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Founded and staffed by undergraduate students at the University of Alabama at Birmingham, *Inquiro* is an annual research journal produced as an outlet for the publication of undergraduate scientific research. UAB is an excellent undergraduate research university, and with the addition of a journal such as *Inquiro* in which to publish their findings, the package is complete. Any undergraduate student at UAB, as well as any student participating in a summer program at the university, is eligible to submit research. The rights to every paper published in *Inquiro* are retained by the author, leaving each individual free to submit to and publish in a larger national journal or magazine. Students are invited to submit research papers, short reports derived from posters or research narratives throughout the year.

letter from the editor

The journey of curiosity begins early.

Minds are naturally molded to thirst for knowledge, to experience, to interpret, to inquire. All beings, in some way, are a part of this journey. An infant wonders about this new world in the simplest manner; who is that curious figure looking back in this shiny object? A dancer searches to translate emotions into gestures, evoking feelings through choreography. A ship captain seeks to master the mysteries of the sea. An anthropologist strives to understand beings through culture and patterns. A scientist endeavors to inquire about the mechanisms of the natural and physical world.

I showed the signs of a scientist at a very young age. I was four the first time I looked into a microscope, astonished at the tiny cells that make up our body. My parents, both Anatomic Pathologists, inspired the act. I vividly remember watching them when I was young as they meticulously examined slides for hours, dictating the most obscure of words: carcinoma, leukoderma, lymphoma. Fast forwarding into my adult years, I developed a passion for communication through music, art, and debate. I loved performing with my jazz vocal group in music festivals, sharing my work in exhibitions, and exercising philosophical discourse to delve into the structure of society. However, the initial curiosity of my first glimpse of cells never ceased to brew inside of me. As a result, I was moved to examine the three levels of cancer in medicine. I shadowed surgeons and oncologists to see treatment, worked in labs analyzing stage transitions to see research, and took interest in my parents' work to see diagnosis. It amazed me that a simple malformation in a small group cells could lead to an entire transformation of lifestyle for an individual. The field of Pathology impacts patient care in the most profound ways. To be able to see and acknowledge this impact is an imperative driving force in pursuing innovation in holistic patient-centered therapy and treatment. Currently, my interest lies in fostering this realization through contribution in diagnosis and clinical research in Pathology. Such was the deep impact of a small inquiry into a microscope.

Although Rachael's journey took a different path, it was also facilitated by a spark of inquiry. Her first encounter with scientific exploration occurred when she saw her father performing a necropsy on a chick. Captivated, she watched as he cut open the tiny chest and shifted internal organs around. The discovery of the infection site fascinated her. During her studies, her interests shifted from her father's expertise, avian diseases, to medicine. The initial experience of witnessing the identification of a chick's cause of death served as the catalyst for her development. Scientific research can achieve many feats: preventing disease, creating new technologies, and improving our understanding of the world, and these successes are a result of our curiosity to understand the ways the world works.

I have cherished my time as Editor-in-Chief of Inquiro, not only because this year Rachael and I have been able to continue the journal's rise to a national scale, but also because I, too, have grown in the process. We are lucky to have played a part in facilitating the interest of undergraduates pursuing research by exposing them to opportunities in publishing their work and understanding the rigors of the reviewing process. I am also lucky to have loved my job. As editors, we are constantly in the middle of reading papers on fascinating discoveries in the natural and physical sciences. In terms of the impact on my personal academic endeavor

in medicine, I have greatly benefited from my involvement with Inquiro. I was able to learn the language of science through direct communication with my peers and was able to make connections with my own cancer research to similar research across UAB and the nation. The interwoven web of innovation in science is a spectacular phenomenon and I am confident that because of these strides, patient care is improving each and every day.

The continued outstanding caliber of Inquiro since its first publication in 2007 serves a testament to UAB's dedication to scientific growth through research. Volume 5 stands as one of the most competitive issues, with one of the smallest manuscript acceptance rates. We have received multiple requests to be part of the journal, either through paper submissions or board membership applications. These requests have come from across the US and the world, including from Singapore and India. However, even with Inquiro's climbing fame, the journal has preserved its integrity through the submission process, placing full faith in our faculty reviewers' expertise and selections. The thought of such a small initiative expanding to have such a great impact was perhaps unfathomable by its founders. UAB's phenomenal commitment to undergraduate student enrichment has made this possible. This commitment is seen through the many professors who serve as academic and inspirational mentors to their students, the administrators such as Mike Sloane who facilitate the journal's growth through guidance, advocacy, and support, and the undergraduates who take an early initiative to expand their knowledge and impact on science. The continuation of Inquiro, then, is a reflection of the values of our institution.

My involvement with Inquiro has fortified my belief that creating a collaborative scientific community is imperative for sustaining progress. Inquiro builds this community for the next generation of innovators with each issue. May Volume 5 be just another building block on the way to an inspired future.

Cheers,

Khushboo Jhala, Chief Editor 2011 – 2012

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NASA: Is it the End or Only the Beginning?

Amiya Ahmed

Earlier this year, the end of NASA's 30-year-old Space Shuttle Program saddened many Americans. The return of the Shuttle Atlantis and its crew seemed to mark not only the end of America's leadership in space exploration but also the end of manned American space travel. For many children whose American dream included becoming an astronaut, the future seemed bleak. However, in spite of the shutdown of NASA's iconic program, human spaceflight has not come to an end. The U.S. will soon start using private spaceflight firms to ferry astronauts to and from the International Space System; until then, the U.S. will depend on the Russian Soyuz spacecraft. Nonetheless, this commercialization of the space industry may serve many positive purposes, contrary to the public's reaction. First, similar to the historic space race between the U.S. and the former Soviet Union, competition between private firms may in fact drive the progress of space technology. Additionally, the use of commercial companies for spaceflight enables NASA to pursue greater pursuits, namely deep space exploration.

Deep space exploration appears to be the new frontier of space exploration, and America will remain a leader in international space exploration. On September 14, NASA revealed plans for a massive deep-space rocket, which would allow astronauts to not only return to the moon but to also visit Mars and destinations much further than previously imagined. Based on the space shuttle schematics developed under the Constellation program, which was ended by the Obama Administration on February 1, this new rocket would require a $10 billion budget through 2017. Although costly, human expeditions to an asteroid by 2025 and to Mars in the 2030s have already been predicted. Additionally, deep space exploration takes place on another front as astronomers discover a multitude of new planets. On September 12, a "Super Earth," 36 light-years away was discovered. This planet, which was discovered along with fifty other new planets, is thought to be the right distance away from its sun to be habitable. This means that that water (and life) could potentially exist on this planet given the correct atmospheric conditions and planetary composition. Another exquisite planet, Kepler-16b, was discovered on September 15th. Nicknamed "Tatooine" after Luke Skywalker's home planet in Star Wars, this planet was found to have two suns, much like the fictional planet.

As NASA continues to search for planets with potential water and possible life with the Kepler Mission, it becomes more plausible with deep-space rocket technology to one day visit such destinations. However, whether or not that occurs anytime soon, a new era of space exploration has undoubtedly begun with deep-space technology.

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Nobel Prizes Awarded

Atbin Doroodchi

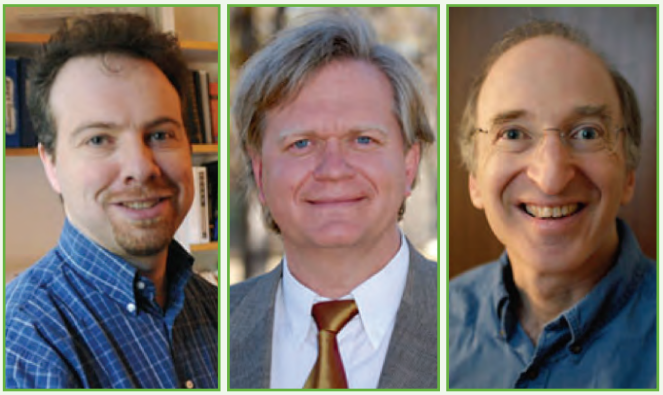
For the Nobel Prizes in Physiology and Medicine for 2011, Bruce A. Beutler and Jules A. Hoffmann were jointly recognized for their discoveries concerning the activation of innate (non-general) immunity and Ralph M. Steinman was recognized for his discovery of the dendritic cell and its role in adaptive immunity. Innate immunity is the non-specific part of the immune system, and it is seen in every animal. Dr. Steinman passed away three days before the announcement of the prize, after suffering from pancreatic cancer.

Bruce A. Beautler, Jules A. Hoffman, and Ralph Steinman

In Chemistry, the Nobel was awarded to Dan Schechtman for the discovery of quasicrystals. Quasicrystals are ordered non-periodic structures whereas crystals are periodic structures. They are solid and used in material sciences.

Dan Schechtman

The 2011 Nobel Prize in Physics was divided for the research of Saul Perlmutter and that of Brian P. Schmidt and Adam G. Riess for the discovery of the accelerating expansion of the universe through observations of distant supernovae. Supernovae are stellar explosions that are extremely luminous.



Adam Riess, Brian Schmidt and Saul Perlmutter

The Aftershocks of L'Aquila: The Indictment of Italian Seismologists for Manslaughter

Helen Lin

April 6, 2009 changed the lives of citizens in the central Italian town of L'Aquila in the Abruzzo Mountain region. At 3:23 am, a 6.3 magnitude earthquake jolted the picturesque medieval fortress-town, killing 308 people and reducing the town to rubble. Consequent additional quakes hindered rescue efforts and further damaged the quiet town.

The citizens of L'Aquila claimed that the assurances of the Serious Risks Commission, a government panel charged with monitoring and assessing the tremors in the months preceding the earthquake, persuaded them to remain in town. When asked if the public should worry, commission member Bernardo De Bernardis responded no. In fact, when asked if citizens should sit back and relax, he responded, "Absolutely, with a Montepulciano doc [a Tuscan red wine]." In 2010, the Serious Risks Commission came under investigation. The seven members of the Commission sit on many major committees in Italy:

- Enzo Boschi, President of the National Institute of Geophysics and Volcanology (INGV)
- Franco Barberi, Committee Vice President
- Bernado De Bernardinis, former Vice-president of the Civic Protection Department; current President of the Institute for Environmental Protection and Research
- Giulio Selvaggi, Director of the National Earthquake Centre
- Gian Michele Calvi, Director of the European Centre for Training and Research in Earthquake Engineering
- Claudio Eva, Earth Scientist at the University of Genoa
- Mauro Dolce, Director of the Office of Seismic Risk at the Civil Protection Department

In May 2011, Judge Giuseppe Romano Gargarella indicted the seven and ordered them to trial on September 20. The citizens of L'Aquila and the prosecution asked for $67 million in compensations. In response, 5200 seismologists wrote protest letters against the indictment. International scientific groups condemned the charges. The Seismological Society of America wrote a letter to Italy's president calling this trial an "unprecedented attack on science."

Interestingly, the case centers not on a failure to predict the earthquake, but rather the commission's false assurances to the public. Earthquakes are currently impossible to predict because it is difficult to know the exact threshold of stress required to break a fault line. Current tools only allow measurements of ground deformation. Additionally, the L'Aquila fault system is complex with several strike-slip faults. Seismologists have yet to find a conclusive method for predicting earthquakes. A recent study by Giampaolo Giuliani is looking into predicting earthquakes based on the amount of radon gas that leaks through fault lines. Other studies are following to develop accurate means of prediction.

The prosecution in the trials alleged that the experts gave the public an "approximate, generic, and ineffective assessment of seismic activity risks as well as incomplete, imprecise, and contradictory information." Experts had held an emergency meeting six days before the earthquake due to the occurrence of several smaller quakes in the region, one of which registered at a magnitude of 4.1. The seismologists on trial maintain that there was no evidence that a major earthquake was imminent, although it was perhaps probable. It was this advice to the town which gave a false sense of security.

Cases like these are far from unusual. Giuseppe Zamberletti, the former chief of Italy's civil protection agency, was placed under investigation after he ordered the evacuation of several towns in Garfagnana, an area in the province of Lucca following unusual seismic activity. Zamberletti was accused of causing public alarm when the major earthquake never occurred, but his case never went to trial. Alredo Biondi, one the defense lawyers, said, "[In Italy], often there's no punishment, but plenty of criminal charges to choose from."

After a three-hour hearing on September 20, the trial was adjourned until October 1st. But what will this trial mean for the scientific community, especially those in charge of warning the public about natural disasters? Many of these events contain certain elements of unpredictability. As seen with Hurricane Irene, hurricanes can weaken or strengthen during their courses. Tornadoes are even more fickle as they can to destroy one house and not another on the same street in an instant. Perhaps these trials will lead scientists to be overly cautious in their predictions. Is this necessarily a bad thing if it could save lives (assuming people listen, of course)? The question lingers, then, as to what extent the scientific community will be affected by the outcome of the trials. While it is unlikely that the outcome will imminently affect US natural disaster policies, the aftermath is definitely one to follow.

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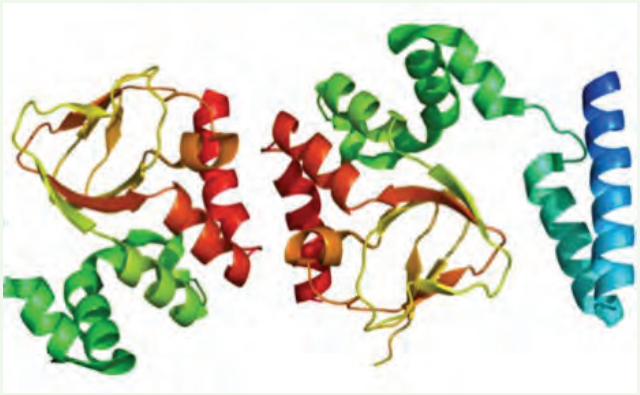
Say Goodbye to Chronic Pain

Sadhvi Batra



According to *Science* magazine, researchers at Cambridge University have identified the gene HCN2, which produces a protein associated with the onset of chronic pain. There are currently an estimated 116 million people living with chronic pain in the United States alone, and five percent of the overall global population is affected by this condition. Because it can lead to serious problems like depression, fatigue, anxiety, and loss of mobility, finding a cure for chronic pain in the near future is imperative.

The discovery of HCN2 provides the potential for curing neuropathic chronic pain, pain arising from injury to the nervous system. In the experimental trials following the isolation of HCN2, laboratory mice were bred to be deficient of the HCN2 gene. From these trials, research personnel observed that the mice continued to respond to sudden pain but did not suffer from neuropathic chronic pain.

This finding is critical to the fields of research and medicine, because it illustrates that the manipulation of a single mechanism can lead to the termination of the entire biological response. By removing the gene HCN2, researchers skillfully eradicated chronic pain but still maintained the crucial pain responses necessary for survival.

According to The Academy of Pain Medicine, healthcare costs to cover chronic pain in the United States range from $560 billion to $635 billion annually. Still, current medications are not effective in treating this condition. By isolating the HCN2 gene, researchers provide hope for a future cure for neuropathic chronic pain. This is sure to alleviate the suffering of millions of people.

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Professor Lee Berger with the Australopithecus sediba *fossils.*

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The Missing Link

Kavita Nadendla

According to papers released in the latest issue of Science magazine, remains recently discovered in a South African cave appear to be a new member of the human family tree. As a candidate transitional species between our ape-like ancestors and modern human beings, the fossils may yield new insights into the evolution of the human lineage.

Thanks to his then nine-year-old son, Matthew, Lee R. Berger of the University of Witwatersrand found the bones of *Australopithecus sediba* in the fossil-rich cave region of Malapa, near Johannesberg, in 2008. Matthew found the first bone, which was identified as belonging to a male child. Two weeks later, Berger uncovered the remains of an adult female. Scientists have long considered the Australopithecus family, which includes the famous specimen Lucy, as a candidate for a human ancestor. Interestingly, the new fossils show a novel combination of features resembling both pre-human creatures and the genus, Homo.

Reports of the findings detailed the brain, pelvis, hand, and feet of *A. sediba*. Scientifically dated as almost two million years old, the brain is small like that of a chimpanzee but with an expansion behind and above the eyes, suggesting a more human configuration. Like other Australopiths, the pelvis of *A. sediba* had already evolved for walking upright. However, prior to the discovery of *A. sediba*, it was thought that certain features of the modern human pelvis evolved to adapt to an increased brain size at birth. However, the presence of those adaptations in *A. sediba*, who lack increased brain size, invalidates that hypothesis.

Further mixing of traits between Australopithecus and Homo were seen in the hands and feet of *A. sediba*. The fingers of *A. sediba* were curved like those of a creature that climbed trees, but they were also long and slim with the potential to use tools like Homo did. The heel and shin of *A. sediba* remain fairly ape-like, while its ankle and foot arch are similar to modern humans and could allow for walking on the ground. Additionally, there is a large attachment site for an Achilles tendon.

So does all this mean that *A. sediba* is the "missing link?" According to scientists, who prefer the term "transition form," it is a good candidate to represent the evolution of humans. However, the earliest definitive example of Homo is actually 150,000 to 200,000 years younger. But the fact that *A. sediba* clearly indicates a mixture of Australopithecus and Homo is a confirmation of Charles Darwin's evolutionary theory.

The Benevolence of *Rattus norvegicus*

Timothy Fernandez

Whether 6 feet tall or just a few inches in height, heroes come in all sizes. A study done at the University of Chicago showed that even *Rattus norvegicus*, better known as the rat, has the urge to help a friend in need. Humans seem to use their senses of morality to put themselves above the rest of the animal kingdom, but this study showed that rats will often try to free trapped friends.

Humans share over 80% of their genes with the rat, so, from a genetic perspective, this behavioral similarity is not too surprising. Inbal Ben-Ami Bartal, a psychologist at the University of Chicago, conducted the study under the notion that the rats were capable of "emotional contagion," a term indicating that the rats are able to feel each other's pain. One rat was placed inside a clear cage that could be opened from the outside, and another rat was allowed to roam free. The unrestricted rat immediately took action to ease the other's pain. After repeatedly circling, digging, and biting the cage, the roaming rat eventually learned how to liberate his comrade. Strikingly, once the free rat learned how to open the cage, he would almost immediately rescue his friend in need during every trial.

When the cage was empty, the rats largely ignored the restrainer. Even when a stuffed rat was placed in the cage, a similar result ensued. Only five rats out of 40 learned how to open an empty cage; the percentage shown for cages with stuffed rats is fairly comparable. However, 23 out of 30 rats easily learned how to open a cage when there was a fellow rat in distress.

In order to test the rats' benevolence, another experiment was performed that made them choose whether to free a fellow rat, or five chocolate chips. Shockingly, the rats were equally likely to free their brethren first as their most beloved sweets. This came as a surprise to Bartal et al because the rats absolutely adored their chocolates. In fact, the study even shows that the rats shared their chocolate reward with their newly freed companion.

The question remains whether the free rat truly intended to ease the captive's suffering or its own pain of hearing distressed whining. Either way, it is interesting to see that the hero rat responded to the call of duty. It shows that nature has made it rewarding for us to end the suffering of another. Somewhere in the mammalian genetic code is a possible calling to be a hero. Maybe next time tragedy occurs, your savior will be a real life "Mighty Mouse".

