Bowles Center for Alcohol Studies Second Annual Conference

"Fetal Alcohol Spectrum Disorders - Research to Practice"

September 11, 2004
William and Ida Friday Center
Chapel Hill, North Carolina

The Fetal Alcohol Spectrum Disorders (FASD) conference is aimed at physicians, nurses, judges, teachers, social workers, child advocates, addiction counselors and others who work with people affected in-utero by alcohol. The conference will bring national and local experts to Chapel Hill for the one-day conference. Families of affected individuals are encouraged to attend.

The morning session will include presentations describing the disorders caused by exposure of a fetus to alcohol, the extent of the problem in the United States and in North Carolina, the impact of FASD on the legal system, and a personal perspective by a mother turned FASD activist. During the afternoon, attendees can choose to attend two of the following seminars: 1) the basic science of FASD; 2) FASD issues faced by health care professionals; 3) legal and child protection issues of FASD; and 4) the impact of FASD on the education system. A final presentation will cover the North Carolina perspective on the problem.

A traveling laboratory, UNC's science bus, "Destiny", will be available throughout the day, and will allow attendees to experience first-hand a science-based curriculum designed to help prevent FASD.

For more information, contact the UNC School of Medicine's Office of Continuing Medical Education at (919) 962-2118 or visit www.med.unc.edu/cme.

FAS Research Highlights Agents That Can Block Alcohol's Damaging Effects on Embryos

Fetal alcohol spectrum disorders (FASD) affect approximately 40,000 children born each year in the United States (Am J Med Genetics, March 22, 2004). Understanding the complex mechanisms underlying alcohol-induced developmental alterations could greatly aid in FASD prevention. Shao-yu Chen, Ph.D., Assistant Professor at Bowles Center for Alcohol Studies, and his collaborators, including Dr. Kathy Sulik, have focused on two mechanisms that underlie alcohol-induced birth defects: increased reactive oxygen species (ROS) and cell-cell adhesion molecule disruption.

Shao-Yu Chen, PhD

Dr. Chen is a leader in showing that ROS damage contributes to alcohol's fetal malformations. Alcohol (ethanol) exposure increases the generation of ROS. In addition, Dr. Chen has shown that ethanol-induced cell death is diminished in culture with the addition of a naturally occurring free radical scavenger, superoxide dismutase. This agent also is capable of reducing the incidence of alcohol-induced abnormalities in cultured whole embryos. This work has recently been extended to an *in vivo* model. Concurrent maternal treatment with ethanol and EUK-134, an agent that has both superoxide dismutase and catalase-like activity, resulted in less cell death and a significant reduction in major malformations in near term fetuses. The laboratory is taking this work a step further - investigating the incidence and severity of birth defects in mice whose mothers self-administer ethanol and the potential of antioxidants to diminish alcohol's damage.

In addition to a free radical mechanism, alcohol appears to be damaging to a fetus as a result of interference with the L1 cell adhesion molecule (L1). In collaboration with Dr. Michael Charness and coworkers at Harvard University, Dr. Chen has employed the whole embryo culture system to show that low concentrations of octanol are able to antagonize ethanol-induced inhibition of L1 and diminish the adverse developmental effects of ethanol. In addition, Dr. Chen is a co-first author on a recently-published PNAS report that describes structure-activity relationships of the peptides NAP and SAL, both of which can antagonize ethanol's inhibition of L1 adhesion. These peptides are also capable of reducing ethanol-induced developmental damage.

When speaking regarding his commitment to FASD research, Dr. Chen says, "As a parent, I am acutely aware of the importance of having healthy babies. This makes the research work in which I am involved especially rewarding. I am fortunate to have had the opportunity to collaborate with Dr. Sulik over the past ten years. During this time I have learned to appreciate the value of interdisciplinary and collaborative teams of researchers. In particular, I continue to benefit from the strong support from other faculty members in the Bowles Center for Alcohol Studies and the Department of Cell and Developmental Biology."

The Bowles Center for Alcohol Studies
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To become involved in our mission, call Elizabeth Amend at (919) 843-6204 or email amend@unc.edu.

For treatment information call UNC Health Care's Alcohol and Substance Abuse Program at (919) 402-1644.

www.med.unc.edu/alcohol

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Center Line

Bowles Center for Alcohol Studies
School of Medicine, University of North Carolina at Chapel Hill

Our mission is to conduct, coordinate, and promote basic and clinical research on the causes, prevention, and treatment of alcoholism and alcoholic disease.

