EDITORIAL

Glutamine, heat shock protein, and inflammation — opportunity from the midst of difficulty

'When written in Chinese, the word "crisis" is composed of two characters. One represents danger and the other represents opportunity.'

— John Fitzgerald Kennedy, 35th US President

'In the middle of difficulty lies opportunity.'

— Albert Einstein

'I am going to cure this disease and figure out how to treat patients with medicines whose side-effects aren't worse than the disease itself!' With that statement I began my medical career as I lay in a hospital bed at age 15.

In 1985 I was a typical 15-year-old boy, obsessed with playing soccer and trying to figure out what I wanted to be when I grew up. My life changed dramatically when I was told I had inflammatory bowel disease, specifically ulcerative colitis. In the same discussion I was told I would not be able to eat or drink anything for the next few weeks. What a thing to tell a teenage boy — I lost 60 pounds over the next weeks, the steroids I was on failed to slow the progression of my disease and the side-effects were maddening. This led the physicians at the community hospital I was at to throw up their hands and transfer me to the University of Chicago Hospital where I was told I was to be given total parenteral nutrition (TPN) via an intravenous line in my shoulder. What an introduction to the field of nutrition support! I was given TPN for 6 weeks during which time I was obsessed with food, so much so that I read cookbooks and recipes day and night and learned everything I could about nutrition. When I was finally allowed to eat again I was overjoyed, but after my first solid food my physicians did not seem happy. Then a new physician appeared at my door. I thought he was another nutrition doctor, come to tell me I could not eat again and that the TPN would have to be restarted, but that was not to be the case. He was a surgeon; he had come to tell me that my large intestine had dilated and was leaking. I had peritonitis and I needed to have surgery right away. I was amenable of course, and I went to have my colon removed. Following two additional surgeries and a temporary ileostomy, I had an ileal-pouch anal anastomosis performed. Little did I know the future opportunities this illness would present me with.

My ileal-anal pouch was never an ideal solution. It was continually afflicted with a condition called 'pouchitis' for which I was always taking antibiotics. The antibiotics had their own side-effects of course, such as numb and tingling fingers and toes. I entered Valparaiso University as a pre-medical student, knowing I wanted to be a physician and pursue a career in medical research. Ideally, I wanted the opportunity to discover new treatments for the many serious illnesses lacking adequate therapies. Further, it was my hope to investigate therapies that would have minimal side-effects.

To this end, at the end of my freshman year of college, I went back to the University of Chicago and walked into the Gastroenterology Research Center and asked to speak to someone. It was at this point that I met Dr Stephen Hanauer, one of the directors of inflammatory bowel disease research. I told him that I had been a patient in his hospital a few years before and that I wished to do whatever it took to learn to do research in the field of inflammatory bowel disease. He said he couldn't pay me and I would have to volunteer, which I said would be just fine. He also told me that inflammatory bowel disease was a rather large field for an 18-year-old college freshman with no laboratory experience whatsoever to be taking on. So he suggested I study something I knew a little more about. Specifically, he suggested I examine the potential role of gut nutrients in pouchitis of the ileal-anal pouch, as I had chronic pouchitis myself and the ileal-anal pouch was becoming the surgical procedure of choice for ulcerative colitis.

After reviewing the literature, I proposed that a deficiency in short-chain fatty acids might be contributing to the inflammation seen in pouchitis of the ileal-anal pouch. However, the University of Chicago was not able to fund the project for the following summer. In desperation I sent the proposal to Dr Sidney Phillips who was chairman of Gastroenterology at the Mayo Clinic in Rochester, Minnesota as they had the largest number of ileal-anal pouch patients in the world at the time. A number of months went by and I had forgotten I had sent the proposal when I got a phone call from Dr Phillips asking if I would like to come to the Mayo Clinic to perform my study in a group of their pouchitis patients. I left the

next day, and over that summer discovered that pouchitis patients did have a significant deficiency in butyric acid versus patients without pouchitis. This led to a very exciting, but very humbling, presentation at the American Gastroenterological Association (AGA) Meeting in New Orleans; as I stood next to my poster I was continually asked where my father was. I also realised how very little I knew about my own data. I would never be so ill prepared again.

