Medical Center. It is anticipated that this trial will utilize the microporous catheter to dissolve clots and perform simultaneous angioplasty in patients having an acute heart attack.

HERPES SIMPLEX VIRUS

Research being done to understand the HSV and identify the events that cause ocular infection of the eye.

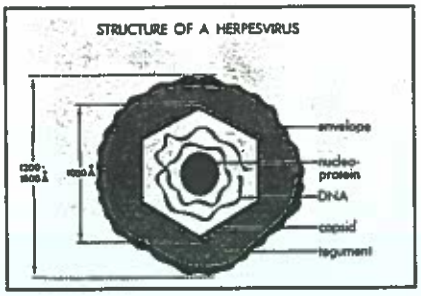
Herpes simplex virus (see figure) is a common human pathogen which can cause infections in the eye and various other parts of the body. When HSV infects the eye the resulting disease can range from being mild with quick recovery, to potential sight damage. Herpes stromal keratitis (HSK) is an example of the latter. In HSK, the virus attacks the cornea, a specialized avascular epidermal tissue which allows light to penetrate the eye. For the eye to function properly the cornea must remain clear. However, when the HSV infection occurs white blood cells migrate from blood vessels into the cornea to fight the pathogen. If the infection is severe enough even the blood vessels themselves may grow into the central cornea. The accumulation and activity of cells and blood vessels can result in a badly inflamed eye. Thus, a host response that is too exuberant in destroying an infectious pathogen can seriously damage sensitive corneal tissue leading to scar formation and possible blindness.

One approach to solving this problem is to identify the critical participants and events that comprise the ocular inflammatory response. Once the mechanism of corneal inflammation is understood it is possible to devise strategies to prevent or limit the damage done to the cornea. It is known that injured cells have the potential to release cytokines and chemokines, small proteins that function as intercellular messengers. Their role in inflammation is to attract white blood cells to sites of infection and activate the migrating cells to clear the virus from infected tissue.

Current efforts by **Drs. Robert Lausch** and **John Oakes**, are being directed toward identifying which cytokines and chemokines are synthesized and released by epithelial cells and keratocytes, two different cell types that are found in abundance in the cornea. Epithelial cells compose the surface of the cornea and rest upon a layer of transport connective tissue called the stroma. Lying within this connective tissue are cells called keratocytes. To date, their studies suggest that only stromal keratocytes synthesize significant amounts of chemokines in response to viral attack.

One potential explanation for this is when the infectious virus spreads deep into the underlying stroma the potential for a life-threatening systemic infection increases, and keratocytes are programmed to release factors that trigger a vigorous inflammatory response. On the other hand superficial infections confined to the epithelial surface may be effectively dealt with by other means, via antiviral substances found in tears.

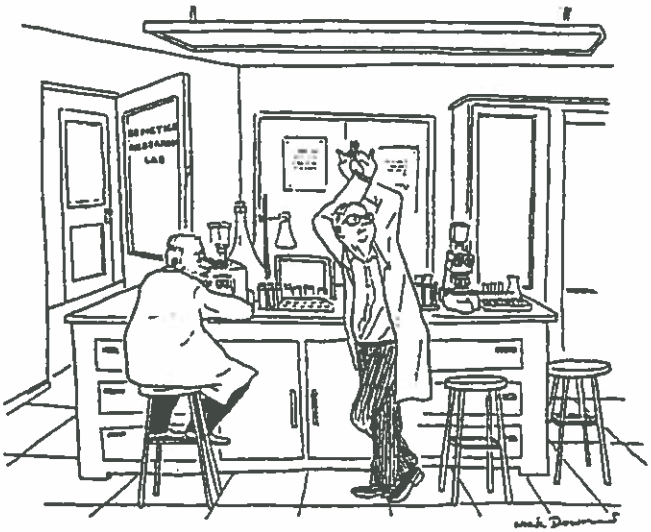
A second line of investigation is to search for reagents which can suppress an exuberant ocular inflammatory response. It is known that while certain cytokines promote inflammation other cytokines seem to be able to suppress this response. Recently, they evaluated the candidate molecule interleukin-10 in an experimental mouse. It was found that systemic or local administration of this immuno-regulatory cytokine could significantly protect against the development of HSK. Such treatment did not interfere with the capacity of the host to clear the virus from infected ocular tissues. These exciting results suggest that a naturally occurring cytokine may be useful in treating destructive inflammatory disease. Further experiments are being directed toward learning how interleukin 10 exerts its protective effect.



The structure of a Herpes virus.

A microscopic view of HSV.