Glow-in-the-Dark Cats

Helen Lin

The potential new frontier for HIV research may be fluorescent cats. While humans are susceptible to HIV, felines suffer from FIV, feline immunodeficiency virus. Despite affecting different species, the viruses cause nearly identical symptoms because most of their basic biochemistry is identical. Previous studies have shown that the protein TRIMCyp may keep humans and monkeys from being infected with FIV. As expected, cats lack this protein. The protein is believed to recognize FIV's protein coat and disable it as the protein tries to invade a cell.

To test whether cats given TRIMCyp would become immune to FIV, scientists at Mayo Clinic created genetically altered cats. They used the technique, gamete-targeted lentiviral transgenesis, a process by which genes are inserted into feline oocytes before sperm fertilization. The technique has worked previously with mice and cows. Researchers created a lentivirus containing the TRIMCyp gene and a fluorescent protein gene as a marker. If the cats began to glow green, researchers would be able to determine that the cats have TRIMCyp. Once the oocytes were infected and fertilized with normal cat sperm, the oocytes were injected into the fallopian tubes. Each cat received 30 to 50 eggs. Of the 22 cats fertilized, five cats became pregnant with multiple embryos. Ten of the embryos contained the target gene, but only three kittens with the genes survived. When the researchers tried to infect blood taken from the kittens, the virus did not replicate well. The cats themselves have not yet been infected with FIV.

These florescent cats will join the ranks of other fluorescent animals in science that include fish, pigs, macaque monkeys, and mice. Scientists hope that experiments like this one will provide additional strategies to address the HIV epidemic.

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faculty spotlight: Dr. Edward Taub

Redefining Recovery: Applying Behavioral Techniques as Rehabilitation

Naveed Farrukh

Dr. Taub serves as a testament to the success delivered by hard work and sincere passion. After numerous awards and great prestige for his work on Constraint Induced therapy (CI therapy), he continues challenging himself. He is a simple man whose interests revolve around his research and hearing his wife, Mildred Allen, a renowned lead opera singer, sing. He fearlessly challenged a medical dogma that had existed for more than half a century, and Dr. Taub remained resolute in the face of stinging criticism by trusting that thorough research can systematically answer questions. His work represents a shining example of translational research – taking laboratory bench data and integrating it into the clinical world. Dr. Carl McFarland, the Psychology chair who hired Dr Taub, describes him as "a guy who really loves what he does. Most days he comes in happy to work." Dr. Taub has brought millions of dollars in grant money to UAB and revolutionized the rehabilitative applications of behavioral techniques. He attributes his success to a modest set of tips and tricks; Dr Taub insists that research success comes from personal effort. His amazing rehabilitative stories reflect all the time, energy, and creativity he has poured into his projects.

Dr. Taub insists that research success comes from personal effort. His amazing rehabilitative stories reflect all the time, energy, and creativity he has poured into his projects.

After Dr. Taub's undergraduate career at Cornell, he entered behavioral analyst training and obtained his Master's degree at Columbia. As one of the premier operant conditioning research centers in the world, Columbia gave him the opportunity to learn from famous mentors such as Fred Keller. He finished his formal education with a PhD at New York University, and he moved to the Institute for Behavioral Research in Maryland, where he carried out experiments on deafferented monkeys. This research led to his discoveries in neuroplasticity and rehabilitation therapy. His early experiments reshaped the field of behavioral neuroscience by disproving Sherrington's Reflexological position, a popular theory held for over 70 years. Nonetheless, Dr. Taub still deems Sherrington a great scientist. He argued that Sherrington's explanations were perfectly reasonable, but skewed because "mother nature isn't a lady." The history of science shows that the most reasonable explanation is not always the correct one.

In Dr. Taub's experiments, the monkeys' dorsal spinal nerves were severed. Although sensations were eliminated, motor-neural pathways controlling arm function remained intact. Dr. Taub's research team explored why the monkeys stopped using their arm. Several years of research showed that deafferentation created a window of disability, but, after a few months, the monkeys should have been able to use their arms again. However, they incorrectly learned that the arm was not usable. This phenomenon, dubbed "learned nonuse," stems from basic reinforcement. Through many different forms of punishment (losing coordination, falling, acquiring food less efficiently, etc.), the monkeys learned to not use the affected arm. Instead, they learned compensatory behavior using only the good arm. These compensatory behaviors were reinforced by partial successes. Together, these two reinforcement components produce a strong conditioning environment that leads to learned nonuse. Dr. Taub applied behavioral techniques that forced the monkeys to use their deafferented arm, and the results showed extraordinary promise.

Almost a decade passed before he could successfully transform these findings into a rehabilitation technique to convert a useless limb into a functional one. Because the laws of behavior are universal in all mammals, he thought he could translate his findings to human rehabilitation following a stroke. For example, patients really can't use an affected limb for a period of time after a stroke. Even later in recovery, when they really could they continue to often feel embarrassed in front of others when trying to use an affected limb. This adversive consequence dissuades usage of that

limb, and successfully using only the unaffected limb positively reinforces the nonuse of an affected limb. Dr. Taub thought that the same behavioral interventions that were successful with monkeys could serve as a human rehabilitation technique. Over the next few years, Dr. Taub moved to UAB and developed on tests the basis of human rehabilitation treatment. This was his first experience with translational research, an uncommon practice at the time. He referred to this new application of his research as "following the string." He emphasized the importance of an open mind, which ultimately led him to ask whether the therapy could be translated from monkeys to stroke patients. Stroke patients mirror deafferented monkeys by presenting similar arm nonuse. Furthermore, these patients had previously been condemned by the clinical world, providing further negative reinforcements. Entering the field of stroke rehabilitation without formal training in the field gave him a fresh perspective, and he challenged the paradigm that stroke patients could not recover limb function more than one year after a stroke.

Dr. Taub's rehabilitation technique is known as Constraint Induced (CI) Therapy. Constraint in this therapy implies a dual meaning physical restraint of the good arm and behavioral constraint of the stroke-affected arm imposed by the training situations used in the therapy. His therapy allows stroke patients to recover significant limb function, regardless of the length of the period of disuse. This therapy centers on one-on-one work with a therapist, during which the patient is encouraged to perform numerous tasks with their affected limb while their unaffected limb is restrained. The program boasts an approximately 95% success rate by "rewiring" certain regions of the brain activating the affected area.

The fundamental basis of this therapy uses positive reinforcement to induce movement. After a patient performs a series of tasks with his affected limb, the therapist records evaluations and shows these to the patient. These help to motivate the patient to continue improving limb function. Dr. Taub described the process as "similar to an arcade game because humans are intensely competitive. They want to improve on their previous best performance, and they also want to reduce the effects of their stroke. They want to get normal." The average treatment lasts two weeks and has three central components: restraint of the unaffected limb, a transfer package (TP), and repetitive practice with minimum rests between exercises. The transfer package includes problem solving, homework, etc. All these exercises promote constant patient immersion in the therapy process for a substantial portion of the day. Some patients call the therapy magical; within two weeks, they regain at least half the former functionality of their limbs after a long period of disuse. Essentially, the therapy represents a behavioral modification to the wrongly learned habit of nonuse. Analysis of CI therapy has shown that the technique can consistently improve a patient's limb functionality; on average, a patient with only 9% functionality in his or her arm can improve to 52% functionality level.

Dr. Taub continues refining and expanding CI therapy. The therapy has been shown to improve patients of all age groups; he and his colleagues have successfully treated an 18-month old child as well as a 92-year old man. Furthermore, Dr. Taub has found that the program could treat any form of learned nonuse, such as patients suffering from traumatic brain injury. Another important application that Dr. Taub found for CI-therapy was to treat Focal Hand Dystonia, where artists suddenly find themselves unable to coordinate hand movements. He has worked with concert artists, such as Leon Fleisher, and they usually achieve almost complete recovery, allowing them to return to their passion. With his therapy, it has been shown that eight out of 10 phantom limb pain patients reduce the painful sensations. At UAB, Dr. Taub worked with speech language pathologists on CIAT II (Constraint Induced Aphasia Therapy). This targets aphasia, impaired language ability, which is usually borne out of stroke. The program was amazingly successful; on average, patients achieved a more than 10 times speech improvement. Through the many modifications of the CI-therapy program, a greater variety of patients have been able to utilize the program in their recovery, including patients with cerebral palsy, traumatic brain injury, and multiple sclerosis.

He emphasized the importance of an open mind, which ultimately led him to ask whether the therapy could be translated from monkeys to stroke patients.

In addition to his works on therapy, Dr. Taub also collaborates with other researchers to explore territorial plasticity and the conversion of different areas of the brain from one use to another; these projects have led to publications in major scientific journals like *Science* and *Nature*. Four of his German clinics use imaging software to further study the relationship between neuroplasticity and CI therapy as does his lab here at UAB. Using EEG and transcranial magnetic stimula-

tion (TMS), it was shown that brain areas representing the arm shrank to 50% of the normal in patients suffering from stroke or traumatic brain injury. After CI therapy, though, this level almost returned back to normal. Just recently in 2008, Dr. Taub and graduate student, Lynne Gauthier, revealed CI therapy can be associated with an increase in the volume of brain matter corresponding to motor areas of the brain and in the hippocampus.

Throughout his illustrious research career, Dr. Taub's centered his central methodology on the concept of Strong Inference. This Baconian form of science explores a phenomenon by asking a question and using a set of possible answers to the question, which are characterized as hypotheses. Scientists try to eliminate all but one answer, which then provisionally stands as the truth. This method harbors no sense of absolute truth, and the last answer only stands until new research disproves it. Within this framework, Dr. Taub pioneered the use of behavioral techniques in the field of stroke rehabilitation, earning him accolades such as the Distinguished Scientific Award for the Applications of Psychology from the American Psychological Association (2004) and the William James Award from the American Psychological Society (1997). The Society for Neuroscience named his CI therapy one of the top ten Translational Neuroscience Accomplishments of the 20th century.

Dr. Taub was keen to offer advice to aspiring researchers. First, he stressed the importance of a good mentor; a researcher's views are strongly influenced by that mentor. He expressed his gratitude to his mentors who now reside as historical figures in psychology: Fred Keller, Joe Brady, and Neal Miller. Dr. Taub hailed Dr. Miller as "the greatest exemplar of strong inference in psychology and neuroscience in the 20th century." Second, he stressed that problem solving is the most important part of science. "That's the fun," mused Dr. Taub. He noted that because it has the same creative impulse as art on the most fundamental level, science has been called the great art form of the 20th century. Dr. Taub described the most important part of creativity in science is the identification and assessment of fruitful phenomenon. He says that once a scientist has learned to solve problems, it becomes easy to find different applications. However, just like art, he emphasized that it is not what one creates, but the process of creation that brings satisfaction. A scientist does not need to be brilliant; he or she just needs work hard and integrate his or her own unique perspective. Dr. Taub added "I work in rehabilitation of humans now, which is just as interesting as deafferentation in monkeys, which is just as interesting as other areas of research I have worked in. They are all interesting – it's what you bring to it in terms of the creative thrust."

After accomplishing such great strides in the field of neuroscience, Dr. Taub joked that he still sings in the shower. "I used to help my wife learn her lines when she sang for the Metropolitan opera. This is true! I used to sing her soprano arias in the shower. One day she came in with tears in her eyes and moved back the shower curtain and said 'Please don't sing. Please, they are all the wrong notes!' I wasn't offended, I knew she was right." Yet, he still continues to help her, just as he has continued his research and his passion for the art of science. He commented that if young scientists work hard, they can make their own luck. He emphasized that sometimes individuals are wrong and sometimes they are right. "[But] don't let being wrong stop you," he insisted, "because when you're right – you'll hit a jackpot."

"I work in rehabilitation of humans now, which is just as interesting as deafferentation in monkeys, which is just as interesting as other areas of research I have worked in. They are all interesting – it's what you bring to it in terms of the creative thrust."

Dr. Taub would like to give special thanks to:
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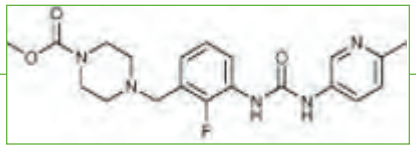
science news

Drug Discovery: A Potential Treatment for Heart Failure Patients

Ashruta Patel

Myosins are important in helping the heart contract through their motor properties. Heart failure results from the proteins being unable to contract. A small molecule, Omecamtiv mecarbil is a direct activator of cardiac myosin and has been considered a potential treatment for heart failure patients. The American Heart Association predicts nearly 300, 000 deaths occur every year in the United States from heart failure. Omecamtiv mecarbil is still being investigated further through clinical trials; however, the possibility of affected patients using it in both intravenous and oral formations can motivate other drug discovery programs.



[Figure 1 – Omecamtiv mecarbil structure]

Researchers at South San Francisco, California-based Cytokinetics have discovered that heart contractions rely on isoforms that have different ATP (adenosine triphosphate) hydrolysis rates. Cardiac myosin hydrolyzes ATP and the energy is used to facilitate large conformational changes in protein necessary for muscles fibers to contract. The fast and slow isoforms assist with contractions in a normally functioning heart. Contractile dysfunction could occur when the amount of these ATP-hydrolyzed myosin isoforms decrease, which eventually leads to heart failure.

This drug helps increase cardiac myosin's rate of ATP hydrolysis which in turn helps elevate cardiac function. Omecamtiv mecarbil binds to an amino acid sequence on the cardiac myosin. This drug is selective for slow skeletal muscle myosin and thus does not affect fine motor movements. Therefore, if Omecamtiv mecarbil were to be chosen as a successful drug for heart failure patients, the high rates of heart disease could be lowered and additional drug discoveries could lead to additional important innovations for various illnesses of concern.

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research narrative

An Evaluation of the SPOONS Program on an Acute Care for Elders (ACE) Unit

Michelle Chang

As I sat in on interdisciplinary rounds at the Acute Care for the Elderly (ACE) unit at the UAB Highlands Hospital, I couldn't help eyeing everything and everyone in the room. Seated at a long table was the team of health professionals who worked on the ACE unit—a physician, a nurse practitioner, a social worker, a pharmacist, a dietitian and a physical therapist. One by one, they discussed the patients currently staying in the ACE unit, including their health status, preferred diet, expected duration on the unit, the extent of the family's involvement in visiting and caring for the patient, and the patient's results for a clock drawing test.

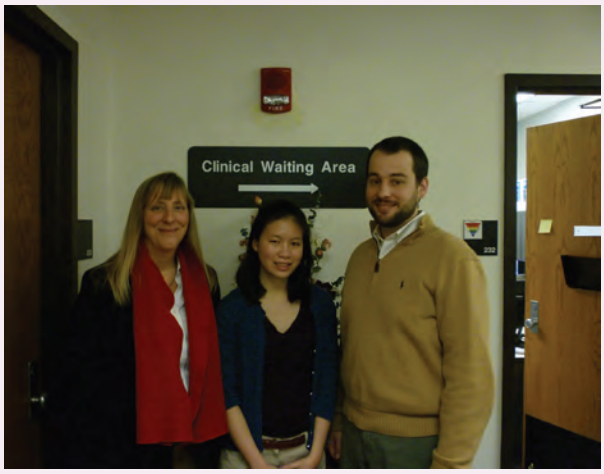
At first, the way that every ACE staff member communicated and coordinated with each other to address the needs of the patients amazed me. Many questions ran through my mind such as, "Why was it important for the social worker to mention that the patient's extended family wasn't visiting her?" And "what was the importance of how a patient drew the hands of a clock?" I later learned that the clock drawing test or a Mini-Cog Assessment is administered as a quick method for determining the patient's cognitive status at that specific point in time. The results may suggest whether a patient has a cognitive disease such as Alzheimer's disease, vascular dementia, and delirium. Overall, I was impressed by the team's discussion. Through collaboration, they were able to provide elderly patients with health care that addresses their biopsychosocial needs.

The SPOONS program is a vital component to the comprehensive care provided at the ACE unit. Unlike the typical hospital volunteer, SPOONS volunteers take a proactive role—providing mealtime assistance and socialization for patients. Upon hearing about SPOONS, I became interested in learning about the effectiveness of individual attention on the care of elderly patients in particular.

After rounds, Professor David Buys, who taught the Introduction to Aging course I had taken that previous spring semester, took me on a quick tour of the unit. Professor Buys, who was working on a nutritional study with Dr. Julie Locher, was at the unit to recruit patients. As he explained to me, recruiting patients for clinical studies, especially randomized controlled trials, can be difficult. Although the research does not put them at any risk or involve significant active effort on their part, finding persons willing to participate is still tough. Moreover, retention of patients who do consent to participate can be a problem as well. From this instance, I gained an understanding of the major challenges that clinical

researchers, in contrast with basic science researchers, encounter; clinical studies cannot just pay for lab rats or chemicals to run experiments on. Clinical studies apply scientific knowledge to observe real people in the medical environment.

After the visit to the ACE unit, I knew that I wanted to pursue a study that examined some aspect of such a special hospital unit. Hence, under the guidance of Dr. Julie Locher, Dr. Kellie Flood, and Dr. Buys, I began a primary literature review of the nutritional decline of older adults and of hospitalized older adults. Undernutrition has been defined in multiple ways: unintentional loss of weight, low anthropometric measurements (i.e. height, weight, body mass index, percentage of body fat), abnormal biochemical markers (i.e. micronutrient status), and poor nutritional intake. Individuals over the age of 65 have an increased risk of undernutrition due to age-related changes and acute or chronic diseases. Studies have even suggested that hospitalization may increase this risk of undernutrition for older adults (Elmståhl et al. 1997; Sullivan et al. 1997; Hall et al. 2000; Schenker 2003). Due to age-related physical or cognitive impairments, patients may not be able to eat meals without support. They may experience difficulty reaching their food or even feeding themselves. As Dr. Flood, medical director of the ACE unit, explained to me, patients afflicted with arthritis, which is inflammation of the joints, require assistance to remove the plastic lids and wrapping covering food. Without any help, the patient has no choice but to let the food sit on the tray, unopened. Moreover, a meal is typically a social activity, and older patients may have a reduced desire to eat their meals if they feel isolated in a hospital setting.



the nursing staff as needing assistance. During that time, a volunteer may assist a patient by setting up the meal tray, feeding the patient with a spoon, and wiping his or her mouths. A volunteer also communicates with the patient during meal assistance—smiling, listening, and speaking clearly to encourage the patient to finish the meal. The service provided by SPOONS volunteers supports the nurses and patient care technicians (PCTs), who may not have one to three hours to spend with each patient for mealtime assistance and socialization.

I currently seek to evaluate the effects of the SPOONS program on the dietary intake of older adults admitted to the ACE Unit. The Minimal Eating Observation Form- Version II (MEOF II) developed by Westergren et al (2009) will be used in this study to observe

Clinical studies cannot just pay for lab rats or chemicals to run experiments on. Clinical studies apply scientific knowledge to observe real people in the medical environment.

Increased risk of undernutrition from hospitalization is a significant concern because undernutrition is associated with poorer health, decreased ability to recover from medical conditions and increased risk of mortality. These associated consequences may lead to prolonged hospitalization or greater risk of re-admission. Therefore, hospitalized older adults should receive some form of nutritional intervention to address their increased risk of undernutrition. In doing so, hospitals can enhance the quality of care they provide in a more efficient manner.