I returned to the Mayo Clinic the next summer, where I proposed a pilot project to create suppositories made of butyric acid in the hope of treating pouchitis and reducing patients' need for continual antibiotic, and at times steroid, therapy. As I researched that proposal I came upon a great deal of literature supporting the potential of another gut nutrient, particularly in the small bowel, which the ileal pouch is created from. That nutrient was glutamine. Little did I know in the summer of 1991 that this simple amino acid would become the focus of much of the rest of my research career. As a result of the fact that glutamine seemed like an obvious alternative nutrient to support a small-bowel pouch I also created a 'glutamine suppository'. We then examined the effect of glutamine and butyric acid in two groups of the most severely ill pouchitis patients at the Mayo Clinic. We discontinued these patients' chronic antibiotic, steroid, and anti-inflammatory therapy, and to our surprise 60% of the patients treated with glutamine were able to remain off their chronic antibiotic and anti-inflammatory therapy and remain disease free. These data were presented the following year at the AGA in San Francisco and this time I was prepared to present my poster and knew my data well.

Glutamine could not, however, spare my ileal pouch from pouchitis and that year I underwent three surgeries that would ultimately lead to the loss of my ileal pouch, my ileum, and a portion of my jejunum. This left me with a jejunostomy, which I have to this day. Luckily, the surgeon I was working with in the laboratory at the Mayo Clinic, Dr John Pemberton, operated on me and I missed very little of my education and research time as a result.

The following fall I was lucky enough to be able to return to the University of Chicago, this time not as a patient but as a medical student at the University's Pritzker School of Medicine. While a student there I began work in the laboratory of Dr Eugene Chang who was interested in cytoprotective mechanisms in the gut. He encouraged me to look at how glutamine might be protecting the cells of the small bowel and be leading to improvement in patients with pouchitis. To this end I began studying a small-intestinal cell line, specifically IEC-18 cells. I performed experiments in which I provided various concentrations of glutamine to the cells and then exposed the cells to either heat or oxidant injury. I found that increasing concentrations of glutamine were dramatically protective of these small-intestinal cells with regard to both injuries, but I did not know why. I initially hypothesised it was purely a 'metabolic effect', but this seemed too simple and I went back to Dr Chang for ideas. His laboratory was working on various new cytoprotective mechanisms and he suggested I go to the library and see if any of these 'hot' new mechanisms might be playing a role. One of these 'hot' new mechanisms was the role enhanced heat shock protein (HSP) expression could play in protecting cells and tissues from stress and injury. I entered glutamine and HSP in *Index Medicus* and got three papers. One had found that glutamine given to drosophila cells before sublethal heating could enhance the expression of one family of these protective proteins. Perhaps glutamine was enhancing HSPs in my small-intestinal cells and that was how glutamine was protecting these cells from lethal oxidant and heat stress? I went back to Dr Chang with my hypothesis and he indicated that his laboratory had the appropriate antibodies for me to perform an experiment in which I simply added varying concentrations of glutamine to IEC-18 small-intestinal cells and then performed Western and Northern blots probing for HSP 70 protein and mRNA expression. We determined that HSP 70 (the 70 indicates the molecular weight of the protein) was the primary protective HSP that should be studied. The first experiment revealed that glutamine was indeed a potent enhancer of HSP 70 protein and mRNA expression, even in the absence of stress. We also determined that glutamine's protective effect against heat and oxidant stress was significantly attenuated if HSP 70 expression was inhibited by blockade of the HSP 70 transcription factor or via anti-sense inhibition of gene.

At this point it seemed likely that I would pursue a career in gastroenterology. However, in medical school I realised that caring for patients with the same illness I had was much too personal and not nearly as rewarding as I had hoped. I discovered that my true clinical love was anaesthesia and intensive care, and therefore I began an anaesthesia and critical care residency at the University of Chicago. I then began to read the critical care literature and discovered that enhanced HSP expression in the tissues of septic animals could significantly improve outcomes from sepsis, acute respiratory distress syndrome, and overwhelming inflammation in rats and mice. However, the only way to induce HSP expression was by heating the animals to sublethal temperatures or by arsenic administration. Obviously neither was practical for clinical application in humans, and many of the journal articles I read on the subject concluded with the caveat that if only we had a clinically relevant method of inducing HSP in humans we may be able to reduce morbidity and mortality from critical illness and injury. It was at this point that the proverbial 'light bulb' went off in my head. Glutamine could induce HSPs in small-bowel cells and protect them from injury. Further, glutamine

had been given safely to a wide range of critically ill and injured patients without adverse effect. I wondered if glutamine could induce HSPs, specifically HSP 70, in the tissues of septic rats and improve outcomes to a similar extent that sublethal heating or arsenic had in previous studies. To answer this question I returned to Dr Eugene Chang's laboratory in 1997 and with the help of Dr Mark Musch (without whom none of the experiments in this laboratory would ever occur) we administered single doses of glutamine to rats and found that glutamine could indeed induce HSP 70, not only in gut tissue, but also in the heart and lung tissue.

