The Ohio Board of Regents recently funded a total of 18 research projects to seek solutions for the toxic algal bloom in Lake Erie (http://utnews.utoledo.edu/index.php/02_06_2015/ut-researchers-to-lead-majority-of-ohio-water-quality-research-projects). Three of these projects will be conducted in the UT College of Medicine and Life Sciences. It is our great pleasure to feature these projects in the March issue of JCCTR Newsletter.

Method development for detecting microcystin toxins in biological samples

Kenneth Hensley, Ph.D.
Associate Professor of Pathology

One summer morning in 2014, Toledoans awoke to the news that their water supply might be contaminated with a dangerous toxin called microcystin, which is produced when certain algae multiply rapidly in the Lake Erie basin. Four five days some 400,000 residents of Toledo and its suburbs suffered fear and uncertainty. How dangerous is microcystin? How much is really in the water? Who has been exposed? Public officials could offer no easy answers to these basic questions because the science for monitoring human exposure risk had not been done.

Through an initiative funded by the Ohio State Board of Regents Sea Grant program, these questions may soon be answered and new scientific tools developed that can help gauge and manage risk for microcystin exposure.

Dr. Kenneth Hensley, Associate Professor in the Department of Pathology, will develop mass spectrometry assays to measure both microcystin and its glutathione metabolite. This is important because microcystin is rapidly conjugated with glutathione in the human liver, so existing assays that measure microcystin in water, cannot measure the product of human exposure in urine or blood specimens. Filling this knowledge gap will enable researchers and crisis managers to more accurately judge whether hospital patients and community members have incurred a health risk related to environmental microcystin. Furthermore, these tools will enable scientists to perform dose-ranging studies in animals to learn what concentration of microcystin in drinking water might be dangerous to healthy persons or to persons with existing liver damage. Such information is crucial to guiding public policy decisions relating to Great Lakes water usage.

Development of Microcystin Detoxifying Water Biofilters

Jason Huntley, Ph.D.
Assistant Professor of Medical Microbiology and Immunology
Dr. Jason Huntley, will be developing new methods to remove microcystin toxins from Lake Erie water. Given that the August 2014 water crisis directly impacted the City of Toledo, the University of Toledo Health Science Campus is strategically positioned to address this urgent human health problem. Dr. Huntley's laboratory studies how bacteria cause disease and recently started studying the role of biofilms (thin layers of bacteria) in pneumonia. Using his expertise in microbiology and biofilms, Dr. Huntley's goal is to create biofilters – water filters coated with non-toxic, living bacteria to degrade microcystin toxins – that can be used by municipal water treatment facilities like the City of Toledo. This “fighting bacteria with bacteria” concept is not science fiction, as evidenced by the proven health benefits of active-culture yogurts and fecal transplants for *Clostridium difficile* infection patients. In addition, biofilters would offer municipal water treatment facilities a safe, efficient, and cost-effective alternative for microcystin removal from Lake Erie water, as opposed to the use of harsh and expensive chemicals. There is some evidence from other microcystin-contaminated lakes across the world that microcystin-degrading bacteria do naturally occur but their abundance in fresh water lakes appears to be quite low and the rate at which they degrade the microcystin toxins can vary widely. Beginning this summer, members of Dr. Huntley's laboratory will work to isolate and grow naturally-occurring microcystin-degrading bacteria from Lake Erie water samples. Once isolated, studies will be performed to screen for the most efficient microcystin-degrading bacterial strains. Next, the Huntley lab will optimize conditions to promote these microcystin-degrading bacteria to form biofilms on a range of commonly-used water filtration filters. Dr. Huntley will coordinate these efforts with Dr. Isabel Escobar, Professor of Chemical and Environmental Engineering and expert in water treatment practices, from the UT Main Campus to test appropriate municipal water filters and filtration systems. Once biofilm formation conditions have been optimized on the most appropriate water filter for municipal water treatment plants, a laboratory-scale water filtration system will be built and microcystin-spiked water will be passed through the biofilter to test microcystin removal rates and biofilter longevity. The results from this project, in consultation with Dr. Escobar and municipal water filtration experts, will be used to design large-scale biofilters for possible incorporation into municipal water treatment systems such as the City of Toledo water treatment facility. These studies are particularly timely given the $264 million, five year renovation and upgrade project currently underway at the City of Toledo water treatment facility. This project has the potential to directly reduce the human health threat posed by microcystin toxins, by developing new methods to safely, efficiently, and cost-effectively removing microcystin toxins from municipal water supplies.

