Bowles Center Researchers Visit Residential Alcohol Treatment Program

Each year, the Bowles Center for Alcohol Studies (BCAS) takes a group of its members, including graduate students, post-doctoral fellows, faculty and staff, for a visit to the Triangle Residential Options for Substance Abusers (TROSA) in Durham, NC. Darin Knapp, PhD, Associate Professor in the BCAS and the Department of Psychiatry, works with TROSA residents and staff to provide Bowles members with a comprehensive understanding of the successful program that serves approximately 300 substance abusers.

Darin Knapp, PhD, coordinates the Bowles Center's visits to TROSA, a unique residential treatment program.

TROSA's President and CEO, Kevin McDonald, recovered from substance abuse and launched the non-profit organization in 1994 to help recovering drug and alcohol abusers change their lives. The unique self-supporting two-year residential program includes vocational training, education, communication, peer counseling, mentoring, leadership training, and aftercare services post-graduation. TROSA's goal is to produce self-reliant and upstanding citizens with life skills and work skills that enable them to find jobs after they graduate from the program.

Many scientists work diligently to understand and solve the problems of addiction, alcohol abuse and alcoholism. It is important for researchers to meet some of the people whose lives will be changed by therapies developed from their research. The BCAS trips to TROSA allow that interaction and exchange of information. "The attention of most of our investigators, post-docs, and students is on basic research into the relevant biological and behavioral mechanisms in models of alcoholism. This research can undoubtedly benefit from consideration of the relevance to clinical application, and we hope that this benefit includes the translation of our discoveries into new and more effective therapies," says Dr. Knapp.

When speaking on the significance of the trips, he says "I believe our trips to TROSA provide a rare and meaningful opportunity to remind ourselves that alcoholism is not just a disorder of neurochemistry, brain circuits and organ toxicity. Beyond the diagnosis of "alcoholic" is a human face, a personality, often a lost soul, a heart wrenching struggle, and a plea for help. We learn at TROSA that those challenged with alcoholism are not unlike us."

The Bowles Center for Alcohol Studies
Tel. (919) 966-5678
Fax. (919) 966-5679

To become involved in our mission, call Melissa Mann at (919) 966-5678 or email melissa_mann@med.unc.edu.

For treatment information call UNC Health Care's Alcohol and Substance Abuse Program at (919) 966-6039 or (888) 457-7457.

www.med.unc.edu/alcohol

Center Line, Vol. 16 No. 2 *Published quarterly to bring greater understanding of alcoholism research and the Center's mission.*
A. Leslie Morrow, Ph.D., Editor-in-Chief; Angela D. Farrior, Managing Editor; Jane Saiers, Ph.D., Science Writer

UNC Bowles Center for Alcohol Studies
CB# 7178, Thurston-Bowles Building
University of North Carolina at Chapel Hill
Chapel Hill, North Carolina 27599-7178

Non-Profit Organization
US Postage
PAID
Permit No. 177
Chapel Hill, NC 27599-1800

This document is supported by subscriptions and donations to the Bowles Center for Alcohol Studies.

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.

ISSN 0738-6567 Volume 16, Number 2, June 2005

Rusyn Laboratory Elucidates Molecular Mechanisms that Contribute to Alcohol-Associated Liver Injury and Cancer

Most people are aware that excessive alcohol consumption can cause life-threatening liver disease that, in its advanced stage, is characterized by the inability of the liver to perform vital functions such as deactivating and eliminating substances that are toxic to the body. Fewer people are aware that excessive alcohol consumption can cause cancer and that alcoholic beverages, like other substances such as tobacco products, are classified by the International Agency for Research on Cancer as known human carcinogens. This lack of awareness is not surprising given the dearth of educational initiatives to raise public awareness of the link between excessive alcohol consumption and cancer. For example, unlike tobacco products, which are labeled with warnings from the US Surgeon General about health risks including cancer, alcohol products do not carry warning labels concerning this grave health outcome. Why is the link between excessive alcohol consumption and cancer not more widely publicized?