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Mechanisms of Alcoholic Liver Disease: UNC Lab Elucidates Molecular Underpinnings of Injury and Restoration of Alcohol-Exposed Liver

Alcohol-related liver disease affects an estimated 2 million individuals in the United States and is the third most common cause of preventable death in middle-aged Americans. Although the course of alcoholic liver disease can be modified, it is currently incurable. The only salvation for patients with advanced disease is a liver transplant, which many hospitals are unwilling to grant an alcoholic patient because of the concern that relapse to heavy drinking will undermine long-term outcomes. Dr. Michael Wheeler and his laboratory within the Bowles Center for Alcohol Studies are working to improve the prospects for alcoholics with liver disease through research that illuminates the mechanisms by which alcohol damages the liver and reveals cellular and molecular targets for interventions to prevent liver damage or stop its progression.

Wheeler Research Team: (left to right) Courtney Munroe, Jamie Milton, Michael Wheeler, Ph.D., Ian Hines, Christy Byrd, and April Black

A heterogeneous condition, alcoholic liver disease is manifested by fatty liver, the deposition of fat in liver tissue; alcoholic hepatitis, or chronic liver inflammation; and cirrhosis, which is characterized by scarring of liver tissue and deformation of the liver. Although liver disease progresses in stages from fatty liver to hepatitis to cirrhosis in some patients, an individual with alcoholic liver injury can have any one or all of these conditions concurrently.

Like the conditions associated with alcohol-related liver injury, the mechanisms of alcoholic liver disease appear to be diverse. However, recent research, including work by Wheeler's lab, implicates the liver's Kupffer cell as playing a central role in numerous aspects of alcoholic liver disease. In the healthy individual, the Kupffer cell helps the liver to fulfill its vital function of deactivating and eliminating toxins that are produced by or introduced into the body. When exposed to excessive alcohol, on the other hand, the Kupffer cell contributes to the initiation and maintenance of toxic processes that cause liver-cell injury and death. Much of Wheeler and his colleagues' research focuses on the mechanisms by which the Kupffer cell mediates liver injury when it is exposed to alcohol.

Wheeler and his lab are breaking new ground by applying cellular and molecular techniques to the study of the mechanisms of liver disease. By examining how alcohol affects the workings of the individual cell at the molecular level, they can answer questions that cannot be addressed with classical biochemical approaches. The power of the cellular/molecular approach adopted by Wheeler and his laboratory is illustrated by their work on oxidative stress and cytokine production mediated by activated Kupffer cells.

A highlight of Wheeler's earlier work was the use of recombinant viral gene delivery to target oxidative stress, thought to mediate tissue damage caused by ethanol consumption. This seminal work clearly demonstrated the importance of oxidant production in the early mechanisms of ethanol-induced liver disease.

TNF-α, a cytokine produced along with oxidants from activated Kupffer cells, is also implicated as a contributor to death of liver cells. As part of an inflammatory reaction by Kupffer cells, TNF-α levels are increased in alcoholics suffering from hepatitis, a finding consistent with the possibility that TNF-α contributes to the liver damage occurring in alcoholism. Several years ago, work by Wheeler and others was instrumental in establishing the importance of TNF-α in alcohol-associated liver damage by demonstrating that mice bred to lack receptors for TNF-α had little liver damage during chronic alcohol treatment, whereas normal mice with intact TNF-α receptors had substantial damage.

While TNF-α seems to be at least one of the critical factors that mediate injury and death caused by ethanol, some of the earlier events in Kupffer cell activation are now becoming extremely important. Wheeler's group has begun to address the very initial changes that take place in Kupffer cell activation. It is now clear that

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Kupffer cells are activated during early alcohol-induced liver injury and that this process involves gut-derived endotoxins. Endotoxin or lipopolysaccharide (LPS) is known to bind a cell surface receptor found on Kupffer cells called CD14. A large body of work from Wheeler's group and others have strongly argued for the role of endotoxin in the progression of alcoholic liver disease. Recent experimental evidence has demonstrated that mice deficient in CD14 endotoxin receptor are resistant to ethanol induced liver injury. Some of Wheeler's work has recently focused on the role of CD14 expression in liver disease. Several groups have recently reported that CD14 expression is increased during long-term ethanol consumption. Thus, targeting the regulatory mechanisms CD14 expression will likely be important in the development of therapeutic approaches for the treatment of alcohol-related liver disease.