The SPOONS program, for instance, is one intervention that seeks to improve the dietary intake of hospitalized older adults. At the ACE unit, volunteers, who have received specific SPOONS training, typically spend at least one hour with an older adult identified by

patients when assisted by SPOONS volunteers and when no assistance was provided. MEOF II consists of three main dimensions of assessment—"Ingestion," "Deglutition," and "Energy and Appetite." Data collected using MEOF II will allow for statistical comparison of the dietary intake between patients assisted by SPOONS volunteers and those who were not. Through this assessment, I hope to determine how the SPOONS intervention can be improved and modified for use at other hospitals and on other units within the UAB system.

Working on this project has taught me much of the intricacies involved in a clinical study. Before conducting the study, it is necessary to do a thorough review of the current literature, to map out the study's controls and variables, to consider any ethical issues that may

be involved, and to develop a practical method for assessment. Now that I've completed these stages of the project, I look forward to carrying out the project and analyzing results that may help address how hospitals can deal with the costly issue of undernutrition.

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research narrative

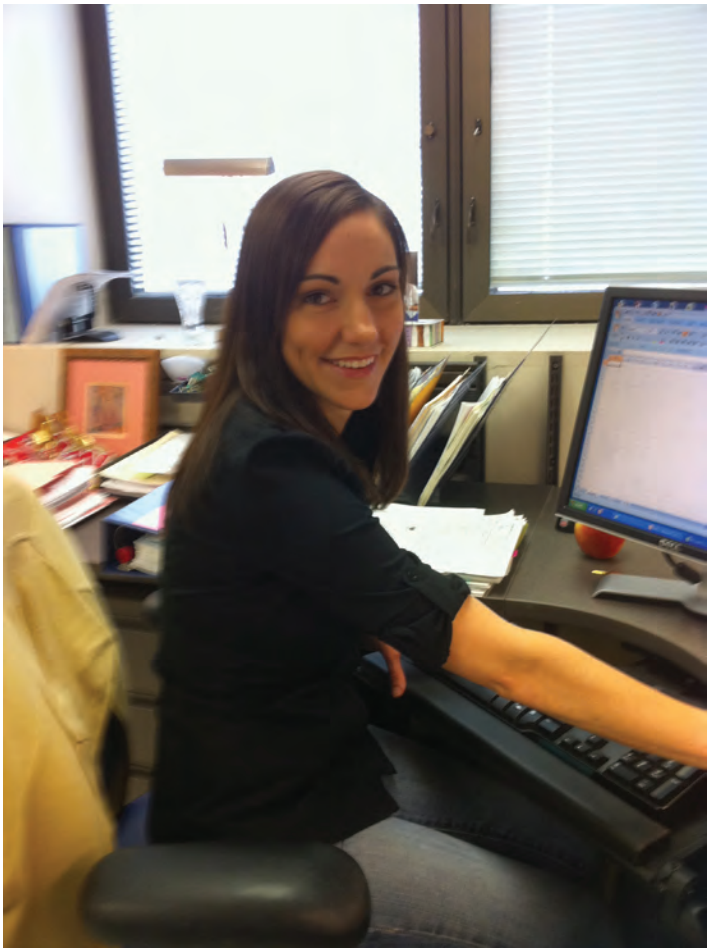
A Fairy Tale

Amanda Melnikoff

For most of us, our image of physicians has been shaped by two primary sources – TV shows and our personal experience as patients. From Dr. Kildare in the 1960s to the current House series, TV serves up endless variations on the theme that doctors rush feverishly through their work days relieving suffering, making extraordinary diagnoses, and solving patients' personal problems with a wisdom that few possess – and doing it all with little more than a stethoscope and a Sherlock Holmes eye for detail. I'm exaggerating, of course, but you know what I mean. TV has a profound influence on our image of physicians.

Then there's our personal experience as patients. We visit the doctor (usually) because something's wrong that the doctor is trained to understand and fix. So we make an appointment and show up and write our name down at the receptionist window. We don't really know why we're required to sit in the lobby so long before our name is finally called, or why the nurse sticks her head out the door and pronounces our names as though it were a question. Melnikoff? Amanda Melnikoff? But at that point we don't care. Why? Because we are hurting and from that moment forward we are hopeful and trusting and eager to find relief from whatever disease we suffer. We bring with us a reverence for that doctor's skill that is otherwise reserved only for ministers and messiahs.

Medical school admissions committees often give weight to an applicant's first-hand knowledge of the medical profession. You've got to be academically accomplished, of course, simply to be considered for admission to medical school, but knowing something about the real duties and experiences of physicians is also a valuable admissions criterion. Why? Because admissions committees understand that TV characters and patient experience alone are poor barometers of the medical profession. Medical school is expensive, and time-consuming. The work is grinding and



the demands are intense. Better to be disabused of fairy-tales on the front end than to be disillusioned and debt-ridden later.

That's why I'm glad for the opportunities I've had to shadow physicians at their offices and work alongside doctors in a clinical drug-study setting. Short of that, I'd be lost in a wonderland of fantasies about what it means to be a physician.

Shadowing helped me understand why I've often had to wait so long in the doctor's lobby before my name was called. I never knew how much a physician might be doing behind that closed

lobby door – reading charts, bouncing down the hall between one patient and another, answering questions, soothing fears, sharing concerns, recording the nature of a patient's situation, managing personnel and office issues as need requires, taking and making phone calls, performing medical procedures with the care that their patients expect, and somehow dealing with those personal issues that interrupt all our workdays at one time or another (arranging transportation for a child's soccer practice, taking a call from the accountant or insurance agent, meeting with the computer software guy or the drug rep, taking a five minute coffee break after four or five unrelenting hours, just to name a few). Knowing what goes on behind that lobby door enables me better to understand why it sometimes takes so long to hear those magic words: Melnikoff? Amanda Melnikoff?

Knowing what goes on behind that lobby door enables me better to understand why it sometimes takes so long to hear those magic words…

Working alongside doctors in a clinical drug-study taught me even more. I learned, for instance, that doctors and patients are dependent on one another in ways I'd never stopped to consider. In a doctor/patient relationship, we typically think that only the doctor has an obligation, namely, do the best you can to help the patient. But what about the patient's obligation?

The medical researchers I worked for were studying Chronic Obstructive Pulmonary Disease. More specifically, they wanted to know if the lungs of patients suffering from COPD might respond to a drug indicated for cardiovascular disease (particularly, heart attack). If their intuitions were correct, the drug might lead to a dramatic reduction in COPD exacerbations, hospitalizations, and mortalities.

Only problem is, if the study is to be instructive, the patients must follow the protocol established in the study. If the study requires that patients take the drug each morning and night, but the patient periodically neglects to do so, or if the patient takes the dosage as prescribed, but waits three weeks after the bottle is empty before returning for follow-up tests, that patient's test results may prove worthless to the researcher. And did I mention the paperwork that both parties must fill out – not once but regularly and in detail – if the results are to be certified? Sometimes patients forget details of their medical history that the researcher needed to know at the beginning, details that the patient may later reveal when filling out paperwork and that may have disqualified the patient from participating in the study had the information been revealed earlier. I didn't realize just how much paperwork is involved in medical research.

Long story short, I've learned a lot from shadowing physicians and working with clinical researchers. My advice to anyone considering applying to medical school is this – if at all possible, get some experience in advance. Be proactive. Call people you know. Let them know your ambitions and ask for their help. You'll be surprised at what might happen. Even more, you'll be surprised at what you learn.

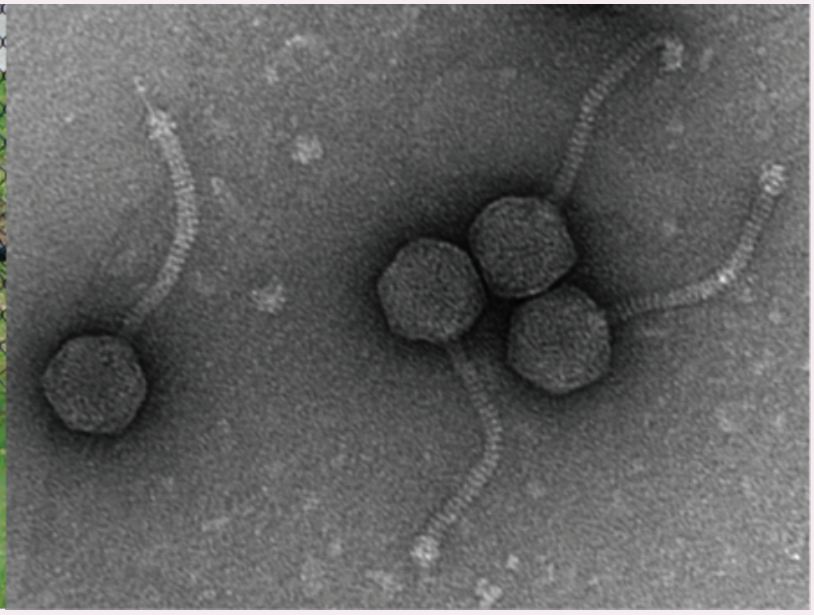
You have nothing to risk but fairy tales.

"Kí lō fé ṣē tōbá dàgbà"

Sope Oguntuyo

From my childhood in Nigeria, to now, adults have asked me, "Kí l fé tbá dàgbà" (translated from Yorùbá: "What do you want to be when you grow up?"). This age old question has been floating around the frontal lobe of my brain and the mind of countless other individuals faced with the same introspective question. Perhaps, others already have or soon will receive the question in another language as well. In my youth, I thought of the possibilities: law, medicine, science, or even engineering; from the sciences, biology and astronomy captured my attention. Ironically enough, it was not until my exposure to the microscope that my view of biology was broadened.

When I was younger, I was able to peek through the eyepiece of a flimsy microscope to observe stationary cells. Although these observations revealed a static image, it set off a dynamic pathway as I quickly absorbed information about the phospholipid bilayer, the nucleolus, and numerous other



Digging for soil samples to isolate a phage

cellular organelles. However, I was only able to retain the names of those cell parts, and not the role they played within the cell. In my earlier days, the microscope was an object of amusement and enjoyment for me; but, in high school, my perspective of this apparatus was put into focus, and I began to develop an appreciation for the valuable instrument. In AP biology, the class was given the opportunity to view live paramecium and algae. Eagerly, I began the quest to find the paramecium, but I became disappointed as the simple organism eluded the microscope's focal point. After instructions from the teacher to adjust the position of the slide, I was able to swiftly find the hairy one-celled organism. This invigorating experience and my overall experience with prior biology courses increased my desire to pursue biology and search for knowledge in this vast and mysterious field. The microscope not only exposed the haven of these diminutive creatures to me, but it also displayed the intriguing world of the scientific field to my eyes, which held a potential career field.

With these positive encounters, I confidently came to UAB as a biology major looking to expand my field of vision in the sciences. With the aide of the Science and Technology Honors Program's Quick Connections, I was able to get in touch with Dr. Sunnie Thompson –the Principle Investigator (PI) of the Microbiology laboratory– through Dr. Marla Hertz. This meeting culminated in getting me involved in a research lab as a freshman on December 1, 2010. At Dr. Thompson's laboratory, I was given the privilege of working on a project regarding the effect of cellular stresses on Internal Ribosome Entry Site (IRES) mediated translational activity, which is another means for translation to occur within an eukaryotic cell. Some cellular mRNAs utilize the mechanism of IRES dependent translation to make some cellular proteins, and several viruses are known to use IRES meditated translation to make viral proteins. Before the conclusion of the spring semester, I was able to present my findings thus far at two poster presentations. Currently, I am performing research regarding Ribosomal Protein S25 (RPS25). RPS25 has been found to be essential for IRES-mediated translational activity. So far, I have been exposed to a plethora of new scientific instruments and techniques, yet there is still a connection that I will always have to the microscope.

This semester, I felt nostalgic using a microscope. More specifically, I used an electron microscope – not a flimsy light microscope – for an experiment in the Phage Genomics course (BY 213). The microscope allowed each member of the class to visualize his/her novel phage that was isolated from a soil sample and worked with throughout the course of the class; moreover, one lucky phage from the class will be fully sequenced and will be analyzed in the spring semester. Regardless, thus far in college, I have drifted from my naïve view that equated biological research with microscopes to one that appreciates other aspects of biology with my research experience in Dr. Sunnie Thompson's research lab and the Phage Genomics course.

However, the microscope will always be a scientific tool that I will always value dearly in regards to the memories held as I peered through the eyepiece and gained knowledge from those observations. My gratitude belongs to Zacharias Jansenn and Anton Van Leeuwenhoek for their individual contributions to the invention of the light microscope. With an early exposure to this instrument and numerous others due to my research experience, I can confidently state that I would like to pursue biomedical research.

Fishing for Success

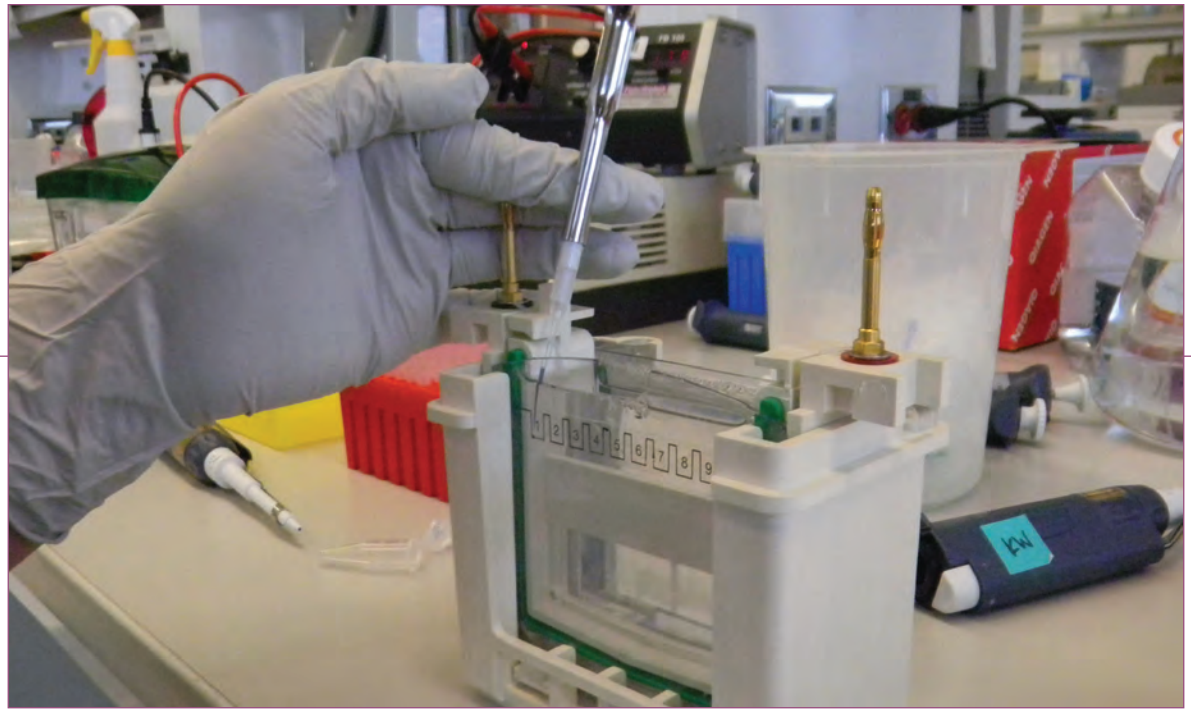
Katherine Beaufait

When I applied for the BioTrain program, I really did not think I stood a chance. Here I was, 19 years old with no research experience or even a college biology class under my belt, applying to a world class research institute. A friend claimed I had a better chance of being struck by lightning than getting the internship. And so, when I eventually did receive a letter stating that the program had an opening for me, I could not contain my excitement. To top it off, the spot was in Dr. Myers' lab - I would be working with the president of the institute himself.

The first week of the internship was titled Biotech Boootcamp, where each intern learns the basic skills needed to thrive in a research lab: pipetting, serial dilutions, plating bacteria, and electrophoresis just to name a few. However, as soon as I met with Kelly Williams, PhD, of Dr. Myers' lab, I began to learn more complex techniques. For example, I was taken down to the fish room where I learned to feed the fish and to change the system's water to ensure they were kept in optimal conditions. Very quickly, I was pulling out tanks and moving fish around to set up crosses, which is the term for breeding. Initially, I have to admit, it was quite frightening as a fish accidentally fell out of the net, and in shock, all I did at first was stare at it flopping on the table. However, it did not take long to overcome the fear and feel confident to move the fish around like the other two technicians.

Meanwhile, up in the lab, I continued to acquire new techniques and procedures, like the BCA and acetylcholinesterase assays I would be using as a major data source for my poster presentation later that summer. My favorite procedure was definitely the Western blotting technique, even when we could not match a primary anti-body that worked well with our samples. It seemed strange at first to put up with a two-day process only to find out at the end that it did not work as intended, but I soon realized these struggles are just part of the whole research experience. As with any kind of scientific discovery, to make progress, you must fail many times first.

Upon returning to UAB, I was so grateful for the internship opportunity. Not only was I put into a renowned research facility, but I was also put in to a lab that cared about my learning and wanted me to grow as a scientist. As I continue my research on campus, I often think about the people I got to spend my entire summer with. Every one of them treated me as an equal, despite my lesser knowledge on the research matter. They also helped me understand how and why I was performing the experiments. I still talk to most of the lab technicians and post-doctoral fellows I worked with during the summer of 2011, and I plan on going back to visit once or twice this year. I am so proud to call the Myers lab my first research lab experience and if possible wish to return in the coming summer.



Undergraduates and Research: From the Side of the PI, Dr. Sadanandan Velu

Miranda Collier

Dr. Sadanandan Velu has served as a mentor to no fewer than 20 undergraduate students since he joined the UAB Department of Chemistry as a faculty member in 2004. His students have gone on to present their work at regional conferences, publish in various journals, win scholarships and awards, and pursue research-oriented careers. Inquiro set out to discover what Dr. Velu seeks in undergraduate research students, how they contribute to his lab, and how involvement in research at an early stage prepares them for the future.

Dr. Velu received his doctorate in synthetic organic chemistry from the University of Madras in India. He was not involved in research until his graduate studies. "In India, we never had opportunities as undergraduates to do research," he explained. "I tell my students at UAB they are fortunate to have these opportunities." He came to UAB in 1997 to conduct research in the Medicinal Chemistry division of the Center for Biophysical Sciences and Engineering. Since 2004, he has been in the Department of Chemistry conducting research in the area of drug discovery.

"There are different approaches to this type of research," he explained. One method used in his lab is to identify molecules with medicinal properties from natural products, specifically from chemicals existing in marine invertebrate animals, such as mollusks and sponges. As a marine natural product chemist, his goal is to synthesize these compounds and their analogs and identify new lead compounds for drug discovery. Why marine animals? "These compounds are very bioactive," he explained. "Often they have anti-cancer properties. These organisms live in a unique environment under the ocean. Their bodies make adaptations for survival. They use these chemicals – unique compounds that are not found in terrestrial organisms."