The Rusyn Research Team: L to R, Sitting: Oksana Kosyk, Christine Powell, Alison Hege, Akira Maki; Standing: Courtney Woods, Svitlana Shymoniak, Blair Bradford, Ivan Rusyn

Part of the explanation lies in the nature of the evidence supporting the carcinogenic potential of alcohol. Almost all of this evidence comes from epidemiologic studies showing that individuals who were heavy drinkers were more likely than those who were not heavy drinkers to have liver cancer and that the amount of alcohol consumed directly correlated with degree of cancer risk. Although the epidemiologic evidence for a link between heavy alcohol consumption and cancer is strong, very little is known about the biological mechanisms by which excessive alcohol consumption can lead to cancer. With tobacco-containing products, in contrast, both epidemiologic and mechanistic studies strongly establish the cancer link and provide a basis for public-health educational initiatives such as Surgeon General reports and warning labels.

Dr. Ivan Rusyn and his colleagues at the University of North Carolina's Department of Environmental Sciences and Engineering at the School of Public Health are working to discover the molecular and cellular underpinnings that explain the alcohol-cancer link. Rusyn uses animal and cellular models to elucidate the molecular mechanisms of cancer caused by various substances, including alcohol. With two degrees, an MD and a PhD, Rusyn approaches his research from both clinical and scientific perspectives, and he continually seeks to ensure that his basic-science endeavors are relevant to human health. Rusyn is motivated to discover the mechanisms of alcohol-associated liver cancer by the possibilities that his research will uncover targets for interventions to prevent alcohol-induced liver damage and carcinogenesis and will provide evidence to support efforts to educate the public about the hazards of excessive drinking.

In one series of experiments published in *Hepatology* (February 2005), Rusyn and his colleagues attempted to determine whether the molecular events that cause liver injury during excessive alcohol consumption also contribute to mechanisms involved in carcinogenesis. "Damage to DNA—changes in the genetic code—are necessary in order for cancer to develop," Rusyn explains. "We know from previous research that excessive alcohol consumption causes the body to generate harmful intermediates known as oxidants (or reactive oxygen species) that may be important in causing alcohol-mediated liver injury—that is, fatty liver, fibrosis, and cirrhosis. It is also known that oxidants are capable of damaging DNA. In these studies, we sought to determine in an animal model whether the oxidants generated during chronic consumption of alcohol can cause DNA damage consistent with that associated with cancer. We

Continued on next page

Ivan Rusyn, MD, PhD

Experience

Assistant Professor and Director, Laboratory of Environmental Genomics, Department of Environmental Sciences & Engineering, UNC School of Public Health; Member, Bowles Center for Alcohol Studies

Education

M.D. (with Honors), Medicine, Ukrainian State Medical University, 1994; Ph.D., Toxicology, University of North Carolina at Chapel Hill, 2000

Recent Publications

Bradford, B.U., Kono, H., Isayama, F., Kosyk, O., Wheeler, M.D., Akiyama, T.E., Bleye, L., Krausz, K.W., Gonzalez, F.J., Koop, D.R., and Rusyn, I. Cytochrome P450 CYP2E1, but not NADPH oxidase is required for ethanol-induced oxidative DNA damage in rodent liver. *Hepatology* 41:336-344, 2005.

Hays, T., Rusyn, I., Burns, A.M., Kennett, M.J., Ward, J.M., Gonzalez, F.J., and Peters, J.M. Role of peroxisome proliferator-activated receptor-alpha (PPARa) in bezafibrate-induced hepatocarcinogenesis and cholestasis. *Carcinogenesis* 26:219-227, 2005.

Rusyn, I., Asakura, S., Pachkowski, B., Bradford, B.U., Denissenko, M.F., Peters, J.M., Holland, S.M., Reddy, J.K., Cunningham, M.L., and Swenberg J.A. Expression of base excision DNA repair genes is a sensitive biomarker for *in vivo* detection of chemical-induced chronic oxidative stress: Identification of the molecular source of radicals responsible for DNA damage by peroxisome proliferators. *Cancer Res* 64:1050-1057, 2004.