Subsequently, we administered glutamine and endotoxin concomitantly and found that glutamine could potently induce HSP 70 in multiple organs. More importantly, we found that glutamine markedly improved survival when given at onset of experimental sepsis. An unexpected and interesting finding of this study was that glutamine administration was potently anti-inflammatory, as indicated by significant attenuation in tumour necrosis factor-alpha (TNF-α) and interleukin-1-beta in glutamine-treated animals following experimental sepsis. Further literature review revealed a possible association of HSP 70 expression and attenuation of pro-inflammatory cytokine expression.

So glutamine could in fact induce HSP 70 in multiple tissues of a whole animal model and improve survival following endotoxin shock. Additionally, glutamine could attenuate inflammatory cytokine release, a totally unexpected finding, but perhaps related to HSP expression. The logical next step was to evaluate whether glutamine could enhance HSP expression in human cells. Further, did glutamine have an effect on cytokine expression in human monocytes? These are the fundamental questions that led to the inspiration for the research project for which this award was given.

This research project essentially examined the effect of glutamine on human peripheral blood mononuclear cells (PBMCs) stimulated by endotoxin. We found that as human immune cells are exposed to increasing concentrations of glutamine and then stimulated with endotoxin they increased their expression of HSP 72. We also found that increasing concentrations of glutamine attenuated the release of TNF-α from human PBMCs stimulated with endotoxin. This study showed for the first time that glutamine could manipulate HSP in human cells. Further, it showed that glutamine's anti-inflammatory properties could be observed in human PBMCs.

This study was the culmination of work done by quite a number of investigators both at the University of Chicago and the University of Colorado Health Sciences Center. I initiated the trial at the University of Chicago during my residency and fellowship and then took a faculty position in anaesthesiology at the University of Colorado, where I serve as the Director of Nutrition Support Services. Included in the contributors to this research was an undergraduate student from my undergraduate alma mater (Valparaiso University), Jacob Reihm, who worked with us at the University of Chicago; Dr Mark Musch, who as I mentioned previously aided in virtually all the work I have done up to this point; Hongyu Ren, who was my indispensable lab assistant at the University of Chicago; Dr Madelyn Kahana, who was my friend and mentor in anaesthesiology and intensive care medicine, and who nurtured many facets of my life throughout medical school and residency; Dr Eugene Chang, who was my research mentor for the better part of 10 years and to whom I owe a great debt for giving me the opportunity to work in his lab; and finally, Kristen Singleton, who is now my right and left hands in the laboratory and a good friend here at the University of Colorado. She helped to complete this project and without her I am fairly sure my lab in Colorado would cease to exist. I owe all of these individuals a huge debt of gratitude.

In the last year I have revisited the operating room five additional times for intestinal resections and stoma revisions. It has been a challenging year, but the news that our laboratory received the 2003 John M Kinney International Nestlé Award for General Nutrition could not have come at a better time. This award has provided an enormous morale boost both to my research team and to me personally. This new motivation is particularly important for we are entering a very challenging and exciting time in our laboratory as we begin to translate years of bench research into what we hope will become clinical benefit. We are in the process of initiating clinical trials of glutamine in critical illness and in patients following cardiac surgery, which we hope will make a significant difference in the clinical outcome of these very sick patients. As for me, I am still dedicated to a career studying the use of nutraceuticals, particularly glutamine, to attempt to improve patient outcomes using medicines with side-effects that aren't worse than the diseases themselves. Further, I hope to continue to find unique opportunities in the difficulties I am faced with, both in life and in the laboratory.

Paul Wischmeyer

Department of Anesthesiology
University of Colorado Health Sciences Center
Denver, Colorado
USA