Once useful compounds are identified, he tries to develop synthetic methods of making them in the lab. "We can't rely on marine resources to get it. You get very tiny amounts, and it can be ecologically unfavorable if you try to harvest large amounts of marine organisms from the ocean. The practical solution is to make these compounds in the lab." In addition to environmental issues, there are other advantages to synthesizing compounds as well. "You can modify the chemicals to optimize their favorable properties and diminish any unfavorable properties," said Dr. Velu. Currently, his lab is using these methods in anti-cancer, anti-bacterial, and anti-parasitic drug discovery projects, among others. "Design of these molecules, optimization, structural activity relationship studies…the main part of all this is synthetic organic chemistry, and in my opinion, students are very comfortable working in the kind of research I do," said Dr. Velu regarding the aptitude of undergraduates for his lab's work. Students who have completed organic chemistry courses are able to join his lab and apply what they have learned to practical situations. "They are very excited about having a hands-on experience of conducting organic chemistry reactions in the lab. This area is definitely suitable for undergraduate organic research."

For Dr. Velu, the most important quality in a student researcher is enthusiasm. "They should not take a research course just because they want to fill something in their resume. There should be passion and interest for it," he said. He appreciates the energy and the inquisitive attitude undergraduates bring to his lab and to their weekly lab meetings. In addition to enthusiasm, discipline is another important trait that he looks for in interested students. "Often, students are used to taking classes and making 'A' grades. Research is not like that," he asserted. "There is uncertainty involved. You could very well be spending a lot of time working toward something and have unfavorable results. You should be prepared to handle that kind of disappointment and overcome it."

While he expects decent grades, especially in Organic Chemistry I and II, they are secondary to the personal attributes necessary in a competent researcher. In his experience, Dr. Velu knows that grades, passion, diligence and research affinity are not necessarily

always connected. "I have seen a lot of students with lower grades doing very well in research, and also students with very high grades not doing as well," he cautioned, evidencing that each student's aptitude for research is unique.

According to Dr. Velu, the primary benefit to being involved in research as an undergraduate is gaining experience and becoming familiar with the diligence and commitment associated with the field. "The success of the research is secondary," he said. "I want them to learn. It is good to get a feel for this area early on before they start graduate school. If it leads to publications and presentations, all of that will help them further." He believes it is important that students work in different labs to find a type of research that interests them. "They may not be able to solve one of the world's big problems. This time is too short," he explained. "The ultimate goal is to get the experience. Then they can make a decision: is research the best option for me?"

When undergraduates participate in research, it not only benefits them, but their mentors, departments and schools as well. "It helps me that they are flexible," said Dr. Velu. "With post-docs and graduate students, it is often difficult to explore a new idea. But with undergraduates, I really like being able to try out the feasibility of novel projects." He highlighted the positive impact of accomplished undergraduates on the school's reputation. "When our undergraduate students are winning awards, publishing papers, making presentations…we are all benefited. The department and UAB as a whole."

He believes that both student and mentor have responsibilities to uphold. "I ask that students be prepared. Research is not like taking a course. It's a little more involved," he explained. "There has to be an adjustment to be more disciplined, more committed." As a mentor, he takes care to set reasonable goals and expectations for undergraduates. "I want them to be successful, but I must remember that they are young. They are trying to see if research is a career option."

When asked how interested students should begin the process of becoming involved in research, Dr. Velu offered specific advice. "The first thing to do is check out the websites of research-active faculty. See their publication profiles," he said. "Then make a short list of people who do research in the areas you are interested in." The next step is to send out e-mails. "Ask if they are available for a short chat. They can clarify if they want undergraduates in the lab." If a student follows these steps, Dr. Velu believes they will be able to find possible mentors in areas of research they want to pursue. To students who may not be interested in any specific area, he advised, "Go read! You may find something on a faculty member's website that stands out to you. That grabs your interest and gives you some direction."

Overall, Dr. Velu regards undergraduate research students and their work very highly and encourages everyone to become involved. "I would not rate the efforts of undergraduates any less than anybody. They can make significant contributions," he said. "They can switch labs and explore. Do I like biochemistry? Drug discovery? Or something else?" He believes that undergraduates should attempt to discover which path is right for them, and each student who follows his or her passions to the lab is one step closer to finding an answer.

"It opens up all sorts of opportunities you would never have had otherwise. People doing high profile research have contacts all over the world…"

Further benefit to undergraduates comes from the relationship between a student and his or her mentor. "In research, you get to know the student very well, in a one-on-one basis," said Dr. Velu. "We can write very strong letters of recommendation for students, about how disciplined the student is in the lab, how methodical he or she is in carrying out the research, how they interact with others. Those are all important aspects people look for in applications." In addition to letters of recommendation, research has extensive networking potential. "It opens up all sorts of opportunities you would never have had otherwise. People doing high profile research have contacts all over the world," he pointed out.

A Mechanistic look at the Regulatory Functions of Peroxisome Proliferator-Activated Receptor γ (PPARγ) Coactivator 1α (PGC-1α) and Estrogen Related Receptor (ERRα)

Ashruta Patel

Introduction

Proliferator-activated receptor γ coactivator 1α (PGC-1α) is a transcriptional co-activator involved in the regulation of energy production and utilization in metabolic tissues. Target genes are induced through both PGC-1α co-activators and their interactions with nuclear receptors and additional transcription factors at specific regulatory sites. Estrogen related receptor (ERRα) is an orphan nuclear receptor that controls metabolism. Studies have established that the activation of ERRα by PGC-1α induces genes with roles in lipid transport, fatty acid oxidation, TCA cycle, oxidative phosphorylation, mitochondrial biogenesis, mitochondrial dynamics, and oxidative stress defense. ERRα is a potential intermediate in PGC-1α action. PGC-1α co-activator properties allow it to control gene expression through specific DNA binding transcription factors at the promoters of target genes.

PGC-1α is a potent co-activator of ERRα, and it is likely that ERRα is an important transcriptional regulator of PGC-1α induced genes. Lower basal levels of PGC-1α expression are detected in the brain, suggesting a low preference for fatty oxidation under normal conditions. The brain has efficient OXPHOS machinery to support impulse transmission and expresses a significant level of PGC-1α.

Potential Therapeutics for Drug Discoveries

Kaempferol ($C_{15}H_{10}O_6$), a polyphenolic non-steroidal plant compound commonly found in dietary flavonoids, has been isolated in many plant sources including, tea, broccoli, and grapefruit. Studies have been able to determine that Kaempferol functions as an inverse agonist for estrogen related receptors alpha and gamma (ERRα and ERRγ). Kaempferol binds to ERRα and ERRγ and blocks their interaction with co-activator peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α).

Inhibition of ERRα activity by its inverse agonist impairs the ability of PGC-1α to induce expression of energy metabolism genes and enhances mitochondrial biogenesis and oxidative capacity. XCT 790 is one inverse agonist for ERRα that disrupts the interaction between ERRα and PGC-1α. Bioinformatics analysis confirmed that the TCAAGGTCA motif serves as the main ERR binding site (in vivo). XCT 790($C_{23}H_{13}F_9N_4O_3S$) acts as a selective inverse agonist of ERRα that demonstrates ≈90-100% inhibition of ERRα constitutive activity in previous studies. Past experiments indicate XCT790 most likely binds in the ligand-binding pocket of ERRα.

There have been many proposed mechanisms that explain what possible effects of the interactions of PGC-1α and ERRα in different environments. One scenario could be the interaction of PGC-1α and ERRα expressing their target genes. These target genes could be hindered from carrying out a specific function in the presence of an inverse agonist, such as XCT 790. ERRα could be activated through other coactivators, which could express both PGC-1α and ERRα target genes. Further investigation of the transcriptional pathway could help determine what types of drugs could assist with neurological diseases.

Medical Significance in Neurological Diseases

ERRα and PGC-1α can regulate the target gene expression cooperatively. PGC-1α also plays a crucial role in metabolic regulation and reduced amounts have been hypothesized to cause neuronal vulnerability and mitochondrial defects in Huntington's Disease (HD). The abundant evidence of disruptions in neuronal metabolism and mitochondrial respiration in HD reinforces this hypothesis. Consequently, PGC-1α has been suggested as a potential therapeutic target in HD. PGC-1α also plays a crucial role in metabolic regulation, and reduced amounts may cause neuronal vulnerability and mitochondrial defects in HD.

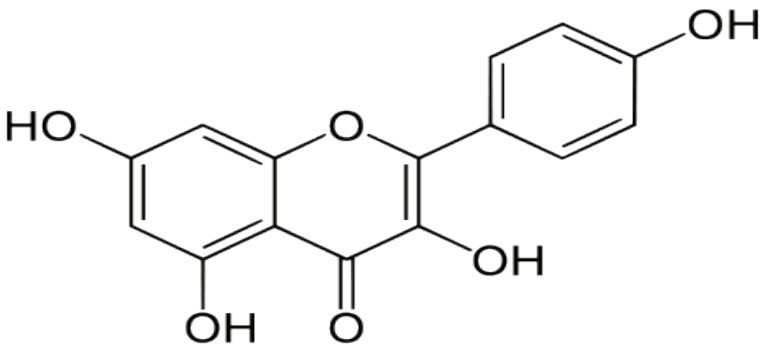


Figure 1: Kaempferol ($C_{15}H_{10}O_6$) structure

Various metabolic diseases might be treated with ERRα ligands. For example, an ERRα agonist might be able to prevent the loss of PGC-1α expression by increasing ERRα activity and other target genes, which can potentially lead to an increase in energy production and insulin sensitivity. Thus, targeting PGC-1α and ERRα in HD could increase metabolism and improve mitochondrial function.

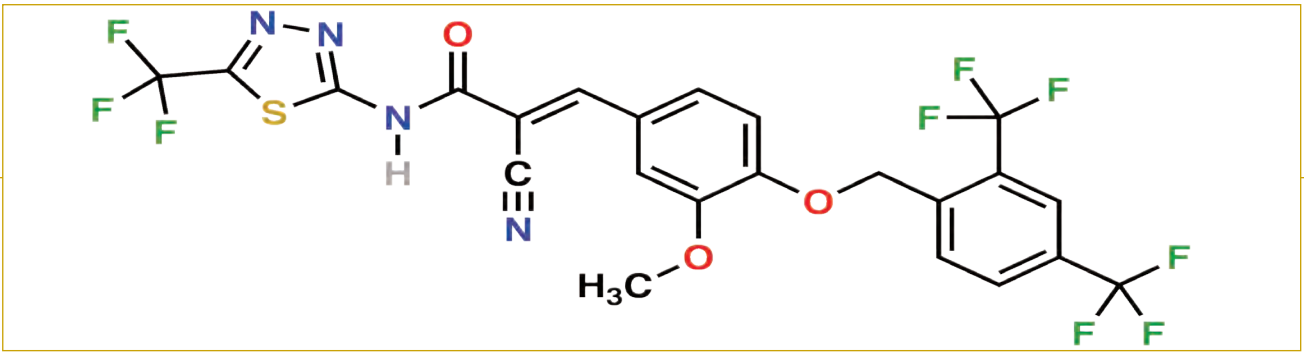


Figure 2 - XCT 790($C_{23}H_{13}F_9N_4O_3S$) structure

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faculty interview: pharmacology and toxicology

Interview with Stephen Barnes, Ph.D.

Miranda Collier

Dr. Barnes is a Professor in the Department of Pharmacology and Toxicology with secondary appointments in the Departments of Biochemistry and Molecular Genetics, Environmental Health Sciences, and Vision Sciences. He is the Director of the Targeted Metabolomics and Proteomics Laboratory. Inquiro sat down with Dr. Barnes to talk about his experiences, his research and his advice to students who are interested in research.

Where did you attain your undergraduate degree and what subject did you study?

The University of Surrey in England – Applied Chemistry.

Where did you attend graduate school? What did you study to attain your PhD?

Imperial College of Science and Technology, University of London – Biochemistry – the application of radio gas chromatography to the study of carbohydrate metabolism.

Who is the most interesting person you have worked with, and what area of study did it involve?

Dr. Alan Hofmann – I began working with him in 1974 on bile acid metabolism and we are still talking about it today. When I meet with him, he has so many ideas. I'm exhausted at the end of it. I worked with him when I first came to America at the Mayo Clinic. He is somebody whom you can talk to for hours and hours. He and I have had a 36-year conversation about the evolution of the chemistry of the bile acids – how they're formed, all the forms you see amongst the species, and most particularly, how they conjugate with glycine and taurine. Issues such as, do elephants make bile acids? The grand questions of life.

When and why did you decide to come to UAB?

1977. It followed my visit to the Mayo Clinic with Dr. Hofmann. It was a difficult time in England and I decided that there were better opportunities for development in the USA. I had an offer on the table from Dr. Basil Hirschowitz to join his group.

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What would you say about the atmosphere of UAB's scientific community?

When I first arrived, there was a lot of opportunity to do cross-disciplinary research and few barriers because of the "young" status of UAB. It was a creative period. The atmosphere has changed because it's bigger. Bigger organizations get less personal, it's inevitable. Before, UAB was somewhat compressed. You would meet people much more easily. Now the senior people are more likely to meet outside UAB at an airport than inside UAB, where we are usually so busy. It's amazing how many people assemble at the Delta Airlines counter.

What research is your lab currently focused on?

We're interested in proteins, how they function and defining them using mass spectrometry. These issues have found application in breast cancer prevention, lens cataract disease and liver function.

How do you connect the dots from the biological observation to a discrete chemical explanation?

In the case of breast cancer, we've been trying to understand what the rationale is for the way early exposure to certain dietary compounds can prevent adult breast cancer. It seems that an exposure may occur 30 to 40 years before the first signs of cancer that regulates the extent of cancer risk. We want to know what is doing this – what proteins function as enzymes or signaling molecules?

Our lens research focuses on the biochemistry behind the formation of cataracts. Some proteins in the lens function as chaperones to keep other proteins from precipitating, and when these chaperones fail or are truncated by proteases, proteins precipitate and cataracts seem to be associated with this. What specific proteins lead to this result? Can it be prevented? We've found that external factors such as UV light exposure and diet can affect lens protein composition as well.

The liver function that we study is the conjugation of bile acids. This is important for everybody because these bile acids are the natural detergents in the body. If you can't make enough bile acids or do not conjugate them correctly, you end up with a deficiency of vitamin D, which can lead to rickets, or a deficiency of vitamin K, which will lead to inappropriate blood clotting. So sometimes kids with cystic fibrosis, for instance, who can't secrete enough bile acids, get rickets. It's curable – you give them conjugated bile acids. If you can supply them, the problem goes away. With genome sequencing, we can see where the enzymes evolved from.

What are the benefits to you of having undergraduates in the lab, and what are the benefits to those students from working in a high-activity research lab such as yours?

It's rewarding to be able to identify students with a strong interest in research. I was one myself! I like being part of their development. The right student can have the opportunity to use state-of-the-art equipment and discover if doing research is what makes them tick.

Describe how you feel about the relationship between undergraduate research students and their mentors.

Undergrads are usually good listeners; therefore, it is often an effective teaching experience for both parties. From the mentor's point of view, the undergraduate is usually technically very competent without being experienced. As a mentor you get a lot out of it, because in a short period of time, you can take somebody from a situation where they don't know how to do a thing to becoming quite expert. I know they enjoy the experience, because I enjoy the experience.

What should students consider during the search for a research mentor?

Choosing somebody who is going to teach you both technically and philosophically is very important. You want somebody who is technically able to take you down a research path and work with you to get you over different humps. But is a mentor there to strictly determine what you do? Or is your best course looking for somebody who gives you a bit of space and encourages you to think out of the box? To stop and think about a problem from a very different, elevated point of view. Most of the time, you look at the problem from up close. But can you get the bigger picture? Is the mentor clearly giving you the tools you need when you finish that period of your research training, such that you can effectively go to the next one?

Mentors can encourage thinking a little bit differently. They often won't correct you every time you make a mistake. Part of the learning is to recognize your mistakes and not be afraid of error. Error happens. If you focus only on being perfect, you end up not doing the experiment that your instinct wants to do.

What advice would you give to aspiring research students, or to students who are considering a career in research?

Research is very exciting and there are several ways to make a career in research. Becoming an independent investigator takes a lot of hard work and dedication, not just being bright. Successful investigators combine risk-taking along with systematic investigation. As much as research is needed, it should also be realized that it is a privilege to be allowed to work on your own ideas. Society is letting you do what it won't let other people do. Society is willing to pay for you to try. You are carrying out experiments that perhaps will have a reward for people in general. But maybe not. This was impressed on me, and I use it time and time again with students. When you have to work really hard to get something done, and it's difficult, just remember society is giving you a privilege.

short report

Effects of Sulforaphane and Cisplatin Treatments on A2780-CP20 Ovarian Cancer Cell Line

Sadhvi Batra • Shweta Patel • Syed Meeran, Ph.D. • Trygve O. Tollesfbol, D.O./Ph.D
Department of Biology • University of Alabama at Birmingham

Abstract

Sulforaphane is an active component found in cruciferous vegetables, such as broccoli, that has been shown to exhibit anticancer properties. For example, in a breast cancer model, sulforaphane reduced the viability of breast cancer cells. Moreover, cisplatin is an anticancer drug used to treat ovarian cancer. However, the numerous side effects of cisplatin can be unbearable and some ovarian cancers are resistant to cisplatin treatment. Therefore, identifying the effects of sulforaphane on ovarian cancer cells will potentially lead to a better strategy. In this study, we determine the effects of sulforaphane, cisplatin and a combination of sulforaphane and cisplatin treatment on the cell proliferation and morphology of A2780-CP20 cancer cells that are cisplatin resistant. Results of this study show that sulforaphane is a good component that also targets growth of ovarian cancer cells. The overall trend from the data gathered from cell viability assay, cell morphology analysis, and colony forming assay showed A2780-CP20 cells are dose-dependent with respect to viability and morphology. Further experiments can now be performed to determine the effects of these compounds on ovarian cancer cell proliferation and to understand the mechanisms involved.

Introduction

Ovarian cancer is the uncontrollable growth of cells near the ovaries—as there have been indications of possible extra ovarian sites in the fallopian tubes—that causes older women to experience pain in the lower abdomen[3]. Lack of treatment or early diagnosis of ovarian cancer can result in a premature death. According to Centers for Disease Control and Prevention, in 2007, approximately 20,749 women in the United States were diagnosed with ovarian cancer of which 14, 621 died[1]. (It is to be noted that the diagnosed women are not the same who died in 2007). Current primary treatment regimes second to surgery for ovarian cancer include chemotherapy with cisplatin.

Cisplatin is an anti-cancer drug that binds to the DNA of cancerous cells and inhibits the cell cycle, causing reduced growth and apoptosis (Figure 1)[3]. Although cisplatin provides a treatment mechanism for ovarian cancer, the side effects of the drug are a major problem for a subset of patients because the drug does not differentiate targeting between normal and cancerous cells. Chemotherapy with cisplatin includes, but is not limited to the following symptoms: hair loss, suppression of the immune system, and nausea[2]. As such, it can be concluded for a subset of patients with significant side effects, cisplatin must be prematurely terminated making it unsatisfactory as a mono-therapy[3].