Wheeler, M.D., Smutney, O.M., Check, J.F., Rusyn, I., Schulte-Hermann, R., and Thurman, R.G. Impaired Ras membrane-association and activation in PPAR(alpha) knockout mice following partial hepatectomy. *Am J Physiol* 284:G302-312, 2003.

Website

http://genomics.unc.edu/rusyn/rusyn_dw.htm

Research supported by NIEHS and NIAAA.

also sought to identify the specific mechanisms responsible for causing DNA damage during chronic consumption of alcohol. We asked the question of whether the same mechanisms that cause alcohol-associated liver damage are also involved in DNA damage consistent with that in carcinogenesis."

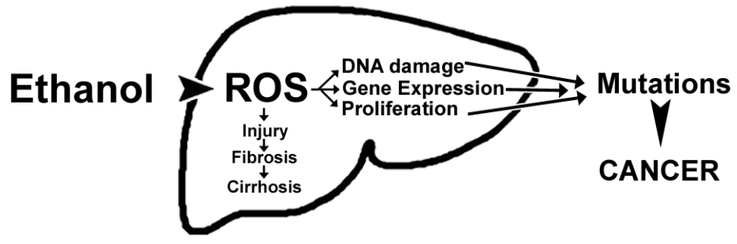
In support of the possibility that chronic alcohol consumption causes DNA damage, several markers of DNA damage were found in liver tissue of rats and mice treated with alcohol for 4 weeks. Rusyn and his colleagues hypothesized that this DNA damage would prove to be mediated by the same molecular events that are involved in alcohol-induced liver injury. Their results surprised them. They found that the oxidants previously shown to be critical for mediating alcohol-associated liver injury and those that are required for mediating alcohol-associated DNA damage are not coming from the same cell in liver. In the animal model that was used, alcohol-associated liver injury depends on the oxidants produced by activation of a molecular pathway, including the enzyme nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase), in Kupffer cells in the liver. On the other hand, alcohol-associated DNA damage in this model was found to be dependent on the production of oxidants by activation of a distinct molecular pathway that included the enzyme cytochrome P450 2E1 (CYP2E1) in liver cells known as hepatocytes. In a strain of genetically engineered mice that lack CYP2E1, no oxidative DNA damage was observed, whereas mice having normal levels of CYP2E1 did show oxidative DNA damage.

Rusyn and his colleagues suggest that the results support a potentially important role for hepatocyte-derived CYP2E1 as a source of oxidants that cause DNA damage during chronic exposure to alcohol, a finding that may lead to better understanding of a link between alcohol drinking and cancer. The data also suggest that the mechanisms involved in alcohol-associated liver injury and those involved in alcohol-associated carcinogenesis may differ. According to their working hypothesis (Figure), excessive consumption of alcohol leads to the production of reactive oxygen species, DNA damage, and increased proliferation of liver cells and ultimately can result in mutations and development of hepatocellular carcinoma.

Rusyn and his laboratory have recently turned their attention to studying genetic factors that may determine susceptibility to alcohol-associated liver injury and carcinogenesis. Their research, some of which is funded through the UNC Bowles Center for Alcohol Studies Pilot Grants program, attempts to identify specific genes or modifications to genes that make some individuals more susceptible than others to the adverse effects of alcohol on the liver. Using the latest advances in genetics and molecular biology, Rusyn and his colleagues are attempting to define a "liver toxicity susceptibility state" that characterizes individuals at risk of alcohol-induced liver damage. Identification of genetic and other biological markers for susceptibility to toxicity may facilitate the development of diagnostics and other tests that can be used to identify at-risk individuals in the clinical setting and the development of interventions for alcohol-associated liver injury and carcinogenesis.

The typical experimental approach of using one animal strain in a model of human alcoholism cannot be applied in these experiments, which attempt to identify determinants of alcohol susceptibility that

Continued on next page



Alcohol and liver cancer: Working hypothesis. Ethanol consumption leads to production of reactive oxygen species (ROS), DNA damage and increased cell proliferation in liver. This ultimately results in mutations and development of hepatocellular carcinomas.

