Research Update From the Department of Anesthesiology

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The Department of Anesthesiology at the University of Toledo has been blessed to have had two pioneers in the specialty of anesthesia as faculty members. John (Tom) T. Martin, M.D. was the second Chairman of the Department and a world expert who literally wrote the book on Positioning in Anesthesia and Surgery and associated nerve injuries. He was a prolific writer authoring or co-authoring 48 articles in professional journals, 31 book chapters and edited hundreds of anesthesia research articles over the years. He became the Chairman and later trustee emeritus of the International Anesthesia Research Society and the editor of its flagship journal, Anesthesia and Analgesia, the oldest publication in the specialty of anesthesia. He continued writing well after retirement and a resident achievement award of which I am a proud recipient was established in 1991 in his honor.

Vladimir (Vlado) Nigrovic, M.D., Ph.D. was a long standing member of the department internationally known for his work on muscle relaxants. He also held the rank of Professor within the Department of Pharmacology. His research resulted in over 100 scholarly publications and was twice the recipient of the B.B. Sankey Award for progress in the field of anesthesia. He was a devoted educator, colleague and mentor to many of our former residents and current faculty. A Vladimir Nigrovic Endowed Research Fund was established by his family to support continued research. The legacy of these two significant individuals lives on within the department to this day. It is no accident that
Nerve injuries are a well-recognized complication of anesthesia. Those of us who trained under Dr. Martin became disciples of his teaching and take pride in our attention to positioning but injuries still occur. An analysis of the American Society of Anesthesiologists (ASA) Closed Claims Project in 1990 reported an incidence of 15% for anesthesia-related nerve injury of which ulnar nerve injury accounted for 34% of those claims. A subsequent follow-up study in 1999 showed little change in anesthesia-related nerve injuries as the incidence was 16% of which 28% were ulnar nerve injuries. Certain co-morbidities and demographics have been identified to be more frequently associated with nerve injury.

Recommendations to prevent ulnar nerve injury are limited because the mechanism for most injuries remains complex, multifactorial and incompletely understood. The ASA updated practice advisory for the prevention of perioperative peripheral neuropathies in 2011 offers few new strategies to prevent ulnar nerve injury under anesthesia during surgery. Studies have shown that often many patients have previously undiagnosed ulnar neuropathy. Therefore, it is conceivable that some patients undergoing surgical procedures with previously undiagnosed neuropathy may be worsened during the prolonged immobile state that occurs during anesthesia and surgery. As a prelude to improving our understanding of ulnar nerve dysfunction that occurs during anesthesia and surgery, we would like to determine the incidence of ulnar neuropathy in a preoperative patient population. We are in the process of enrolling 300 subjects, 50 years of age or older, of either sex being scheduled to undergo a surgical procedure at UTMC.

Two other studies currently under way involve pyridostigmine, an acetylcholinesterase (AChE) inhibitor traditionally used for the treatment of myasthenia gravis. It has also been used as prophylaxis against chemical warfare during the Gulf War being given to soldiers prior to deployment. Recently the clinical role of pyridostigmine has expanded and it appears to be a promising therapeutic agent for the treatment of postural tachycardia syndrome (POTS), neurogenic orthostatic hypotension and orthostatic hypotension associated with Parkinson’s disease. Also a second generation of cholinesterase inhibitors is currently approved for the treatment of mild-to-moderate Alzheimer’s disease.

In one study we are looking at Electromyography (EMG) with Repetitive Nerve Stimulation in Patients on Chronic Pyridostigmine Therapy. Where acute administration of cholinesterase inhibitors as used to reverse the effects of muscle relaxants may enhance muscle function, the chronic use appears to impair it. If the impairment of the neuromuscular junction caused by pyridostigmine is significant then there may be a decreased margin of safety for patients undergoing anesthesia. A total of 10 patients have already been enrolled with a total of 20 anticipated.

A related study is looking at the Dose-Response Relationship of Rocuronium in Patients Taking Pyridostigmine Pre-Operatively Compared to Those Not Taking Pyridostigmine. Despite the widespread use of muscle relaxants and availability of monitoring devices, a significant proportion of patients receiving muscle relaxants display signs of muscle weakness in the recovery room which has been shown to be associated with an increased incidence of post-operative complications. It is possible that some patients demonstrating prolonged weakness following the administration of muscle relaxants probably have an increased sensitivity to the effects of muscle relaxants. Anecdotal evidence in our department suggests that patients taking pyridostigmine show increased sensitivity to the effects of non-depolarizing muscle relaxants (NDMR). There appears to be no systematic human investigation of the dose-response relationship of rocuronium (a NDMR) in patients chronically taking pyridostigmine. We intend to study the sensitivity of patients taking pyridostigmine to the effects of this commonly used non-depolarizing muscle relaxant.