How this translates to the human condition is a critical question. In humans, a correlation exists between the severity of alcoholic liver disease and CD14 levels. In addition, it was reported that serum from human patients with severe alcoholic hepatitis contained higher levels of soluble CD14 than healthy controls. These data pose some critical questions in our understanding of ethanol-induced liver injury. Specifically, what role does the increase in CD14 expression due to acute ethanol have in chronic ethanol toxicity? Certainly, understanding the regulation of CD14, considering its association as a risk factor in humans for severe alcoholic liver disease, is potentially important for the development of therapies for alcoholic liver disease.

In earlier work that Wheeler was involved in, it was found that Kupffer cells from female rats fed ethanol had a significantly greater response to LPS than Kupffer cells from male rats. Interestingly, CD14 expression was higher in Kupffer cells isolated from female mice. Taken together, these and other studies emphasize the importance of CD14 in the activation of Kupffer cells during chronic alcohol consumption.

Wheeler has also been intrigued by the seemingly disparate "downstream" actions of TNF-α in the liver. It is clear that TNF-α causes injury in most models of liver disease, but it has also been shown to play a role in the regenerative phase as well. Wheeler applied molecular techniques to determining the mechanisms underlying its varied actions. He reasoned that the disparate actions might be determined by various signals generated by TNF receptor 1 that lead to specific cellular events such as death or proliferation. He focused on a protein known as Ras because it is known to be activated by TNF-α and to be important in modulating cellular responses to TNF-α. Ras has been likened to a molecular switch that, when activated, initiates a cascade of events that participate in cellular proliferation. Wheeler and his lab found that Ras levels were significantly increased in the livers of mice exposed to a diet of alcohol unless the mice were bred to lack the TNF receptor type 1. Furthermore, while the increase in Ras levels was associated with proliferation of liver cells in animals having intact TNF-α receptors, cellular proliferation was inhibited in mice lacking the TNF-α receptor type 1. These results strongly implicate TNF-α-mediated activation of Ras in cellular proliferation occurring in the alcohol-exposed liver.

Says Wheeler, "These results show that TNF-α stimulates injury and proliferation via different mechanisms. For proliferation, TNF-α stimulates Ras activation. For cell injury, TNF-α stimulates oxidative stress that leads to cell death. Now we need to take this research one step farther. We want to know why one cell responds to alcohol-induced activation of TNF-α by dying while another cell responds by proliferating. The answer is in the signaling pathways. By getting down to the molecular level, we will be able to find the answer." It is possible that it is a case of survival of the fittest. Cells that are dying when exposed to alcohol may

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Michael D. Wheeler, Ph.D.

Education: Ph.D. in Pharmacology, University of North Carolina, Chapel Hill, 2000; B.S. in Chemistry, Appalachian State University, 1996

Experience: Assistant Professor, Department of Medicine and Pharmacology, University of North Carolina at Chapel Hill, 2002 – present; Post-doctoral Fellow, Curriculum in Toxicology, University of North Carolina at Chapel Hill, 2001-2002; Post-doctoral Fellow, Dr. R. Jude Samulski, Gene Therapy Center, University of North Carolina at Chapel Hill, 2000-2002

Honors and Awards: Pre-doctoral Fellow, National Institutes of Health (NIAAA), 1999-2000; Pre-doctoral Fellow, Bowles Center for Alcohol Studies, 1998-1999; American Chemical Society, Undergraduate Research Award, 1996; Sigma Xi Undergraduate Research Award, 1996

Web page:
http://www.med.unc.edu/alcohol/faculty/wheelerm/wheeler.htm

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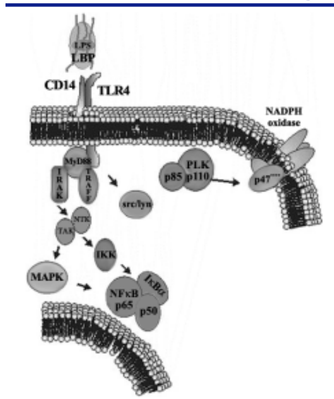

CD14 Scheme: LPS signaling pathway in macrophages. LPS couples with soluble factor LPS binding protein to interact with the LPS receptor CD14. Since CD14 has no transmembrane-spanning region it links to toll-like receptor 4 (TLR4) to initiate intracellular signaling. The NFkB pathway has been well-characterized and is known to be downstream of CD14:TLR4 activation. However, the signaling pathway involved in the activation of NADPH oxidase has not been clearly defined. Our work has begun to elucidate some of the molecules such as src kinase and PI3 kinase that may be involved.