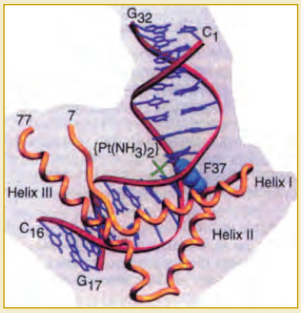

*Figure 1: Cisplatin binding of the DNA of cancerous cells is depicted. Cisplatin molecules are the small blue stick models bound to the DNA double helix.**

Research findings have indicated that sulforaphane (SFN), a dietary compound found in cruciferous vegetables and a potential chemotherapy drug, can be used to treat and prevent breast cancer[6]. For example, as seen in Figure 2, SFN has been shown to regulate histone-modifying enzymes, resulting in a relaxed chromatin structure that allows for a more direct access to DNA[5]. Also, in a previous breast cancer model, SFN decreased the viability of cells[5].

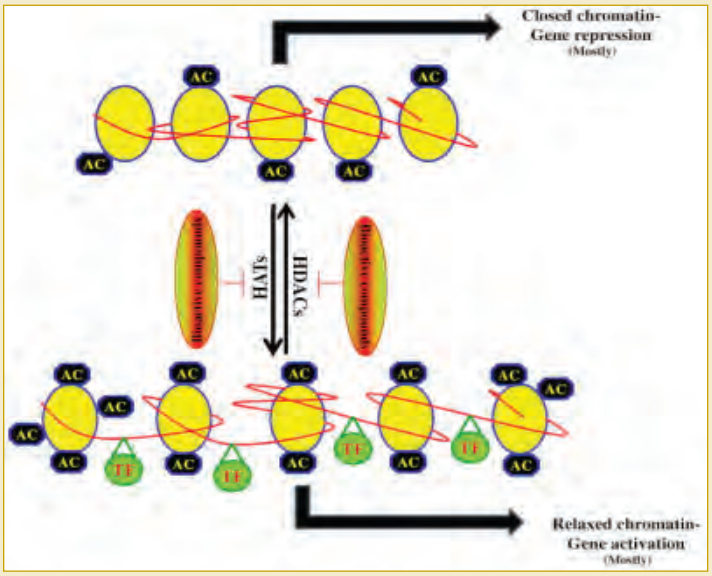

*Figure 2: Histone modifications caused by SFN as found in preliminary data. Figure has been linked to show SFN's anti-cancer properties.***

The purpose of this research is to determine the effects of sulforaphane on the A2780-CP20 ovarian cancer cell line. The A2780-CP20 cell line is estrogen receptor-negative, a subtype of cancerous cells. An estrogen receptor-negative cell line lacks the expression of estrogen receptors on the cells[9]. As such, estrogen receptor-negative cancer cells do not respond to hormonal treatments that are receptor-targeted, making the cancer more difficult to treat[9]. Nonetheless, some ovarian can

cer cells have been shown to respond to chemotherapy drugs such as cisplatin that do not target hormonal receptors as another mode of treatment[9]. Unfortunately, clinically treatment has shown A2780-CP20 to be resistant to cisplatin treatment. However, it is to be noted that the cisplatin-resistant nature of the cell line is not an all-or-none phenomena. Cisplatin resistance is based on many factors, including "cisplatin uptake and efflux, inactivation by thiol-containing molecules, or could be caused by the sensitivity of the target cell to DNA damage caused by cisplatin"[3]. If the effects of sulforaphane can be thoroughly understood with a combination treatment with cisplatin, a more natural treatment method can be used to treat cisplatin-resistant and estrogen receptor-negative ovarian cancer.

Methods and Materials

Cell Culture

A2780-CP20 estrogen receptor-negative, cisplatin resistant human ovarian cancer cell line was cultured in 37°C and 5% CO_2 with RPMI medium supplemented with 10% Fetal Bovine Serum and 1% penicillin/streptomycin[8].

MTT Cell Viability Assay

A MTT cell viability assay was performed to determine the effects of sulforaphane, cisplatin, and a combination treatment of the two drugs on the cell viability of the ovarian cells. Initially, a cell count was tabulated using a hemacytometer (ratio of cell to trypan blue stain 1:1). A total of 10,000 cells per well were plated for each dose. The cells were allowed to adhere onto the wells of microtiter plate for 24 hours and then were treated with increasing concentrations of sulforaphane (0, 1.0, 2.5, 5.0, 10.0, and 20.0 μM) and cisplatin (0, 1.0, 1.5, 3.0, and 10.0 μM). The combination treatment included the following concentrations of sulforaphane and cisplatin, respectively: 1.0 + 1.5, 2.5 + 1.5, 5.0 + 1.5, 1.0 + 3.0, 2.5 + 3.0, and 5.0 + 3.0 μM. Cell viability was measured after 72 hours of treatment using a microplate reader to detect MTT absorbance of the living cells.

A 50 μL working stock MTT (50 μg/mL) was added to each well and incubated for approximately 2 hours. Then 150 μL dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan precipitate formed by the MTT. The microtiter plate was shaken for 10 minutes and the absorbance of MTT due to the purple color change of the cells was read using the microplate reader in a stepwise function at 540 nm wavelength with a reference wavelength of 650 nm.

Colony Forming Potential Assay

A colony assay was utilized to view the effects of sulforaphane, cisplatin, and a combination treatment on the potential for these cells to form colonies and confirm results gathered from MTT assay[7]. As cells tend to grow in colonies, the ability to form a colony suggests the way the cells grow was not affected by treatment. This assay was different from the cell viability assay as it was used to more readily see the cell growth, not quantitatively like in the case of the cell viability assay, but qualitatively. The colony forming assay's main purpose is to view changes in cell proliferation.

The most ideal and effective doses shown by MTT data were used in the colony formation assay. A cell count was determined using aforementioned method. Using a 6-well cell culture plate, 500 cells were adhered onto each well for 15 days after treatment with sulforaphane, cisplatin, and a combination of the two substances. The sulforaphane concentrations used to treat the cells were 0, 1.0, 2.5, 5.0, 10.0, and 20.0 μM. The cisplatin concentrations were 0, 1.0, 1.5, and 3.0 μM. The combination treatment included the following concentrations of sulforaphane and cisplatin, respectively: 0, 1.0 + 1.5, 2.5 + 1.5, 5.0 + 1.5, 1.0 + 3.0, 2.5 + 3.0, and 5.0 + 3.0 μM. Cell colony formation was measured after the 15 day treatment period by fixing the cells with 70% ethanol and staining the colonies formed with a trypan blue solution. The number of cell colonies (>50 cells/colony) that remained viable were counted.

Cell Morphology Analysis

Cell morphology analysis was performed to view the effects of drug treatment on differences in cell shape. A cell count with a hemocytometer was performed to determine the exact number of cells to pipette into a plate. Equal number of cells $1X10^5$ cells per plate were added to 12 separate culture dishes in order to view the effects of 12 different drug treatments with sulforaphane, cisplatin, and combination treatment. The doses used were obtained from MTT data and were as follows: sulforaphane (0, 2.5, 5.0, 10.0 μM), cisplatin (0, 1.5, 3.0, 10.0 μM), and combination of sulforaphane and cisplatin, respectively (0, 2.5 + 1.5, 5.0 + 1.5, and 2.5 + 3.0 μM). After a 72 hours treatment period, pictures of cell shape were taken at 100X magnification using a camera. The pictures that were selected best represented the overall image of the cells with the most clarity.

Results

In order to characterize the effects of sulforaphane and cisplatin treatment, a 72 h MTT assay was performed and all drug treatments were compared to the untreated control (Figure 3). Treatment with sulforaphane and cisplatin showed a dose-dependent trend in cell viability: as the drug concentration increased, the drug toxicity to the cells also increased. For the combination treatment, however, a doses-dependent response was not observed. Individual sulforaphane and cisplatin optimum doses, respectively, are 20 and 10 μM. The optimal combination dose was 5.0 + 1.5 μM.

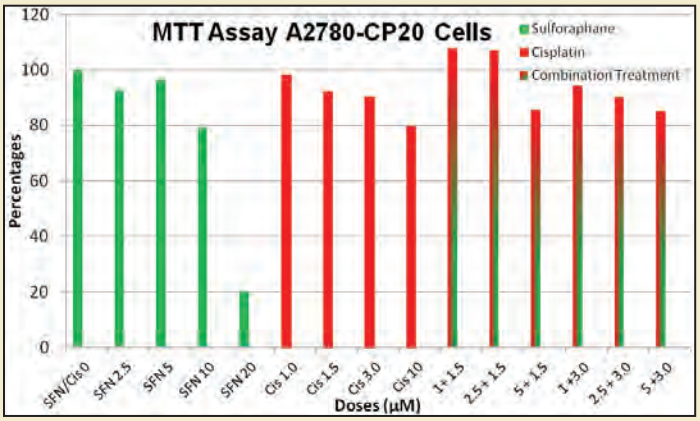

Figure 3: MTT cell viability assay (72 h) showed that sulforaphane and cisplatin have a dose-dependent trend in cell viability; combination treatment of the drugs showed that the optimum doses of sulforaphane and cisplatin were 5.0 and 1.5 μM concentrations.

The results of the MTT analysis were confirmed with a colony forming assay (see Figure 4). The colony assay did not show as gradual of a dose-dependent result of the effect of sulforaphane on the cell line; rather, it showed a more drastic decrease from SFN 5 μM to SFN 10 μM. Also, this experiment and the replicate were only conducted once for preliminary data gathering and does need to be repeated in order to confirm the results shown below.

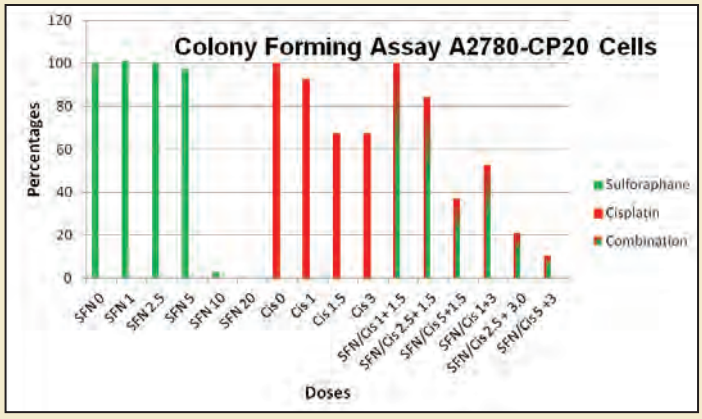

Figure 4: Colony forming assay showed that the colony also has a dose-dependent response to drug treatment.

It was predicted that the drug treatments may affect the morphology of the A2780-CP20 cell line. As such, a cell morphology analysis was performed in which the cells were treated with doses found effective by the MTT and colony forming assays (Figure 3). With increasing concentration, the cell growth was visibly shown to be inhibited for the individual doses of sulforaphane and cisplatin. However, the combination treatments did not show as much response to changes in cell morphology or the physical shape of the cells.

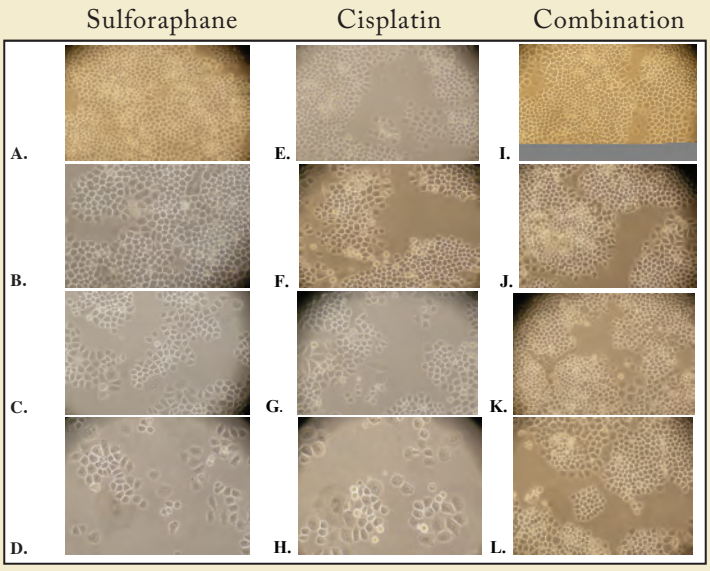

Figure 5: Cell Morphology Analysis of the Effects of Sulforaphane, Cisplatin, and Combination Treatment. Photos A, E, and I show untreated A2780-CP20 cells for sulforaphane, cisplatin, and combination, respectively. Photos B-D show the following sulforaphane dosages respectively: 2.5, 5.0, and 10.0 μM. Photos F-H show the following cisplatin dosages respectively: 1.5, 3.0, and 10.0 μM. Photos J-L show the following combination dosages: 2.5 (sulforaphane) + 1.5 (cisplatin), 5.0 (sulforaphane) + 1.5 (cisplatin), and 2.5 (sulforaphane) + 3.0 (cisplatin) μM.

Discussion

Previous studies with a breast cancer model have shown that sulforaphane causes a decrease in cell proliferation[5]. It was predicted that sulforaphane may yield similar results in an ovarian cancer model. The MTT analysis showed that sulforaphane produces a dose-dependent yield in estrogen receptor-negative ovarian cancer cells with the optimum dose observed to be 20 μM. Cisplatin showed a similar trend with an optimum dose of 10 μM; however, during the combination treatment the optimum dose was found to be 5.0 μM sulforaphane and 1.5 μM cisplatin. As stated earlier, cisplatin treatment is not an all-or-none phenomenon. Within the cell line certain cells showed less resistance to the treatment than others, yielding a dose-dependent response. Unlike the individual doses, a dose-dependent response was not observed with the combination treatments for MTT. The results led us to believe that the two drugs may not be working together to have a combined effect.

The colony forming assay generally confirmed the results shown by the MTT analysis; however, the colony forming assay did not show a gradual dose-dependent trend with sulforaphane treatments as did the MTT analysis. A more drastic decrease is noticed between sulforaphane treatments of 5 and 10 μM. Also, the colony results shown are from one trial; therefore the assay needs to be repeated several times to confirm the results.

The morphological affects of the drug treatments were viewed with the cell morphology assay. As the MTT analysis suggested, the increase in dosages of sulforaphane and cisplatin led to a decrease in cell viability. Optimum doses of both sulforaphane and cisplatin were concluded to be 10 μM. The morphology analysis suggested that the optimum combination dose is 2.5 (sulforaphane) + 3.0 (cisplatin) μM. No change in cell morphology was observed; however, a dose dependent decrease in cell proliferation was noticed.

Collectively, the data showed a dose-dependent response for the individual treatments but not a combination treatment. It is possible that either the sulforaphane or cisplatin alone may be the driving force for the slight decrease in cell viability observed with the combination treatments. Statistical analysis still needs to be performed to determine the degree of significant change in cell proliferation. In addition, sulforaphane treatments of A2780-CP20 cells shows promise for decreasing the growth of ovarian cancer cells, leading to the possibility of using a natural approach to chemoprevention. With neglible to no sideeffects from sulforaphane treatment, this natural approach would be chosen over the cisplatin treatment clinically. To ensure the lack of sulforaphane toxicity to normal cells, this study needs to be performed on normal ovarian HIO-180 cells in the future.

Conclusion

Finding a better treatment method for ovarian cancer cells can allow for more effective treatment with less deterring side effects. Sulforaphane is a natural component with anticancer properties that would help provide a treatment regime for cancer cells, as it has been shown to diminish cell viability. Results of this study show that sulforaphane is a good component that also targets growth of ovarian cancer cells. As such, further study and data analysis needs to be completed in order to fully understand the biological mechanisms that cause the affects of sulforaphane on cell viability in an ovarian cancer model.

Acknowledgements

I would like to thank my mentor, Shweta Patel, for helping me perform this project in a timely manner and for constant encouragement and support. I would like to give an extended thanks to Dr. Syed Meeran for helping establish the structure of my project. Also, I would like to thank Dr. Trygve Tollefsbol for giving me the opportunity to work in his laboratory and Dr. Charles Landen for providing the cell line. This work was supported by a grant from the Norma Liscription Foundation.

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short report

A Proposed Model of Apoptosis of Pancreatic Insulin-Secreting Beta-Cells

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Introduction

Diabetes mellitus type 1(T1DM), or insulin dependent diabetes, results from autoimmune beta-cell destruction.[1] Beta-cells secrete insulin, the primary regulator of glucose homeostasis. When beta-cells die, it results in a decrease in insulin secretory capacity leading to harmful increases in blood glucose levels. The Ca^{2+}-independent phospholipase A2 (iPLA2β) enzyme participates in beta-cell death by a programmed pathway, or apoptosis, that involves generation of ceramides.[2] Ceramides are lipids, which are known to cause apoptosis in several cell systems.[3] The established pathway of ceramide generation is via the hydrolysis of sphingomyelins (SMs) by sphingomyelinase (SMase). It is believed that the neutral form of sphingomyelinase (NSMase) plays a key role in the apoptosis of beta-cells. Figure 1 describes a proposed mechanism for beta-cell apoptosis.[2] In this model, endoplasmic reticulum (ER) stress, induced by depletion of ER Ca^{2+} stores by thapsigargin, activates iPLA2β.

When activated, the iPLA2β enzyme hydrolyzes the sn-2 substituent in membrane phospholipids in the absence of Ca^{2+}.[4] As shown in Figure 2, activation of iPLA2β leads to the hydrolysis of the fatty acid arachidonic acid (AA) and a product devoid of an sn-2 substituent. These are designated as lysophospholipids (LPCs). When they contain choline as the head group in the sn-3 position, they are referred to as lysophosphatidylcholines (LPCs).