These are a few of the ongoing investigations within our department. Outside of our department we have joint studies with the Department of Surgery, Department of Orthopedics and with the College of Business and Innovation on the main campus. There are also four clinical trials being conducted by our division of Pain Medicine under the direction of Dr. Atallah. He will report on these in a future issue of the JCCTR.
Newsletter. As a department we welcome any opportunity to collaborate with other departments on investigations of mutual interest. Also a special thanks to Denise Zeller our research coordinator and the JCCTR who have helped us tremendously.

New Publication

Locking the wheels on glioblastoma: Exploring the actin cytoskeleton as an anti-invasive glioblastoma therapeutic target

Kathryn M. Eisenmann, Ph.D.
Asst. Professor, Dept Biochemistry and Cancer Biology

Glioblastoma (GBM) is the most frequently diagnosed brain tumor in adults; in 2010, there were 22,000 cases in the U.S. Survival statistics for patients diagnosed with GBM are grim. According to the Central Brain Tumor Registry of the United States (CBTRUS), in GBM patients treated between 1995 and 2010, approximately 4-17% survived 5 years beyond their initial diagnosis (American Cancer Society; cancer.org). In reality, people with GBM often live fewer than 15 months following diagnosis. This is because, despite surgery, radiation and chemotherapy, individual cancer cells escape and invade healthy surrounding tissue, making additional treatment attempts increasingly difficult.

The standard treatment for GBM is surgical resection, accompanied by chemotherapy (e.g., temozolomide) and radiation. Surgical resection and radiotherapy, however, are incomplete treatments, as individual cells are found migrating centimeters from the primary tumor [1]. These migrating cells ultimately lead to refractory disease that can compromise critical nervous system functions [2, 3]. Complicating GBM therapy further is the observation that invasive GBM cells resist radio- and chemotherapy [4, 5], relative to their non-invasive counterparts. Interestingly, GBM appears to expose a therapeutic “Achilles heel”, in that an inverse correlation exists between cell motility and proliferation. Migrating GBM cells, through epigenetic mechanisms, down-regulate genes promoting cell cycle progression and proliferation [6]. Hence, therapeutically targeting GBM invasion may in effect sensitize cells to conventional therapies [7]. Currently, however, few anti-invasive therapies exist for the treatment of GBM.

The cell skeleton, or cytoskeleton, is a critical structural network essential to maintaining cell structure, intracellular trafficking, cellular division and cell motility. Cytoskeletal regulators such as the Rho GTPases Rac1, Cdc42, and RhoA and their downstream effectors play a critical role in cellular motility and invasion in general [8] and GBM in particular [9-12]. Because Rho GTPases critically regulate GBM invasion, they are also potential targets for anti-invasive strategies in GBM. However, despite development of compounds targeting Rac (e.g., EHop- 016) and Cdc42 (e.g., AZA197) showing promise as anti-invasion therapies in breast and colon cancers, respectively [13, 14], therapeutically targeting Rho GTPases has its limitations as Rho GTPases are involved in a variety of important physiologic processes. Thus, targeting these proteins may have additional and difficult-to-predict effects in addition to their effects on invasion in GBM. Downstream Rho effector proteins that specifically propagate cellular motility signaling pathways may serve as better targets of anti-invasive therapies. Potential downstream targets include the mDia formin family of cytoskeletal regulators.

Mammalian Diaphanous (mDia)-related formins (mDia1-3) are cytoskeletal regulators that affect both the actin and microtubule cytoskeletons. mDia formins are regulated by Rho GTPases [15], and once activated, generate linear actin filaments by nucleating actin and promoting processive filament elongation. Specifically, the formin homology 2 domain, FH2, is required for mDia-mediated actin assembly [16]. The FH2 domain both nucleates actin, elongates, and in some cases bundles F-actin [17]. mDia formins also stabilize microtubules [18]. Through their effects on the cytoskeleton, they establish and maintain polarized cell adhesion, migration, and division in normal and malignant cells [19].

mDia formins play a role in GBM cell migration and invasion [20-22], and thus are intriguing targets for anti-invasive strategies in GBM. We recently completed a study to assess the role of mDia formins in GBM cell migration and invasion. Our study, published in the journal Molecular Biology of the Cell [20], expands upon an earlier discovery by my long-time collaborator, Dr. Arthur Alberts from the Van Andel Research Institute. Dr. Alberts discovered a bioactive peptide called DAD and developed small molecules called intramimics (IMMs) that behave like DAD. DAD acts as an mDia agonist, promoting the actin nucleation and polymerization activity of the mDia FH2 domain. This contrasts from other well-published mDia antagonist
reagents, such as SMIFH2, which inhibit mDia FH2 activity and prevent F-actin polymerization and migration, in some studies. Both antagonists and agonism independently inhibited cancer cell migration in select systems. One objective from our study was to answer a simple question: Is mDia antagonism or agonism a superior anti-invasive approach in GBM?