The Director's Column

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

Alcohol abuse is known to be toxic causing increased risks of cancer and birth defects. California passed Proposition 65, the "Safe Drinking Water and Toxic Enforcement Act of 1986," that included language stating no person in the course of doing business shall knowingly expose any individual to a chemical known to cause cancer or reproductive toxicity. Alcohol was immediately listed as a reproductive toxicant and two years later was added as a "Chemical known to the State to Cause Cancer." As a result, pubs and restaurants in California have posted warning signs indicating that a carcinogen is served there.

Many laboratories, including my own, have found that alcohol (ethanol) induces enzymes associated with increasing oxidative stress and promoting cancer. However, there are likely many mechanisms and the direct cellular processes are not linked, only hypothetical. The liver metabolizes alcohol and liver disease increases risk of cancer. Liver cancer may increase as hepatitis increases and the interaction with alcohol tremendously increases risk for the often fatal liver cancer.

Epidemiological studies have identified chronic alcohol consumption as a risk factor for cancer of the oropharynx, larynx, esophagus and liver. There is also increased risk of large intestine and breast cancer with alcohol consumption. Breast cancer will occur in 1 of 8 women and even moderate alcohol consumption doubles the risk of breast cancer in some high-risk women. Longnecker (Cancer Causes Control, 5:73,1994) calculated that 4% of newly diagnosed breast cancer cases in the US are primarily due to alcohol. One study on throat (esophagus) cancer found that consumption of about a bottle of wine a day increased relative risk of cancer by 18 fold, while smoking alone more than a pack a day increased risk 5 fold. Together risk increased by 44 fold (A. Tuyns, Alcohol: Health and Research World 2:20; 1978). The effort to reduce smoking related cancers appears to be much greater than efforts on alcohol related cancers. One weakness in the data relating alcohol and cancer is the lack of mechanistic understanding of how alcohol leads to increased risk of cancer.

Ivan Rusyn and others within the Bowles Center are helping to unravel this relationship which would explain why alcohol does not increase all cancers and how we can prevent, diagnose and better treat cancers. More research is needed on alcohol and cancer. Among the National Institutes of Health, the Cancer Institute is the largest ($4.7 Billion). Alcohol might contribute to 10% of cancers. If the Cancer Institute (NCI) devoted 10% of its budget to understanding alcohol and cancer, it would more than double the Alcohol Institute's (NIAAA) budget ($0.4 Billion). I predict we would know how alcohol leads to cancer really fast if that happened. It will happen, but resources will determine how fast. I think California will be there first.

Continued from previous page

may vary from individual to individual. Individual animals within a given so called "inbred" rat or mouse strain are identical, genetically speaking, and are not suited for studying interindividual differences. "It's like testing the same individual over and over again," Rusyn says. To better model the enormous genetic diversity in the human population, Rusyn and other researchers at UNC use panels of inbred mouse strains to understand the extent of variability in response to a specific toxicant as well as to elucidate how the genetic diversity confers susceptibility or resistance. In pilot studies, he and his lab have shown that different strains of mice exposed to the same amount of alcohol show dramatically different degrees of liver damage, and they have begun to identify genetic factors and molecular pathways that contribute to sensitivity and resistance to alcohol-associated liver damage. "There is no such thing as an animal model that always applies—and the standard approach in toxicology of using one strain of animal for testing is not the optimum strategy," states Rusyn. "Using several strains of mice in an attempt to model the genetic diversity we see in the human population, we hope our research will contribute to advances in the recognition and treatment of alcohol-associated liver disease in humans. Knowing the genetic determinants of vulnerability to liver toxicity will help us to develop treatment interventions and to identify those most in need of intervention."

Professor Grant Receives 2005 Bowles Lectureship Award

Drs. Crews, Grant, and Morrow

Kathleen Grant, PhD, received the 2005 Bowles Lectureship Award on April 18, 2005. The Award is given annually to an individual who has made significant contributions to our understanding of the causes, prevention, and/or treatment of alcoholism and alcohol abuse.

Dr. Grant, a professor of pharmacology at Wake Forest University School of Medicine, has done seminal work in non-human primate research, drug discrimination, pharmacotherapy, social behaviors and stem cells of non-human primates.