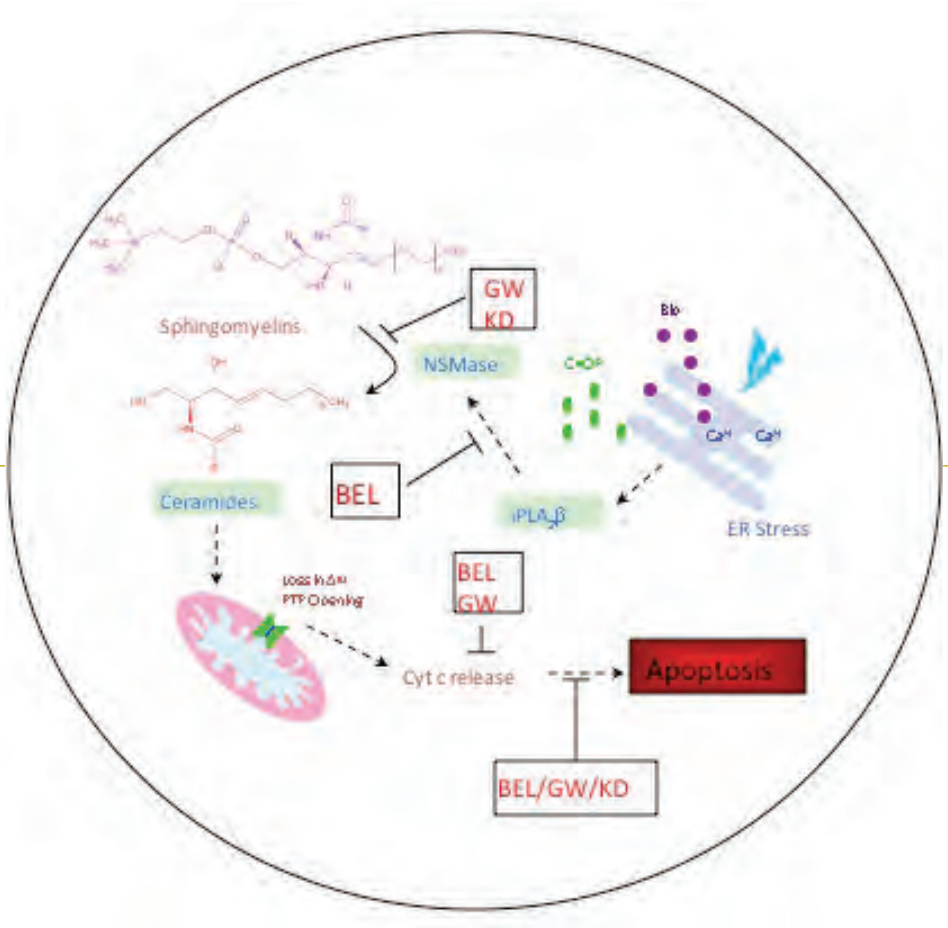

Figure 1. Proposed Model by which iPLAβ/ Ceramides Induce Beta-Cell Apoptosis

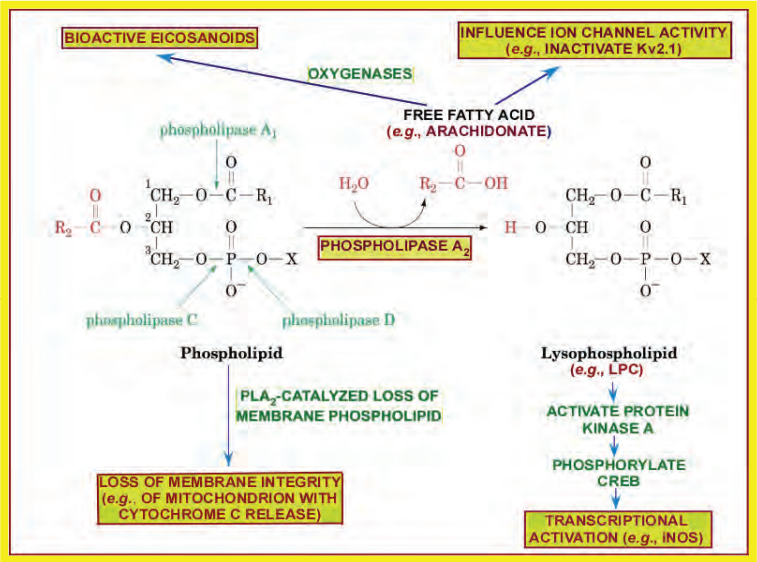

Figure 2. Summary of Phospholipase A2 Activities

Objective

The objective of this project was to identify which lipid products of iPLA2β activation impact NSMase expression and at what concentrations. In initial experiments, two variables were considered: the concentration of each lipid and the duration of exposure. INS-1 insulonoma cells that over expressed for iPLA2β were exposed to one of three products of iPLA2β activation: arachidonic acid (AA), lysophosphatidylcholine 18:0 (LPC 18:0), or lysophosphatidylcholine 16:0 (LPC 16:0). It should be noted that LPC 18:0 and LPC 16:0 are lysolipids that contain different fatty acid substituents at the sn-1 position. Thus, LPC- 18:0 contains stearate with a carbon chain length of 18 and zero double bonds and LPC-16:0 contains palmitate with a carbon chain length of 16 and zero double bonds The differing ratios describe the carbon chain length and number of double bonds. LPC 18:0, also referred to as pomitate, contains an eighteen carbon chain with zero double bonds; LPC 16:0, also referred to as stearate, contains a 16 carbon chain with zero doubles. Preliminary findings suggested that LPC and AA induce NSMase within 6 hours in a concentration-dependent manner. Thus, in this experiment, the cells were exposed to AA, LPC 18:0, or LPC 16:0 for a 6 hour interval at 37°C.

Methods

The experiment involved isolating RNA from the INS-1 cells treated with the three lipids. From these RNA samples, cDNA was generated and then processed for Real-Time PCR to quantitate NSMase mRNA expression of NSMase. The following set of procedures was repeated weekly:

Three 6-well plates were seeded with over-expressing INS-1 cells, an insulinoma cell line that is widely used to study beta-cell function. Each well contained 999 µl of growth medium and 1 µl of lipid solution, totaling to a volume of 1 mL in each well. The control, or vehicle, used was 0.01% dimethyl sulphoxide (DMSO).

The set up was as follows:

Vehicle	Vehicle	1.1-AA
1.2-AA	2.1-AA	2.2-AA

Vehicle	*0.01% DMSO*
Vehicle	*0.01% DMSO*
1.1-AA	*5 µM AA*
1.2-AA	*5 µM AA*
2.1-AA	*100 µM AA*
2.2-AA	*100 µM AA*

Vehicle	1-LPC18:0	2-LPC18:0
3-LPC18:0	4-LPC18:0	5-LPC18:0

Vehicle	*0.01% DMSO*
1-LPC18:0	*5 µM LPC 18:0*
2-LPC18:0	*10 µM LPC 18:0*
3LPC18:0	*25 µM LPC 18:0*
4LPC18:0	*50 µM LPC 18:0*
5LPC18:0	*100 µM LPC 18:0*

Vehicle	1-LPC16:0	2-LPC16:0
3-LPC16:0	4-LPC16:0	5-LPC16:0

Vehicle	*0.01% DMSO*
1-LPC16:0	*5 µM LPC 16:0*
2-LPC16:0	*10 µM LPC 16:0*
3LPC16:0	*25 µM LPC 16:0*
4LPC16:0	*50 µM LPC 16:0*
5LPC16:0	*100 µM LPC 16:0*

Figure 3. Six-Well Plate Set Up for Treatment

RNA was harvested from the cells using the Qiagen RNeasy Mini Kit and Protocol. The RNA samples were then quantified using a spectrophotometer. Results were obtained from duplicates of each RNA sample. If the average 260/280 ratio for each RNA sample was within the range of 1.9 and 2.1, the samples were prepared for cDNA synthesis. If not, the experiment was then repeated with a fresh batch of INS-1 cells. Next, in preparation for Real Time-PCR, first-strand cDNA was generated. A 96-well RT-PCR plate was set up in quadruplets. The first set of the wells were used to determine mRNA expression of 18S, a house-keeping gene which serves as a control. The remaining set of wells was used to determine mRNA expression of NSMase.

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	A	A	B	B	B	B	C	C	C	C
B	D	D	D	D	E	E	E	E	F	F	F	F
C	C1 through D12 are empty wells.											
D												
E	A	A	A	A	B	B	B	B	C	C	C	C
F	D	D	D	D	E	E	E	E	F	F	F	F
G	G1 through H12 are empty wells.											
H												

Legend	
18 S mRNA expression	
NSMase mRNA expression	
A	*Vehicle (DMSO)*
B	*Vehicle (DMSO)*
C	*5 μM AA*
D	*5 μM AA*
E	*100 μM AA*
F	*100 μM AA*

	1	2	3	4	5	6	7	8	9	10	11	12
A	16A	16A	16A	16A	16B	16B	16B	16B	16C	16C	16C	16C
B	16D	16D	16D	16D	16E	16E	16E	16E	16F	16F	16F	16F
C	18A	18A	18A	18A	18B	18B	18B	18B	18C	18C	18C	18C
D	18D	18D	18D	18D	18E	18E	18E	18E	18F	18F	18F	18F
E	16A	16A	16A	16A	16B	16B	16B	16B	16C	16C	16C	16C
F	16D	16D	16D	16D	16E	16E	16E	16E	16F	16F	16F	16F
G	18A	18A	18A	18A	18B	18B	18B	18B	18C	18C	18C	18C
H	18D	18D	18D	18D	18E	18E	18E	18E	18F	18F	18F	18F

Legend	
18 S mRNA expression	
NSMase mRNA expression	
16A	*Vehicle (DMSO)*
16B	*5 μM LPC 16:0*
16C	*10 μM LPC 16:0*
16D	*25 μM LPC 16:0*
16E	*50 μM LPC 16:0*
16F	*100 μM LPC 16:0*
18A	*Vehicle (DMSO)*
18B	*5 μM LPC 18:0*
18C	*10 μM LPC 18:0*
18D	*25 μM LPC 18:0*
18E	*50 μM LPC 18:0*
18F	*100 μM LPC 18:0*

Figure 4. 96-Well Plate Set Up for RT-PCR

Results

Figure 5 portrays the curve of the data collected and analyzed. Real-time Polymerase Chain Reaction (RT-PCR) allows for the detection of PCR amplification during the early phases of a reaction and collects data as the reaction is proceeding. By doing so, RT-PCR allows for easier and more precise quantitation of DNA and RNA. As can be seen in Figure 5, a PCR curve can be broken up into three phases. During the first phase, an exact doubling of product is accumulating at every cycle. At the second phase, the reaction components are consumed and the reaction begins to slow down as products start to degrade. In the last phase, the reaction has stopped and no more products are being made.

As revealed in Figure 6A, NSMase message expression increased as the concentration of arachidonic acid was increased. Additionally, although there was minimal expression observed with concentrations below 10 μM, higher concentrations of LPC 18:0 correlated with increased levels of NSMase expression (figure 6B). However, as the concentration of LPC 18:0 was increased from 10 μM towards 100 μM, NSMase expression decreased. It is suspected that after a certain point, high concentrations of LPC 18:0 have a toxic effect on NSMase. To further elucidate the correlation between the concentration of LPC 18:0 and the mRNA expression of NSMase, future experiments are likely to be focused on concentrations between 10 μM and 25μM. Figure 6B also indicates that lysophosphatidylcholine 16:0 does not appear to have an effect on NSMase expression. Since the activation of iPLA2β leads to release of the fatty acid arachidonic acid and a lysolipid such as LPC, our experiments reveal that a relationship exists between iPLA2β activation and NSMase message expression. Figure 6 portrays a validation of the data collected and analyzed in Figure 5.

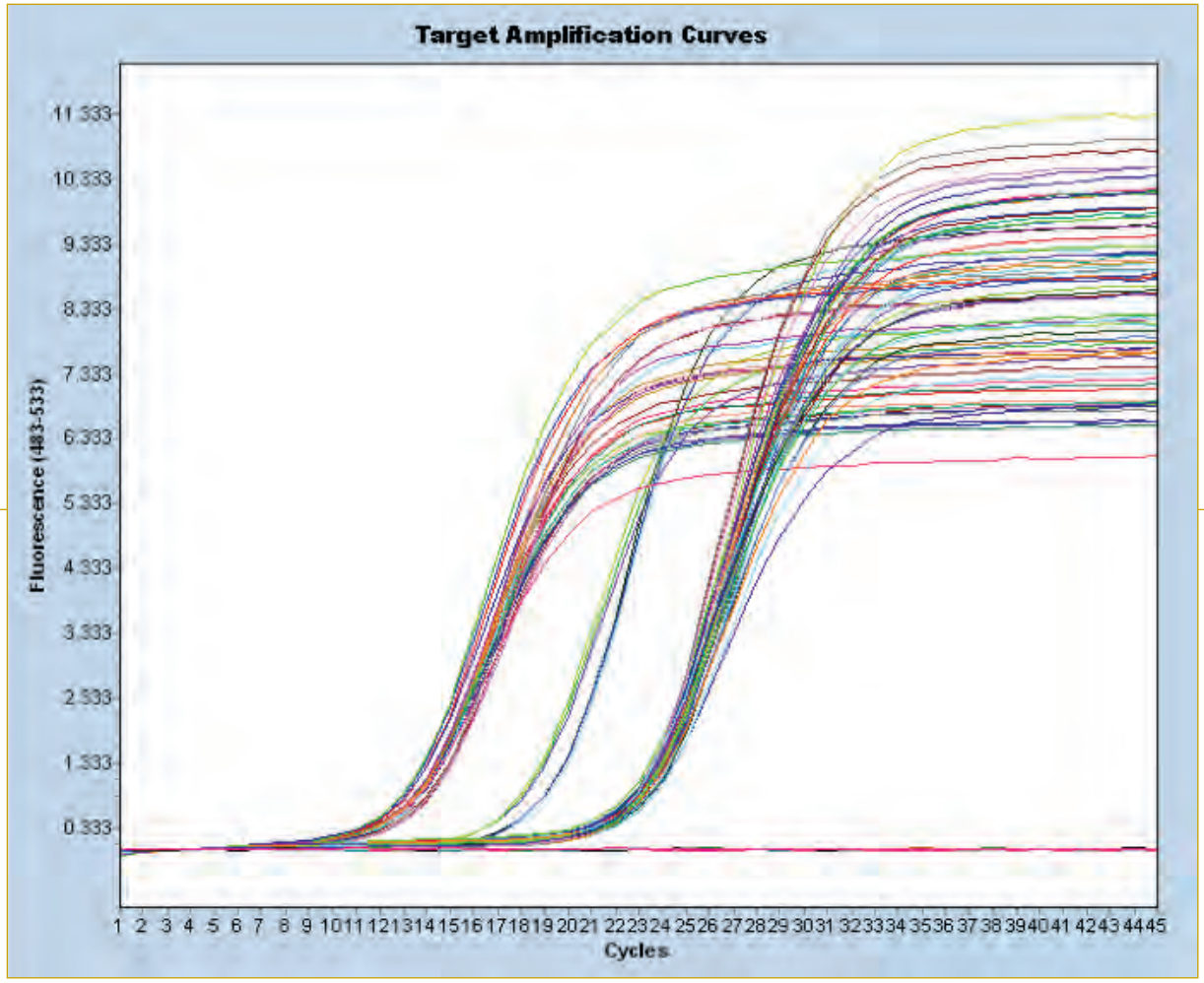

Figure 5. PCR Target Amplification Curves

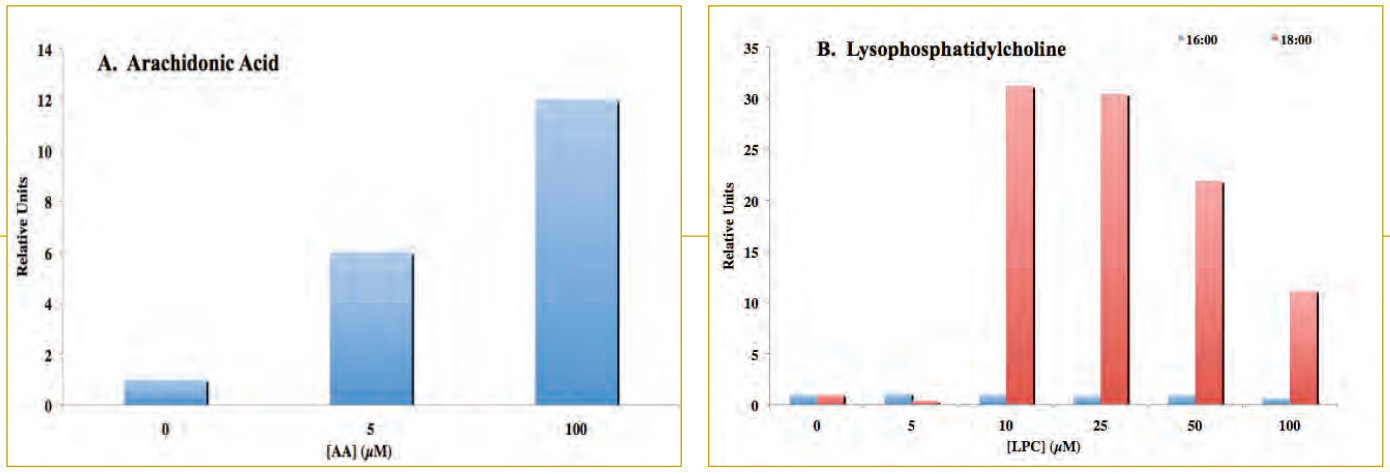

Figure 6. Effects of AA and LPC on NSMase Message Expression ß-Cells

Conclusion

The study shows that arachidonic acid and lysophosphatidylcholine induce NSMase expression. Interestingly, LPC 18:0 induced NSMase whereas LPC 16:0 did not. Thus, it appears that ceramide generation via NSMase-induced hydrolysis of SMs is facilitated by certain, but not all, products of iPLA2β activation in beta-cells. Further studies are needed to confirm these findings and establish statistical significance.

Acknowledgements

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short report

Cyp11a1 Deficiency Retards Cell Movement

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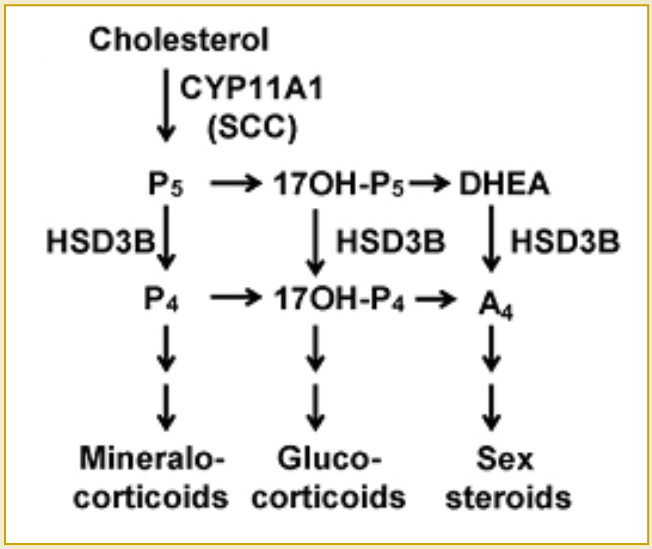
Introduction

Steroids play essential roles in physiological functions such as pregnancy, stress responses, anti-inflammatory responses, and tumorigenesis. They are also needed for embryonic and sexual development. Steroids are synthesized by endocrine glands such as the adrenal cortex and the gonads through steroidogenesis. In steroidogenesis, cholesterol is first converted to pregnenolone (P5) by cyp11a1 (SCC) (Fig. 1). After P5 synthesis the pathway diverges to produce mineralocorticoids, glucocorticoids, and sex steroids.



SCC has been shown to play a major role in zebrafish embryogenesis. In post-fertilization zygotes, blastomeres above the yolk undergo rapid cleavage, during this rapid cleavage SCC is expressed in the cytoplasm of blastomeres. During epiboly, blastomeres move downward from the animal pole to envelope the yolk. As the zygote undergoes epiboly, SCC expression is downregulated in the blastomeres. At the same time, SCC is upregulated in the yolk syncytial layer (YSL), which is an extra-embryonic layer at the forefront of the moving blastomeres. The loss of SCC expression in the YSL does not prevent epiboly but results in epibolic delay. Epibolic delay can be partially rescued by SCC and its product P5.

Figure 1: Steroidogenesis Pathway. Cholesterol is converted to pregnenolone (P5) by CYP11A1 (SCC). After P5 the pathway diverges to produce various other steroids.

P5's ability to rescue epiboly suggests that P5 or its downstream metabolites are involved in epiboly. In fact, P5 has been shown to stabilize yolk microtubules which are necessary for epiboly. P5's role in microtubule stability may also contribute to or control cell movement in other cell types.

It is not known whether the functions of SCC and P5 are conserved from zebrafish systems to mammalian systems. We showed through SCC knockdown that SCC and P5 are important for cell migration. In addition, we also began production of antibodies against zebrafish SCC protein to allow for further SCC studies.