We tested both SMIFH2 and IMMs for their ability to halt migration using two-dimensional wound healing or “scratch” assays. Our results clearly showed that mDia suppression still allowed cells to move. Migration paths for individual GBM cells were randomly intrinsic and wandering, as opposed to directional and towards the wound, as in control cells. When total distances of the migration paths were compared to controls, the results were identical. Essentially, cells were moving, yet steering out of control, as opposed to a clear and directed path. In stark contrast, however, mDia agonism with IMMs completely abrogated both directional and random intrinsic migration, indicating complete inhibition of migration. Cells simply didn’t move in any direction, or for that fact even start to move. Single cell assays such as transwell migration and three-dimensional invasion assays also revealed that mDia agonism was superior to antagonism for halting GBM invasion. We then extended our studies into complex spheroid invasion assays. Thousands of GBM cells are formed into spheroids, which behave in a more physiological manner in their invasive and proliferative properties to tumors, relative to single cells. Once again, IMMs and IMM analogues were superior in halting GBM invasion in spheroids, relative to antagonism. Interestingly, IMMs specifically target invasion and not proliferation, and the effects of IMMS were reversible, as demonstrated with drug washout experiments. Finally, with assistance from Drs. Nicolas Chaia and Marthe Howard from the Department of Neurosciences, we developed a rat brain slice GBM invasion model. Spheroids pretreated with IMMs or SMIFH2 were placed upon cultured brain slices, and allowed to invade for 48 hrs. The depths of GBM cell invasion were quantified with assistance from Dr. Andrea Kalinoski of the UT Advanced Imaging Core Facility. Once again, it was clear that agonism was a far superior approach than antagonism to halting invasion.

The sum of our experiments showed that cancer cells, like any moving vehicle, have a steering mechanism and the steering and wheels must be pointed in the direction that the vehicle must travel. mDia antagonism allowed the cell to travel and the wheels of the cell pointed in the right direction, but the cell was being steering in a wild and uncontrolled fashion. In contrast, mDia agonism locked the wheels of the car in opposing directions, and thus completely abrogated invasion in any direction.

So what is the next step for this work? We are currently developing collaborations with researchers within UT and in Cleveland Clinic to further test IMMs in patient-derived xenograft studies, and to further characterize the mechanisms by which these compounds work. We are testing IMM analogues for toxicity and efficacy in a zebrafish model of GBM invasion. GBM is lethal because it so effectively escapes and evades therapy. Our hope is this discovery will prove to be an anti-tumor strategy and one that will be safe and effective for patients.

Reference List:


**Clinical Research Snippets**

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

Intermittent claudication, leg/calf pain caused by reduced blood flow to the legs during exercise, is associated with impaired quality of life. This affects at least 8 to 12 million Americans. Treatment includes medications, a supervised exercise program, and/or surgical revascularization to correct narrowing of arteries in the leg. This month we review a randomized controlled clinical study that compared the effectiveness of early endovascular revascularization with supervised exercise to supervised exercise alone.
This study was conducted in the Netherlands at 10 sites between May 17, 2010, and February 16, 2013. Patients were randomly assigned to supervised exercise (n = 106) or endovascular revascularization plus supervised exercise (n = 106). The exercise program involved treadmill walking to near-maximum claudication pain. Endovascular Revascularization was performed by an experienced interventional radiologist or vascular surgeon with selective stenting.

Maximum treadmill walking distance improved significantly in both groups, but the improvement was significantly greater in the combination therapy group (with a mean difference of 566 m at 1 month, 409 m at 6 months, and 282 m at 12 months). There was consistent improvement in Quality Of Life (QOL) as measured by global rating and SF-36. Early percutaneous revascularization improves lower extremity blood flow, facilitates subsequent exercise and allows the patient to profit from the long-term benefits of an additional supervised exercise program.

In discussing the limitations of the study, the investigators acknowledged that the results are generalizable to patients with stable claudication and established stenosis of Fem-Pop or Aorto-Iliac disease. The study was limited to 12 months follow-up, and reasons for the decreasing difference in the walking distance between the two groups remains unclear. The authors did not analyze the effects of other factors.

We feel that despite these limitations, this was a well-designed study that establishes the superiority of early endovascular revascularization combined with supervised exercise, in improving walking distance and QOL for at least the first 12 months.

### New Clinical Trials

A031201: Phase III trial of enzalutamide (nsc # 766085) versus enzalutamide, abiraterone and prednisone for castration resistant metastatic prostate cancer
Dr. Jain - Urology

RTOG 0848: A phase ii-r and a phase iii trial evaluating both *erlotinib (ph ii-r) and chemoradiation (ph iii) as adjuvant treatment for patients with resected head of pancreas adenocarcinoma
Dr. Reddy - Radiation Oncology

RTOG 0924: Androgen deprivation therapy and high dose radiotherapy with or without whole-pelvic radiotherapy in unfavorable intermediate or favorable high risk prostate cancer: a phase iii randomized trial
Dr. Reddy - Radiation Oncology

### IRB Corner

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