A third line of investigation focuses on the issue of how HSV is cleared from an infected cornea. The general consensus has been that immune T cells are the principal mediator of recovery from virus infection. However, it is well documented that neutrophils are commonly found in the infected cornea, earlier and in larger numbers than T cells. The role of the neutrophil is being evaluated in an experimental infection model. Recent studies have shown that when animals are depleted of these cells, the virus is able to grow to higher titer and persist longer in the host. These results indicate that, in addition to T cells, neutrophils can also help to clear virus from infected tissue. Precisely how they do this is currently under study.



"Very good Michaels - you're a DNA molecule. Now, get back to work"

BIOTECHNICAL SERVICES

These are several core laboratories that provide a variety of services and analyses to faculty in the College of Medicine.

BIOPOLYMER LABORATORY
Dr. Tin Cao, Director

The Biopolymer Center provides the following services:

Protein Sequencing

The sequencing of peptides is performed on an Applied Biosystems 473A Protein Sequencer. This instrument is the latest version of ABI sequencers. It combines in one system all the sequencing capabilities: pulse liquid or gas-phase chemistry, microgradient PTH amino-acid analysis, and computer data handling. Newly developed sequence analysis algorithms available on the 610A software program allow for more confident and accurate interpretation of the sequence.

Peptide Synthesis

Peptides of defined sequence are assembled on an Applied Biosystems 431A Peptide Synthesizer. This instrument is specially designed for Fmoc solid phase synthesis and provides ample amounts of material to raise antibodies.

DNA Synthesis

DNA synthesis is accomplished with an Applied Biosystems Oligonucleotide Synthesizer Model 381A. DNA of defined sequence is custom-made automatically, using the solid phase/phosphoramidite chemistry. Synthetic oligonucleotides are being used as probes for gene screening, primers for DNA sequencing and to generate directed site mutagenesis.

DNA Sequencing

The automatic sequencing of DNA is being carried out on an Applied Biosystems DNA Sequencer Model 370A. It is a complete system composed of an electrophoresis and detection unit with hardware and software for data display. Genes are subdivided through cloning into series of overlapping templates of DNA and submitted for sequencing.

Special Analysis and Biotechnology

Upon request, the Biopolymer Laboratory performs special research operations to introduce the facility's users to the technical capabilities available. This program is also designed to provide individualized biotechnical solutions to identified projects. Participants in this program receive regular assistance in the conception and completion of their research.

The Biopolymer Laboratory is located on the main campus in CSAB, Room 347. You can contact the Center by phone at 460-7274.

RESEARCH CYTOMETRY LABORATORY
Dr. Raymond B. Hester, Director

This facility provides a variety of cell analysis services. A FACS flow cytometer is available for identification and quantitation of sub-populations of cells identified by fluorescent antibodies or probes, purification of sub-populations by cell sorting, and cloning of cells of interest by sorting single cells in 96-well plates. Both sorting and cloning may be performed aseptically. Commonly used fluorochromes include fluorescein (FITC) for single probe determination and FITC plus phycoerythrin for simultaneous, two-color/probe analysis. FACS analysis and sorting require that the sample consists of a single-cell suspension. For the analysis of cells that grow attached to culture dishes, this lab has an ACAS - anchored cell analyzer and sorter. The ACAS is a fluorescence image analyzer that is ideal for kinetics studies such as intracellular calcium responses. It also has confocal capabilities for sharp, clear fluorescent images.

The laboratory is located in CSAB, Room 359 and can be contacted by phone at 460-6029.

MASS SPECTROMETRY LABORATORY
Dr. F. Aladar Bencsath, Director

The Mass Spectrometry Core Facility offers a wide array of services including protein structure determination by MS/MS spectroscopy, structural elucidation of other molecules, molecular weight determinations, molecular quantitation down to picograms, and stable isotope measurement. Mass spectra are generated from microgram-submicrogram amounts of samples, and both positive and negative ion spectra can be recorded. For the determination of elemental compositions, high resolution and accurate mass measured spectra are created. The main instrument is a hybrid tandem mass spectrometer (MS/MS), a combination of a high resolution magnetic sector and a dual quadrupole spectrometer, an excellent union for producing daughter ion spectra from individual ions of the primary mass spectrum. Electron, chemical, fast atom bombardment, and thermospray ionization modes are presently available for spectrum generation, associated with diverse sampling methods and chromatographic sample introduction techniques. The instrument is equipped with an extensive computer support. Plans are currently underway to upgrade the capability to perform MS/MS with greater sensitivity (sub-picomole), determine molecular weights in excess of 150,000 daltons (5-10 picomoles), and perform on-line micro-HPLC sample processing. The new equipment involves a Quattro-BQ dedicated atmospheric pressure chemical ionization/electrospray (APCI/ES) mass spectrometer.