Aims

1. Synthesize antibodies to confirm cyp11a1 protein expression and allow further experimentation.
    A. Produce recombinant cyp11a1 protein
2. Determine id Cyp11a1's and P5's role in cell movement conserved from zebrafish to mammalian systems.
    A. Knockdown Cyp11a1
    B. Cell Migration Assay

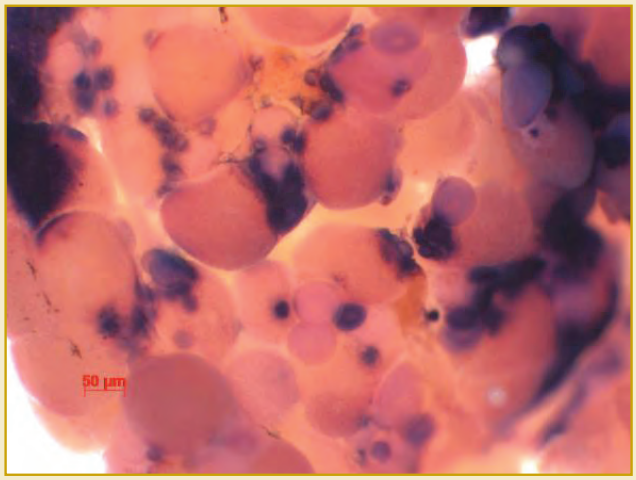


Figure 2: Zebrafish Ovary. In situ hybridization of zebrafish ovary. SCC can be found in oocyte cytoplasm. Signal becomes diluted as oocyte matures and cytoplasm increases. Location of SCC mRNA is not a guarantee that SCC is expressed. Testes and brain also show SCC in situ hybridization. Because of sample contamination, in situ staining of testes and brain was weak.

Antibody Synthesis

Recombinant Protein Induction

While wholemount in situ can provide information on the location and expression of SCC mRNA, it is not a guarantee that SCC protein is being expressed (Fig. 2). To verify protein expression, antibodies against zebrafish SCC protein are required.

A pET expression system was used to culture SCC protein for antibody production. SCC segment fragments N1, N2, and N3 were cloned into a pET29a(+) expression vector (Table1, Fig. 3). E. coli bacterial strains ER2566, DE3, pLys, and R1L were tested for ideal expression. The bacteria were transformed with recombinant SCC and grown to an OD600 = 0.8 and then induced with isopropyl β-D-1-thiogalactopranoside (IPTG) for 4 hours. After induction, proteins were separated by SDS-polyacrylamide gel electrophoresis.

The bacterial lines ER2566, DE3, and R1L showed protein expression in both the induced and uninduced conditions. The bacterial line pLys was the only bacterial line found to express protein solely in the induced condition (Fig. 4).

Optimal induction temperatures were then tested for SCC in pLys. Incubation temperatures of 16°C and 37°C were tested. The bacteria were transformed with recombinant SCC and grown to an OD600 = 0.8 before induction. During sample preparation, both the pellet and the supernatant fractions were collected from both temperature conditions to determine protein solubility. No SCC expression was observed at the 16°C condition. The SCC recombinant protein expression was found only in the 37°C temperature condition and largely in the insoluble portion (Fig. 5).

SCC and P5 Important for Cell Movement

To determine whether SCC's and P5's role in cell movement was conserved from zebrafish to mammalian systems, SCC was knocked-down in mouse Y1 adrenocortical tumor cells. Because of SCC's conversion of cholesterol to P5 in steroid production, knocking-down SCC limits SCC expression and prevents or decreases P5 expression as well.

Knockdown of SCC and P5

Knockdown was achieved by using shRNA sequences complementary to SCC mRNA. The shRNA was introduced into Y1 cells via lentivirus. Once infected the shRNA is processed by the cell to become siRNA, which binds the SCC mRNA. This leads to mRNA degradation and ultimately gene silencing.

Five shRNA sequences were synthesized: m1, m2, m3, m4, and m5. Only shRNA m1 and m4 were found to knockdown SCC expression while shRNA m5 only partially decreased shRNA expression (Fig.6).

Cell Migration

Y1 cells were infected with shRNA m1, m4, m5 and Luc shRNA against luciferase as a control. Each condition was imaged at five minute intervals for three hours. The images were compiled into a movie. Control cells infected with luciferase shRNA exhibited normal cell migration patterns. Cells infected with m1, m4, and m5 exhibited abnormal cell migration patterns. Y1 cells with reduced levels of SCC and P5 exhibited much slower movement than control cells. Reduced levels of SCC and P5 also resulted in frequent direction changes unlike their control counterparts. These differences in movement suggest that SCC and P5 do affect cell movement in mammalian systems.

Discussion

In this study we show that the role of SCC and P5 in cell movement is conserved from zebrafish to mammalian systems. SCC knocked-down cells exhibit abnormal cell movement. Because each cell within the field for all conditions came in contact with another cell in its migration path, it was not possible to perform a free cell migration quantitative analysis. Qualitatively, the data supports SCC and P5 having a role in cell migration.

Because SCC knocked-down cells exhibited frequent changes in migration direction, we posit that P5 may affect cell movement through cell polarity. P5's role in microtubule stability supports this hypothesis. The loss of P5 could negatively affect microtubules and disrupt cell polarity. A loss of cell polarity regulation may explain the constant changes in migration direction.

To further studies of zebrafish SCC, we began efforts to synthesize zebrafish SCC antibodies. Induction experiments found that the expression bacterial pLys was the only tested strain to show SCC recombinant protein expression in the induced condition. Once determined as an ideal bacterial line, temperature conditions and solubility were tested within the pLys system. It was found that the SCC recombinant proteins expressed at higher concentrations at 37˚C and were largely insoluble. Once synthesized, these antibodies will allow us to visualize SCC expression in zebrafish embryos and tissues with greater confidence than with in situ hybridization.

Materials and Methods

Cell culture and Infection – Viruses were cultured in 293FT cells plated with 9x105 cells per 6cm plate grown in 37˚C in 5% CO2. After 24 hours the cells were transfected with pMD2.G (750µg per 6cm plate), psPAX2 (250µg/6 cm plate), and shRNA

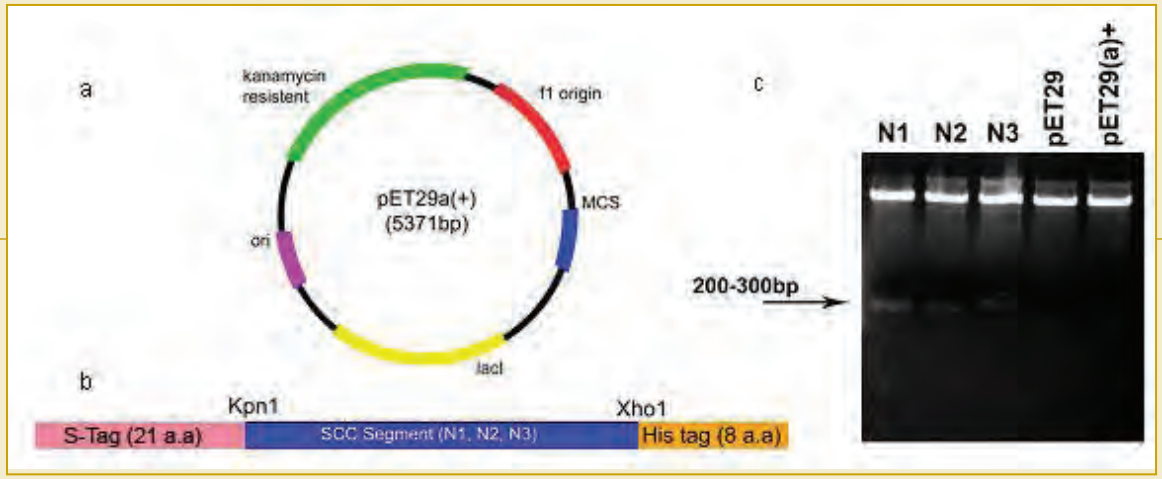


Figure 3: Zebrafish SCC protein segments (a) Segments were cloned into the pET29(a)+ expression vector with a S-Tag and a His tag for protein purification. (b) The segments were cloned in using the Kpn1 and Xho1 restrictions sites. (c) The recombinant protein visualized on 0.8% agarose gel: N1, 246 bp; N2, 248 bp; and N3, 323 bp.

Amino acid region	peptide sequence
1-82 a.a. (N1)	MARWNVTFARLDQSLSSLKNLLQVKVTRSGRAPQNS TVQPFNKIPGRWRNSLLSVLAFTKMGGLRNVHRIMVH NFKTFGPIY
81-163 a.a. (N2)	IYREKVGIYDSVYIIKPEDGAILFKAEGHHPNRINVDAW TAYRDYRNQKYGVLLKEGKAWKTDRMILNKELLLPK LQGTFVPL
210-317a.a. (N3)	ERLGLLLDNIDPEFQHFIDCVSVMFKTTSPMLYLPPGLL RSIGSNIWKNHVEAWDGIFNQADRYIQNIFKQWKENPE GNGKYPGVLAILLMQDKLSIEDIKASVTELM

Table 1: Recombinant SCC protein. Segments of zebrafish SCC protein were cloned into pET29(a)+ expression vector using Kpn1 and Xho1 restriction sites respectively.

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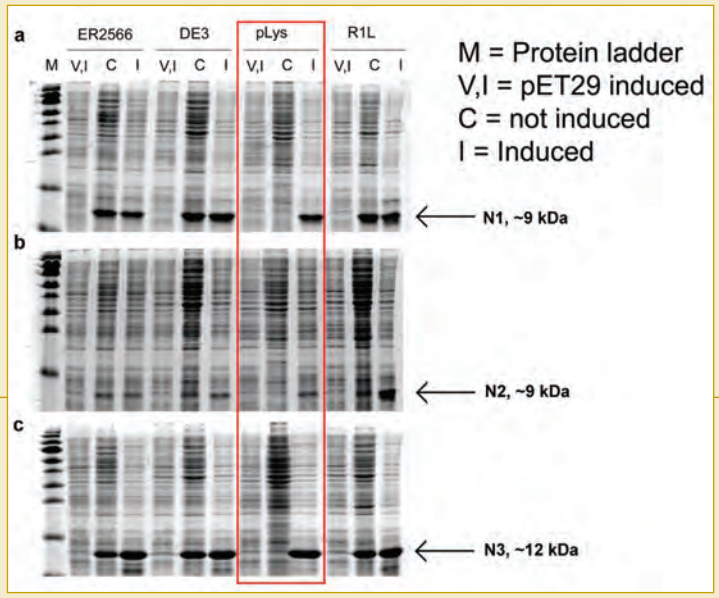

Figure 4: pLys expresses SCC segments. E. coli expression strains were transformed with SCC protein segments. The bacteria were cultured to an OD600 = 0.8 and then induced with IPTG for 4 hours. Only the pLys strain expressed recombinant protein in the induced state for all three segments. (a) N1 (b) N2 (c) N3

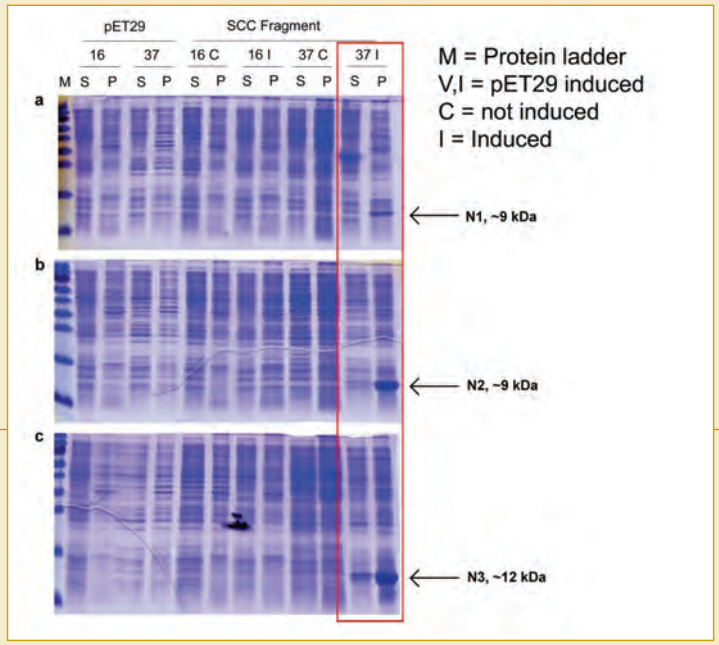

Figure 5: pLys induction conditions. Expression strain pLys was transformed with SCC protein segments. The bacteria were incubated at 16˚C and 37˚C until OD600 = 0.8 and then induced with IPTG for 4 hours. The soluble and insoluble fractions were collected. SCC is best expressed at 37˚C and found largely in the insoluble portion. (a) N1 (b) N2 (c) N3

(1µg/6cm plate) premixed in 50µl OPTI for 5 minutes and then 6µl of LMPF2000 for 15 minutes. At transfection, the medium was replaced with fresh medium. The medium was replaced again after 16 hours. Viruses were collected 48 hours post transfection. Mouse Y1 adrenocortical tumor cells were maintained in Dulbecco's modified Eagle medium (DMEM)-F12 medium supplemented with 10% fetal bovine serum. For infection, Y1 cells were plated with 5x104 cells per 6cm plate. After 24 hours the cells were infected with a viral cocktail (500µl virus, 500µl F12-FBS, and 1µl polybrene). After 24 hours of infection, the medium was replaced with puromycin (2µg/ml). After 48 hours of infection, the cells were harvested for Western blotting or filmed for a cell migration assay.

In situ hybridization – Embryos between fertilization and 48 hours post-fertilization were captured in Petri dishes, chilled on ice, and then fixed overnight in 4% paraformaldehyde/PBS buffer at 4 ˚C. Fish were chilled on ice until unconscious and then sacrificed by severing the spinal cord. Tissues were removed and fixed in 4% paraformaldehyde/PBS buffer at 4˚C. Both embryos and tissues were fixed again in 4% paraformaldehyde/PBS buffer and then washed 3 times in PBST (PBS with 0.1% Tween 20). Samples were digested in proteinase K and then washed 3 times with PBST before prehybridization in HYB+ buffer (60% formamide, 5× SSC, 500 µg tRNA, 50 µg/L heparin, and 0.1% Tween 20) for 4 hours at 70 ˚C. The P450scc(cyp11a1) probe (200 ng/mL) was incubated with the samples for over 16 hours at 70 ˚C. Samples were washed with 75%, 50%, and 25% HYB+/2× SSC buffer for 15 minutes each, followed by 2× SSC, 0.2× SSC buffer, and PBST for 15 minutes each. They were then blocked in 5% albumin in PBST for 2 hours and incubated in a 1:5000 dilution of anti-digoxigenin antibody (Fab, conjugated with AP, Roche Applied Science, Indianap-

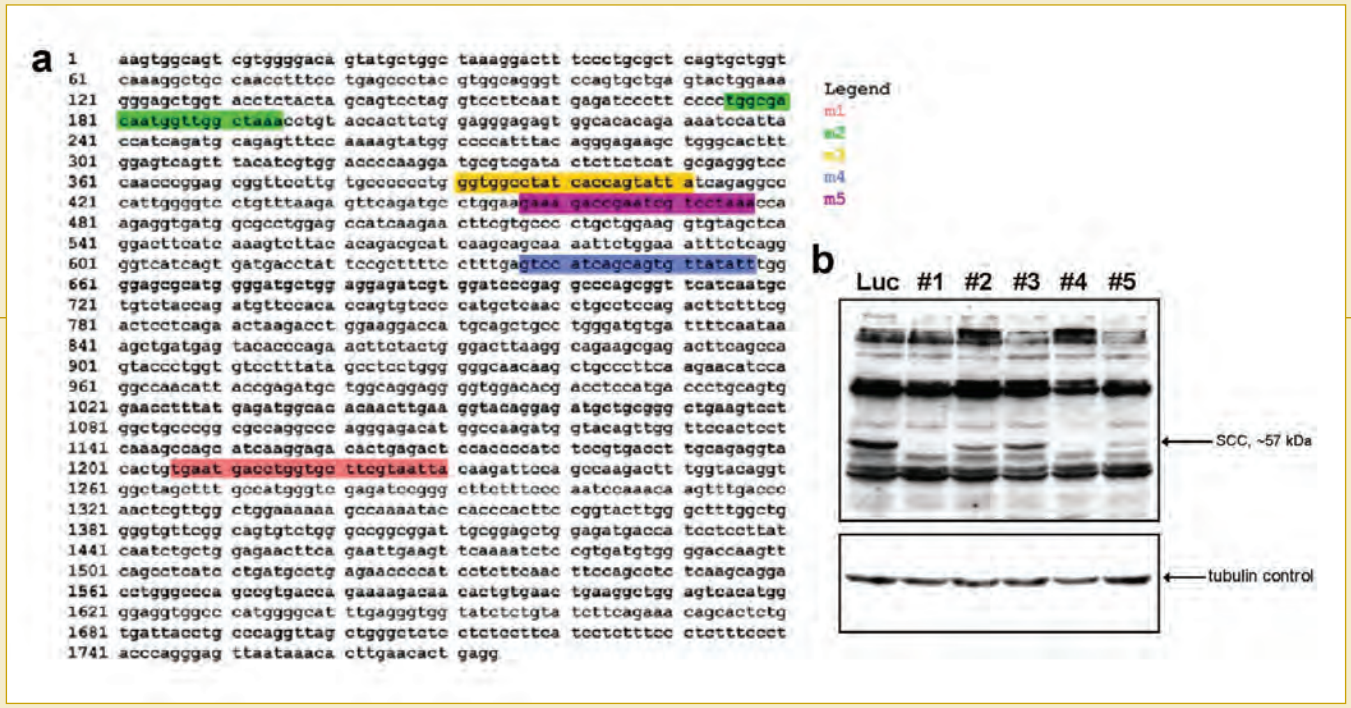

Figure 6: SCC shRNA (a) Five shRNA against SCC were generated. (b) Luc knocked-down luciferase, not normally found in Y1 cells. It was found that m1 and m4 shRNA knocked-down SCC expression with m5 only partially knocking-down SCC expression.

olis, IN, USA) at 4°C overnight. Antibodies were then removed and washed 3 times with PBST and 3 times with staining buffer (0.1 M Tris, pH 9.5, 100 mM NaCl and 50 mM MgCl2, and 1 mM levamesol). The samples were finally stained in NBT/BCIP (Roche Applied Science, Indianapolis, IN, USA) or TNBT/BCIP buffer (Chemicon, Billerica, MA, USA).

Immunoblotting and Antibodies – Cells were harvested and lysed in lysis buffer (50mM Tris (ph=7.5), 5mM EDTA, 1% Tx-100, 300mM NaCl, 50x protease inhibitor) 3 days after siRNA transfection. Equal amounts of total proteins were separated by gel electrophoresis, and then transferred to ImmobilonTM-P membrane (Millipore, Billerica, USA) via semidry transfer. Membranes were incubated with monoclonal antimouse-SCC antibodies and polyclonal anti-Flag or anti-Hsp70 antibodies in 5% milk overnight at 4°C and then with HRP conjugated secondary antibody in 5% milk for 45 minutes at room temperature. Signals were detected by chemilumiescence assays.