The Director's Column

Fulton T. Crews, Ph.D.
Director,
Bowles Center for Alcohol Studies

This issue highlights two different areas of research: alcoholic liver disease and fetal alcohol syndrome. Interestingly, ethanol induced oxidative stress is common to the pathology in both liver and fetus. In addition, alterations in cell proliferation and how cell adhesion and proliferative signals are altered by ethanol are common to these pathologies. Understanding the molecular mechanisms will elucidate the complex factors that drive the progressive pathology as well as providing insight into opportunities for new therapies. These commonalities represent a strength of our Center's efforts to bring together diverse faculty interested in alcohol and how it changes the body. My own laboratory has found that ethanol increased oxidative stress likely contributes to alcoholic brain damage. A common mechanism such as this greatly increases the scholarly interaction as well as the sharing of methodology and insight into potential therapies that might reverse or delay the progression of pathology.

Oxidative stress is not a simple thing to study and it can originate through a number of different processes. It is also difficult to measure. Reactive oxygen species last for very short periods, e.g. milliseconds, before they oxidize something nearby or spontaneously degrade or are neutralized by enzymes. Anti-oxidants appear to play a role in general health and may represent an environmental factor, e.g. anti-oxidants in diet, that impact alcoholic pathology in a variety of ways. The Center's faculty are following these discoveries with more detailed experiments that may lead us to one common element of multiple alcoholic pathologies, but more likely will lead us to multiple elements that sum to increase pathological oxidative stress. Discoveries take time, but we are moving forward.

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be old, sick cells. The ones proliferating after exposure to alcohol may be younger or more vigorous cells—better candidates for supporting regenerative processes in the liver

Remarking on the significance of this work, Bowles Center for Alcohol Studies Director Fulton Crews states, "Dr. Wheeler and his lab are at the cutting edge of research in this area. Wheeler's research has begun to show us exactly how an immune response can mediate the opposing processes of cellular death and cellular proliferation." Wheeler adds, "It is likely that the immune response has a lot more control over things we've never paid attention to." Wheeler is beginning the think about how immunity and cytokine production may control fat metabolism, energetics, fibrosis, hepatocellular proliferation, and even hepatic stem cell differentiation- all facets of liver disease. That knowledge will help to expand the options for effective intervention so that alcoholic liver injury can be treated or prevented.

ABC Stores Now Display Fetal Alcohol Syndrome Warning Posters

Dr. Kathy Sulik and Rep. Martha B. Alexander stand in front of an FAS warning poster.

North Carolina is determined to prevent the fetal effects of alcohol. Leaders of the Alcohol/Drug Council of North Carolina (ADCNC), faculty of the UNC Bowles Center for Alcohol Studies, and the members of the House of Representatives worked together to require the posting of North Carolina's first FAS warning posters in Alcoholic Beverage Control (ABC) stores. The posters read, "WARNING: Pregnancy and alcohol do not mix. Drinking alcohol during pregnancy can cause birth defects." The posters are printed in English and Spanish.

Mothers drinking alcohol during pregnancy can lead to Fetal Alcohol Syndrome (FAS) in their children, a condition that can cause lifelong disabilities such as learning disabilities, mental retardation, and behavior problems. Dr. Kathy Sulik, a faculty member of the UNC Bowles Center for Alcohol Studies and Department of Cell and Developmental Biology, said, "Maternal alcohol abuse is the leading known cause of mental retardation in this country. Women need to know that if they are pregnant they should not drink, and if they drink, they should not get pregnant."

N.C. Representative Martha B. Alexander stated "I am very pleased with the cooperation we have received from N.C. ABC Commission and local ABC boards and from the FAS Initiative Committee of the Alcohol/Drug Council of N.C., which helped develop the poster."

ALCOHOLISM is one of the most costly diseases to the harmony of our families and the economy of our communities. More than 8.5% of the population ages 18 years and older – nearly 18.5 million Americans – have problems with drinking.

Your gift will support our Center's strong research teams (brain, liver and fetus) and our Alcohol and Substance Abuse Treatment Program (ASAP) to develop new, more effective prevention, education, and treatment programs.

Become a FRIEND of the Center and help us as we work to solve the mystery of who, why, and how people become addicted to alcohol and other drugs.

Yes, I would like to join the effort to cure alcoholism in our lifetime.

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