To make services more accessible, the cost to use the mass spectrometry facility have been reduced to $50 per 8 hours for COM faculty. The laboratory is located in CSAB, Room 365. For additional information, contact Dr. F. Aladar Bencsath or Mr. Bruce Baggenstoss at 460-7275.

BIOSTATISTICS AND EPIDEMIOLOGY CORE UNIT
Dr. Donald Herbert, Director

The Biostatistics and Epidemiology Core Unit will provide professional and technical support to the teaching activities of clinical epidemiology, clinical and laboratory research methods and designs, biostatistical analyses, other analytic modes (e.g., Bayesian analysis, numerical taxonomy, meta-analysis), and computer applications. In addition, the B&E unit will undertake an active role in supporting research activities in these areas through the organization of seminars and courses and dissemination of related statistical information to the College of Medicine faculty.

The B&E unit will also initiate independent research, service, and teaching programs. Specifically, the main goals of the B&E unit are to:

1) provide research designs, as well as biostatistical and other mathematical, epidemiological, computer, consultative, and instructional services to all College of Medicine faculty, staff, and students;

2) encourage and support the use of appropriate biostatistical, mathematical, and epidemiologic methodologies and techniques in the design, analysis, and evaluation of biomedical studies; and

3) develop and maintain a collection of statistical methodologies and procedures, biomedical and health policy databases, handbooks and guidebooks, computer software and hardware (or access thereto) to assist College of Medicine students, faculty, and staff to plan, conduct, and evaluate analyses of data to support research, service, and teaching functions.

Costs for services are negotiated for each individual project. The B&E office is located in CSAB, Room 269. For assistance, contact Dr. Donald Herbert at 460-7064.

"I can't possibly see anyone at the moment."

SUMMER RESEARCH PROGRAM FOR MEDICAL STUDENTS BEGINS IN JUNE

The Summer Research Program for Medical Students will begin in June. First and second year medical students interested in the program will contact faculty sponsors over the next few weeks to select a project. Student Research Day will be held in August. The speaker and date for Student Research Day will be announced in the next issue of *The Beat*. If you have any questions, please contact Dr. Randall Powell, Chair of the Student Research Committee, at 471-7994.

If you would like to submit an article for publication, please forward it to:

Jonathan Charest,
College of Medicine
CSAB 170
or
FAX (334) 460-6073

DNA REPAIR ENZYMES

Research being done by COM scientists to explain DNA enzymes and their role in environmentally-induced DNA damage and its subsequent repair in disease processes.

The molecule of the year, as named in the December 23rd issue of *Science* Magazine, is actually a group of proteins known collectively as DNA repair enzymes. These enzymes survey the DNA, recognize any modifications, remove the damage, and return the DNA to its normal sequence and conformation. These enzymes are essential because the DNA within cells is continually exposed to agents which cause damage.

Spontaneous DNA damage occurs as a result of body temperature and interactions with by-products of cellular metabolism. Additionally, the cellular genome is constantly being damaged by a host of environmental agents. If this damage is not repaired, it can contribute to cell death, mutation, chromosomal damage, aging and carcinogenesis.

Some of the new and interesting findings in the field of DNA repair have actually come from research which is in progress here at USA in the laboratories of faculty members in the Department of Structural and Cellular Biology. Their focus has been on the role that environmentally-induced DNA damage and its subsequent repair plays in disease processes such as diabetes and cancer, and in normal biological processes such as development and aging.

For many years DNA repair studies were limited to evaluations of repair across the entire genome. However, as the complexity of the genome began to be better understood and with the application of molecular biological techniques to study DNA repair, analysis of repair within specific DNA sequences became a reality. Using these sequence-specific repair techniques, **Drs. Glenn Wilson** and **Susan LeDoux** were the first to show that repair of alkylation damage (a very common type of damage which occurs following exposure to both endogenous and exogenous agents) is heterogenous within the genome, with certain sequences being repaired very efficiently and a virtual lack of repair in other sequences. Additionally, they have applied these techniques to the study of repair within the mitochondrial genome.

Although much is known about the repair process in the nuclear genome, the scientific dogma for many years was that when mitochondrial DNA is damaged these damaged genomes are simply degraded and the undamaged genomes replicated. Recently, the group here at USA were able to show that there is excision repair in mitochondria of specific types of DNA damage. The initial work done by Cathy Pettepher, a graduate student, was the first to show that there is repair of N-methylpurines in mitochondrial DNA. Subsequently, Wesley Driggers, another graduate student, modified the procedure and demonstrated repair of oxidative damage within the mitochondrial genome. Both of these studies were published in *The Journal of Biological Chemistry.*

The importance of these findings stems from the knowledge that mitochondrial DNA defects such as mutations and deletions have been associated not only with a group of inherited diseases known collectively as mitochondrial myopathies, but also with diseases such as diabetes mellitus, and Parkinson's disease and in the normal process of aging. Since DNA repair processes are the major cellular mechanism for dealing with the damage that is caused by both exogenous and endogenous agents, an understanding of these processes is fundamentaly important.