Impact of pre-existing liver disease on microcystin hepatotoxicity

Thomas C Sodeman, M.D.
Professor, Chief, Gastroenterology and Hepatology

"I have liver disease, is it safe for me to drink the water?"

This is a question I heard many times during the time of the water crisis, and unfortunately there isn’t a good answer. The studies that were done on animals to determine what a possible safe level for microcystin is were done on otherwise healthy animals. Since microcystin affects the liver, it would be reasonable to assume that someone with liver disease might get into trouble when they are exposed to levels of microcystin that would not affect a healthy person. The question is, are people with liver disease a little more sensitive, or a lot more.

To help answer this question, we will expose mice with liver disease, namely fatty liver, to microcystin, to see how more sensitive to it they are than normal mice. Fatty liver will be a stand-in for any liver disease, as they all have the common effect of causing inflammation, or damage in the liver. Additionally, fatty liver is a significant problem in humans, with around one third of Americans having fat in their livers, and of them around ten percent progress to having damage in their livers due to the fat.

Unfortunately this study will not tell us what the toxic level in a human is, either one with or without liver disease. What it will tell us is there may be some citizens of Northwest Ohio who may be more sensitive than others to the effects of microcystin, and who should potentially avoids the water during times when there are harmful algae blooms.

Another aspect of this study is looking at the damage done by microcystin. Little is known about its effects on the liver, both in health and disease, and this study should begin to shed some light on those effects.
This information, as well as the information on toxicity levels, should be useful to regulators looking to decide what to do about the microcystin threat, both now and in the future. As microcystin affects bodies of water around the world, not just in Northwest Ohio, this information will be found here and helpful here, but also used around the world. For the benefit of others.

New Publication in Nature Communications

Cross-talk between two transcription factors regulates blood pressure

Bina Joe, Ph.D.
Professor, Department of Physiology and Pharmacology
Director, Center for Hypertension and Personalized Medicine

Research led by Dr. Bina Joe in the Center for Hypertension and Personalized Medicine and the Department of Physiology and Pharmacology is published in Nature Communications (http://www.nature.com/ncomms/2015/150217/ncomms7252/full/ncomms7252.html). The article titled, ‘Mutation within the hinge region of the transcription factor Nr2f2 attenuates salt-sensitive hypertension’ is focused on NR2F2, a nuclear receptor transcription factor also known as chicken ovalbumin upstream promoter transcription factor (COUP-TFII). It describes the validation of Nr2f2 as a genetic determinant of blood pressure. The study was supported through grants from the National Heart Lung and Blood Institute of the National Institutes of Health to Dr. Joe and first-authored by Dr. Sivarajan Kumarasamy, a Research Assistant Professor in Dr. Joe’s Laboratory.

Essential hypertension, which is defined as elevated blood pressure with no known cause, accounts for >90% of all forms of hypertension. Hypertension is known to be inherited in families, which means that the evidence for the presence of heritable genetic elements on our genomes that predispose some of us to develop high blood pressure, is strong. However, the single biggest unanswered question concerning the genetics of Essential Hypertension is that the identities of genetic determinants of blood pressure remain ‘largely unknown’. Genome wide association studies (GWAS) of hypertension in humans have detected associations between genes and hypertension, but are not designed to differentiate between genes that are causally linked to hypertension and genes that are not. For gene discovery to be translated into clinical use, an integrated approach that includes experimental validation of genes in animal models is required to alleviate this inherent difficulty associated with human studies.

The scientific background of this specific work is that the Wellcome Trust Case Control Consortium (WTCCC),
which was one of the first large scale genome-wide association studies reported a suggestive association on a region on human chromosome 15 containing Nr2f2 to hypertension. Following this study, several other reports in both humans and model organisms viewed this gene as a highly prioritized candidate gene associated with hypertension. Despite these strong association studies, validation of Nr2f2 as a genetic determinant of BP remained undefined. The current study by Dr. Kumarasamy et al was conducted to directly test the involvement of Nr2f2 in BP regulation. For this purpose, a state-of-the-art zinc-finger nuclease based genetically-engineered rat model was generated, wherein the Nr2f2 protein was mutated in the hinge region. The data collected from this model not only serves as a validation study for Nr2f2 as a genetic determinant of BP, but also points to the structural mechanism by which Nr2f2 influences BP as being due to the hinge region, through which it interacts with another transcription factor, Friend of Gata2 (Fog2). The extent of interaction between Nr2f2 with Fog2 is enhanced when the Nr2f2 protein is mutated in the hinge region, which in turn alters the transcription of specific target genes such as the gene coding for the atrial natriuretic peptide. In addition to this specific target gene, the study also serves as the foundation to explore yet unknown targets of the interaction between Nr2f2 and Fog2, through which blood pressure could be mechanistically impacted. Overall, these data provide the first and direct line of evidence for the extent of interaction between two transcription factors, Nr2f2 and Fog2, as being important for the regulation of blood pressure.