Over the past several years this work has been supported by two grants from the National Institute of Environmental Health Sciences. Recently this group learned that a third grant will be funded by the National Institute of Aging to study the role of mitochondrial DNA repair in the aging process.

NIH GRANT TO STUDY FETAL DEVELOPMENT

The National Institute of Health has awarded a five-year grant for $510,349 to **Lynn J. Groome, M.D., Ph.D.** Associate Professor of Obstetrics and Gynecology to evaluate ANS functioning and the information processing capabilities of the human fetus using noninvasive techniques. "We believe that differences in intrinsic ANS activity and the nature of the cardiac response may ultimately help us identify the fetus at risk for neurobehavioral abnormalities, and thus facilitate the implementation of appropriate intervention protocols early in the course of development," explained Dr. Groome. "The implications of our study are many, but most significant is the ability to develop and assess risk factors for individual fetuses."

The study population will consist of fetuses 38 weeks old and older. The research will focus on fetuses at low-risk for abnormalities of the nervous system, as well as those with known abnormalities. "With...more precise information on individual fetuses, we may be better able to prepare families for the outcome of the pregnancy based on measurements for that particular fetus, unlike today, where the information is given to parents based on group statistics." The research instrumentation used in collecting these data noninvasively capture the fetal cardiac electrical signal (EKG). The fetal EKG signal is obtained by placing standard EKG leads on the mother's abdomen using real-time asonography. Fetal behavioral states are assigned based on heart rate pattern and eye and body movements. The human fetus has four behavioral states. Two of these states are active and quiet sleeping. Both of these sleep states are present in the fetus and continue after birth and throughout life. The infants' neurobehavioral developmental course will be followed up to 2 years of age by Dr. R. Franklin Trimm in the Pediatrics Development Clinic.

USA STROKE CENTER

Stroke is the third leading cause of death in the United States and a major source of neurological disability. Approximately 500,000 new cases of stoke will occur in this country over the coming year. Mobile is located in the "stroke belt", a region where stroke is especially common, and we can expect over 1,000 new cases of stroke in our community in 1995.

The USA Stroke Center has been established to provide the citizens of Mobile and adjacent Gulf Coast communities with state of the art medical and surgical care for stroke prevention and intervention. In addition, the Center seeks to raise the existing standard of care for stoke through its efforts in clinical and laboratory research. Specifically, the Center's physicians and nurses will offer new therapies for stroke prevention and acute treatments of stroke, which are available only at a handful of institutions worldwide.

John E. Rothrock, M.D., recently was recruited from San Diego to develop this new Center and to serve as Chair of the USA Department of Neurology. According to Dr. Rothrock, the USA Stroke Center already is offering services to patients through its stroke clinics and is now ready to begin active participation in six multicenter national or international trials, involving therapy for stroke prevention or acute intervention. "Given the current absence of any treatment of proven value for acute stroke," says Dr. Rothrock, "we are particularly anxious to develop therapies which will stop stroke from worsening once it occurs or, even better, reverse the stroke process and thus reduce or eliminate any related disability. We will be offering such therapy to victims of acute stroke at USA affiliated hospitals and clinics."

The two stroke prevention trials USA is participating in are WARSS, which involves a comparison of warfarin vs. aspirin for secondary stroke prevention following noncardioembolic stroke, and SPAF III, which is a comparison of two treatments for stroke prevention in patients with atrial fibrillation who are at a disproportionately high risk of stroke. The four acute intervention trials are STAT (an investigation of ancrod, an extract of Malayan pit viper venom, for acute stroke), TOAST (examining an experimental low molecular weight heparinoid), ASSIST (examining a glutamate antagonist) and ICAM (evaluating a white cell antagonist intended to reduce reperfusion injury); a trial involving use of tissue plasminogen activator (tPA) for acute stoke also is anticipated.

To be effective, stroke treatment requires an informed public and a rapid response to acute stroke on the part of both the public and the healthcare system. Preliminary data from a survey conducted by the Stroke Center suggest that community awareness of stroke and stroke symptoms in Mobile is relatively low. The Center has initiated the USA Stroke Data Bank Study to determine the precise causes of stroke in Mobile, public awareness of stroke and the most appropriate methods for reducing the time which elapses from stroke onset to potential acute therapeutic intervention.

For more information regarding the USA Stroke Center, or to refer a patient for clinical evaluation or potential involvement in a research study, call 471-7841.

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