Dr. Joe commends her talented team members for their relentless perseverance and bringing together their diverse intellectual and technical skills in functional genomics, radiotelemetry, myography and echocardiography (to mention a few), to complete this work. The study being accepted for publication in a top-tier journal comes on the heels of a similar study on another novel gene implicated in hypertension, which was published by this group in the Proceedings of the National Academy of Sciences (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3528556/). Dr. Sivarajan Kumarasamy, who has been a co-author on both of these studies remarked, “I could not wait to see my article in print! Working on these projects with Dr. Joe and the lab has been a remarkably gratifying and fun scientific journey and I am proud to have reached this milestone in my career.” Harshal Waghulde, a graduate student in the Joe lab is elated to see his work on the vascular function of these custom engineered rats and says “It was fun to work on this exciting project and the ecstasy of this research being recognized in one of the top notch journals is worth mentioning. I am thankful to Dr. Joe for giving me this great opportunity to make best use of my skills and scientific expertise to contribute in this excellent piece of work”. The team appreciates help from Dr. Nitin Puri of the Department (who is acknowledged in the article) for his guidance with the vascular studies. Dr. Eric Morgan, who is a collaborator and co-author of the study adds, “It is a great privilege to collaborate with this dedicated and ingenious group of the Center for Hypertension and Personalized Medicine, and it is truly an honor to be fundamentally involved in exploring the physiologic importance of Nr2f2 on the cardiovascular system. Research efforts like these make it an exciting and rewarding time to work at the cross-roads of genetics and physiology.”

Clinical Research Snippets

Anand B. Mutgi, MD
Sadik A. Khuder, PhD

This month we review an article addressing early intervention in acute stroke to prevent or decrease the expected neurologic disabilities. Neuroprotection remains a possible strategy to improve outcome after acute ischemic stroke. Magnesium sulfate has been used as a cerebroprotective agent in diverse animal models of stroke, exerting both vasodilatory and direct neuroprotective and glioprotective effects. A previous clinical trial suggested potential benefit of intravenous magnesium sulfate in patients treated within the first 3
hours after onset of acute stroke. The present study was designed to examine the benefit of early initiation of magnesium sulfate therapy (within 2 hours after the onset of symptoms) on mortality and disability.

This is a well-designed and executed study and with adequate statistical power. A total of 315 paramedic-staffed ambulances and 60 hospital sites in Los Angeles and Orange Counties, California, participated in the study. Between January 2005 and December 2012, a total of 1700 patients underwent randomization: 857 were assigned to the magnesium-sulfate group and 843 to the placebo group. The study results indicated no significant effect on the degree of disability 90 days after the initiation of the treatment. Additionally, no significant improvement in mortality was noted between the two groups.

The authors attributed the lack of benefit to the delay in magnesium sulfate adequately reaching the affected areas in the brain. The concentration of magnesium in the cerebrospinal fluid peaks 4 hours after parenteral administration in the presence of an intact blood–brain barrier and more quickly in regions of focal ischemia where the blood–brain barrier is disrupted. Magnesium sulfate may not have accumulated in brain tissues quickly enough to yield a benefit despite rapid attainment of increased serum levels. In addition, as the authors stated, “a single neuroprotective agent may not interdict enough pathways in the molecular elaboration of ischemic injury, and combinations of agents or agents with highly pleiotropic effects may be required”. In discussing the limitations of the study, the authors acknowledged that the trial was completed over an 8-year period and the delivery of conventional therapy did evolve over that period of time.

Morbidity and mortality from stroke is significant. Among several modalities of treatment early use of TPA and thrombectomy in ischemic stroke have shown benefit. However, these modalities are limited by the need for brain imaging to exclude hemorrhagic stroke. The current study showed no benefit from early administration of magnesium sulfate in stroke patients despite good clinical hypothesis supported by animal studies and prior small scale clinical studies. However it clearly demonstrated the feasibility of early intervention in stroke victims with neuroprotective agents. There is a need to identify a new neuroprotective agent to be implemented in the early hours of acute stroke.

IRB Corner

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