Expression and induction of recombinant cyp11a1 – Bacterial *E. coli* expression lines ER2566, R1L, pLys, and DE3 were transformed with recombinant cyp11a1 protein fragments were grown in 2-YT medium (tryptone 16g, yeast extract 10g, NaCl 5g in 1L H2O) with 50 μg/mL kanamycin up to an OD600 = 0.6. Cells were then induced with 1 mM IPTG at 37°C for 4 hours.

Acknowledgements

The Chung Lab; Taiwan Tech Trek; Academia Sinica, Institute of Molecular Biology; The Zebrafish Core in Academia Sinica (ZCAS) is supported by grant 100-2321-B-001-030 from the National Science Council (NSC); Chung lab et al; UAB Department of Biology, College of Arts and Sciences. Special thanks to my lab partner, Tsung-Yun Huang. He took wonderful images for in situ hybridization. Also special thanks to Jui-Hsia Weng for mentoring me during my internship and for the beautiful Western blot.

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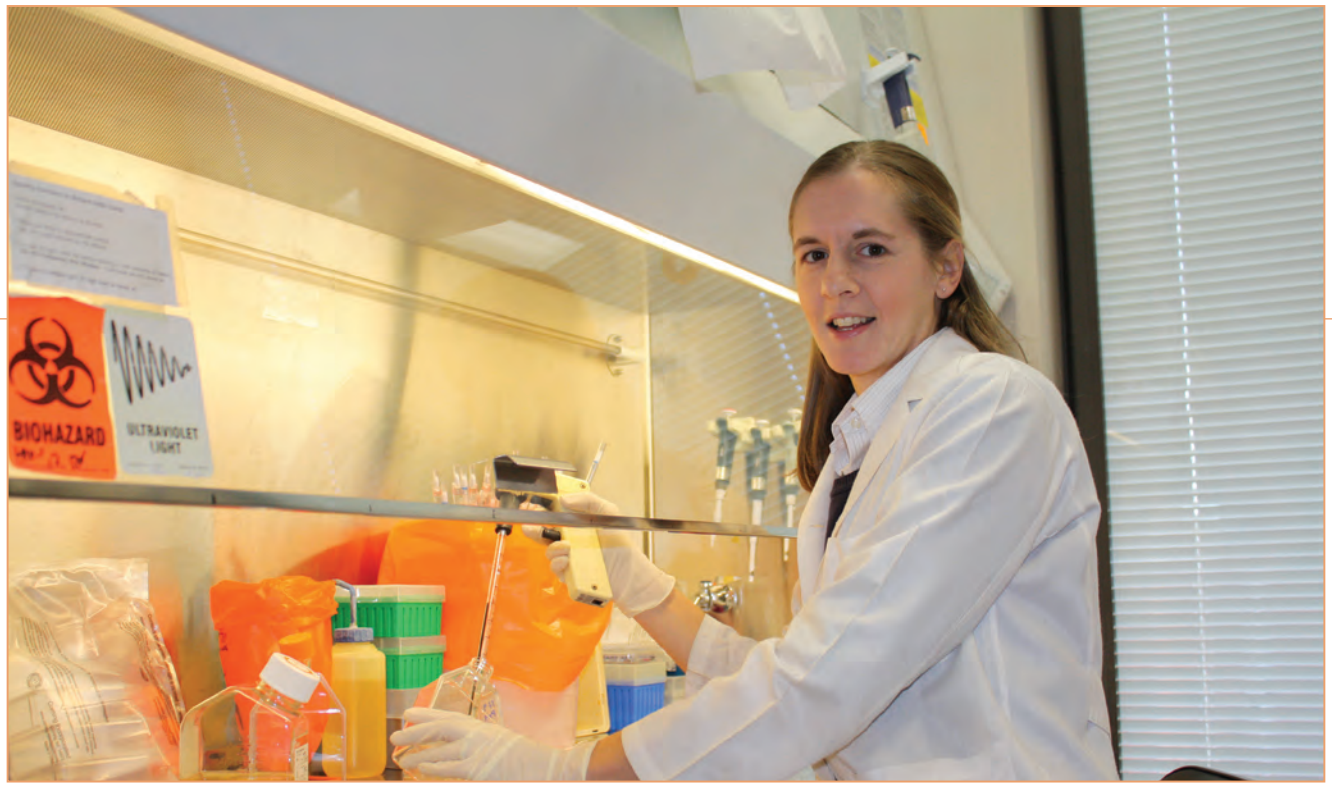
novel research in UAB's novel course

Ramya Singireddy



UAB's Phage Genomics course is a notable addition to the existing science curriculum and further improves the university's standing as a highly active research university. The Howard Hughes Medical Institute, one of the most prominent research foundations in the world, chose to sponsor UAB as one of only twelve universities to offer this course nationally.

Any freshman or sophomore UAB student may apply for a position in the Phage Genomics course taught by Dr. Denise Monti, but only a handful of applicants are selected. Each student isolates a new bacteriophage, a type of virus, and analyzes its genome. The first semester involves wet lab work in which the students use techniques such as streak plating and titer assays to obtain their unique phages. Each student then sends his or her phage to HHMI to be sequenced; they spend the second semester of the course evaluating the gene sequence of these phages.

"The most unique part about this class is that it's a real world experience," says Angelina Joshi, the course's graduate student teaching assistant. "Students are discovering something novel that no one has ever seen and becoming genuine scientists in the process." According to Joshi, this class adds to the pool of new knowledge being discovered about novel genes and their functions, which could potentially be used for gene therapy and other treatments.

Indeed, through Phage, students conduct completely original research in a near-professional lab setting. In addition to learning research techniques, students keep official laboratory notebooks, attend weekly Journal Club meetings, present their results in the UAB EXPO at the end of the school year, and eventually publish their work. At the end of the semester, one student is chosen to present his or her results at the HHMI site in Maryland along with students chosen from the other universities that offer the course.

"I think that Phage will open up many doors regarding research for me. It's a great way to be introduced to the world of research and what it entails," says sophomore Rikita Patel, who is currently enrolled in the Phage Genomics course.

Phage truly is a crucial educational experience for young people at UAB interested in pursuing scientific professions, and its addition to the curriculum solidifies UAB's status as one of the top research universities in the nation.

faculty interview: pediatric neonatology

Interview with Brian Sims, M.D., Ph.D.

Aditi Jani

Dr. Brian Sims is an assistant professor of pediatric neonatology and cell biology at UAB. A UAB graduate himself, Dr. Sims is a dedicated mentor to many budding physician-scientists. I spoke to him at his lab in the Shelby Biomedical Research Building about his education, his experiences in medicine and research, and his views on pursuing an M.D., Ph.D. degree.

Can you tell me a little bit about your education? Where did you pursue your undergraduate, medical, and graduate degrees?

For undergrad, I attended UAB and majored in Biology. I also attended UAB for medical school and my Ph.D. in cell biology. I completed my residency in Pediatrics at Washington University in St. Louis [WashU] and did a Neonatology fellowship back here at UAB. I also did a post-doc at WashU for a year in developmental neurobiology.

If I had asked you where you saw yourself in 10-15 years while you were in high school or undergrad, would you have said "Practicing medicine and doing research?" Why did you choose an M.D., Ph.D. track?

During my junior year of high school, I decided I wanted to do pediatrics, but I didn't have a lot of exposure to research. In college, I did a summer program at MeHarry Medical College where I saw some physician-scientists at work; after that point, I saw myself doing research in the future. Choosing M.D., Ph.D. was more about me wanting to be extensively trained in order to pursue and do basic science research. The M.D., Ph.D. allows you more isolated time to work on research, which is time you may otherwise never get.

Can you talk about your experiences in your education and work at UAB?

It was a very positive experience! In undergrad, I didn't have a lot of the research experiences that are available now, so they were harder to come by but still possible. I had some great instructors here at UAB. I think that whatever environment you're in after high school, you have to be a proactive learner, and I found that I was the rate-limiting step in my education. UAB is a great place to learn, and I wouldn't change anything about my decision to go here.

What's a typical day like for you? Do you spend more of your time in clinical pursuits or research?

I spend about 25% of my time fulfilling my clinical responsibilities as attending in the NICU, and I dedicate about 75% of my time to basic science research. I'm fortunate to be in a very supportive division [neonatology] that values basic science research. As a physician-scientist, you have to find the niche that is best for you and find where the balance is. Also, despite what you want, you have to be in an institution that supports you, so it's a cooperative agreement.

Some people may shy away from an M.D., Ph.D. degree because of the extensive, rigorous course load and the major time commitments of the schooling. What would you say to this?

Any commitment that requires that amount of time requires that the individual be 'all in' and 100% committed. If people are unsure that this is the route they want to pursue, then they may need more time to think about what they want to do. I equate it to marriage – it's not something to take lightly, so investigate and take your time before you make a strong commitment. It's a wonderful life, but it's one that I chose. Everyone has to make that choice for him or herself.

For student who have interests in research and medicine but are unsure about M.D., Ph.D., can you talk about some of your experiences in the benefits of pursuing both?

Ph.D.'s and M.D.'s think differently. Not that that's a bad thing, but the clinical world allows you to see problems firsthand. And that can direct your research interests, and honestly, as a physician, it should be part of the passion that drives your research.

Any words of wisdom or advice for future physicians and researchers?

Take as much time as you can to do rotations, even as an undergraduate. Get as much exposure as you can; it will ultimately make you a better learner, researcher, and physician. It's important to identify what your passion is early. Once you identify it, you will never be bored again. Don't lean towards something for the wrong reason. If you're going to a place you don't want to be, realize that one day you'll be there, so make sure you want to be there!

Can you talk about your research in neonatology?

My major focus is studying the vulnerability of the premature brain. In particular, we study neural stem cells and investigate protective mechanisms to increase cell survival. If the neural stem cells are protected against things like hypoxia and glucose deprivation, in theory, the animals and the human should get the same protection. This could prevent things like cerebral palsy, encephalopathy, hypoxia, and ischemia; so ultimately we have a basic cellular approach to this research.

What is the most rewarding part of your job?

First, dealing with children. Being able to be with families in their most critical time and to see a sick baby be able to go home with his or her parents. And secondly, being able to teach the "future doctors of America."

short report

Effects of the O-acetyltransferase wcjE on the Opsonization of *Streptococcus* pneumonia in a Multiplexed Opsonophagocytic Killing Assay

Pia Cumagun[1] • Juan Calix[2] • and Moon Nahm[2,3]
[1]UAB Pathology Research Experience Program • [2]UAB Department of Microbiology • [3]UAB Department of Pathology

Abstract

Background: Although *Streptococcus pneumoniae* colonizes the nasopharynx, it can also cause pneumonia, acute otitis media, and invasive diseases such as bacteremia and meningitis (1). This Gram-positive bacterium has an extremely diverse polysaccharide capsule that allows it to evade host immune defense mechanisms (3). The genes on the capsular polysaccharide synthesis (cps) locus are responsible for the 93 different capsule types known today (3). Of particular interest in this study was the wcjE allele in the cps locus, which encodes a putative O-acetyltransferase in serotypes 9V and 11A (3, 5). In serotypes 9A and 11E, this gene is inactive (1, 3). Serotypes 9V and 11A are included in the Pneumovax 23 vaccine, whereas 9A and 11E are not included. The primary purpose of this project is to determine the role of wcjE in the non-specific killing (NSK) response of the host against *S. pneumoniae*. In addition, cross-protection against serotypes 9A and 11E in individuals vaccinated with Pneumovax 23 was examined.

Methods: The multiplexed opsonophagocytic killing assay (UAB-MOPA) protocol was followed with slight modifications (2). HL60 cells, normal rabbit complement, and adsorbed rabbit complement were used in order to observe the NSK response in the following serotypes of bacteria: 9V (JC01), 9A (JC02), 9Aα (MB01), 11A (JC03), 11E (JC04), and 11Eα (JC12). To solely investigate the NSK response of the host, no sera was added. To determine whether the vaccine against *S. pneumoniae*, Pneumovax 23, cross-protects against 9A and 11E, 10 sera samples were tested for their ability to opsonize the serotypes 9V, 9A, 11A, and 11E.

Results: Strains that had a disrupted wcjE displayed a higher NSK response than strains with a functional wcjE. Both alpha serotypes that were tested exhibited a higher NSK than strains with an inactive wcjE. In addition, the antibodies in the tested sera samples cross-protected against 9A and 11E. Serotypes 9A and 11E were preferentially killed compared to 9V and 11A.

Discussion: The functionality of wcjE shows a direct relationship with the NSK response of the host. O-acetylation of capsule in 9V and 11A is dependent on the wcjE allele and could be a possible mechanism for these serotypes to evade the host immune defense. Alpha serotypes exhibit partial O-acetylation on their capsules (3). Since 11Eα and 9Aα are intermediates of serogroup 11 and 9, respectively, it was hypothesized that the two serotypes would show an intermediate NSK percentage. Therefore, these serotypes with an intact wcjE could possibly have developed this mechanism of evading the host defense, essentially eliciting a lower NSK response. One possible cause for the preferential opsonization of 9A and 11E is the volatile nature of the O-acetate groups in vaccine PS (4).

Introduction

S.pneumoniae is a Gram-positive diplococcus that colonizes the nasopharynx. It is responsible for causing pneumonia, bacteremia, meningitis, and otitis media (1). Its most important virulence factor is its highly diverse polysaccharide capsule, allowing evasion from the host immune system (3). There are over 90 different capsule types (3). The variability is due to the genes on the capsular polysachharide synthesis (cps) locus as seen in Figure 1 (1, 3). The vaccine, Pneumovax 23, which uses purified capsules of the 23 most prevalent serotypes (i.e. 9V and 11A), has been developed to help combat *S. pneumoniae* (http://www.merck.com/).

O-acetylation of the polysaccharide capsule in serotypes 9V and 11A is dependent on wcjE, a gene located on the cps locus (3, 5). This mechanism is inactivated in 9A and 11E (1, 3). There is partial inactivation in alpha serotypes (i.e. 9Aα and 11Eα) (3). Inactivation of this gene dictates seroswitching (i.e. 11Aα 11E).

Methods and Results

The multiplexed opsonophagocytic killing assay (MOPA) was used to observe the NSK response against *Streptococcus pneumonia* serotypes 9V, 9Aα, 9A, 11A, 11Eα, and 11E. MOPA was also used in order to opsonize 9V, 9A, 11A, and 11E using 10 sera samples from people who received the Pneumovax23 vaccine.

Conclusions

Strains with a functional wcjE (JC01=9V, JC03=11A) showed a lower percentage of non-specific killing than strains with a disrupted wcjE (JC02=9A, JC04=11E). MB01 (9Aα) and JC12 (11Eα) both showed higher non-specific killing than strains with disrupted wcjE. Immunizing with serotypes 9V and 11A cross-protects against serotypes 9A and 11E. This is possibly due to volatile nature of the O-acetate group, especially during vaccine preparations (4).

Future Research

Future research may seek to compare other serotypes with a functional and non-functional wcjE to validate that the inactivation of wcjE causes a higher NSK response in the host. Serogroups 15 and 33 may be beneficial to use. Simultaneously, research may inquire as to how adsorbtion with the capsule reduces NSK. For this, the 11A phenotype may be restored with 11E in the background, and it may be determined if NSK is reduced.

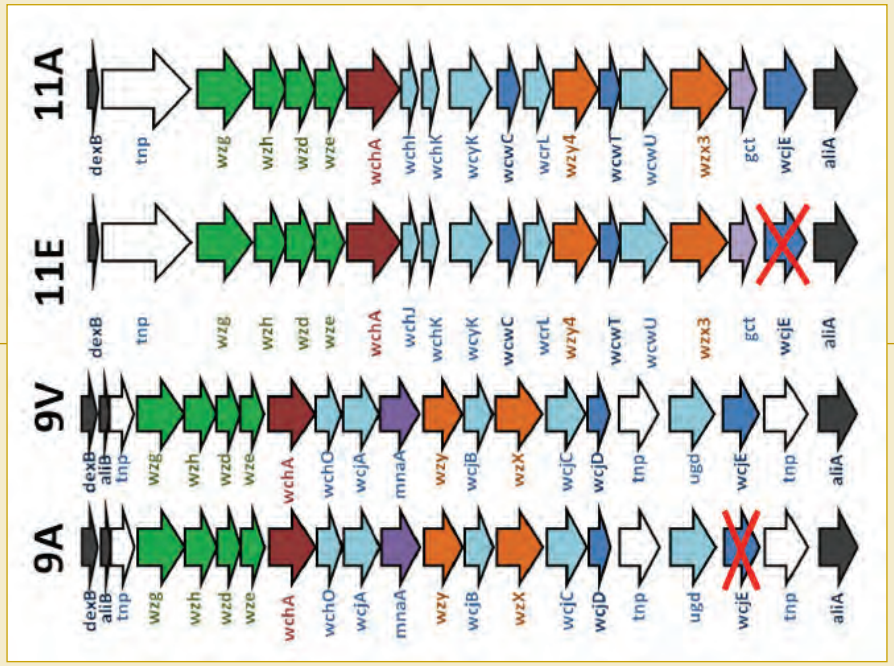


Figure 1. Cps locus. 9V (JC03) and 11A (JC01) are 2 serotypes that contain wcjE (1, 3). The only difference between 9V and 9A, as well as 11A and 11E, is the functionality of wcjE (1, 3).

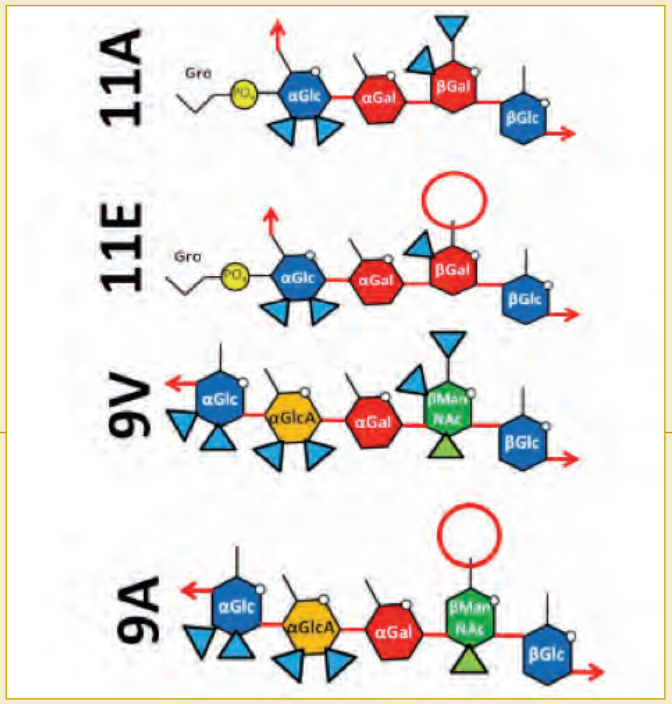


Figure 2. O-acetylation of Capsule. 9V polysaccharide capsule contains O-acetate groups (blue triangle) on the 6th codon (C6) of βManNAc, which 9A lacks (1, 5). 11A capsule also contains the βGal C6 O-acetylation, which 11E lacks (3).

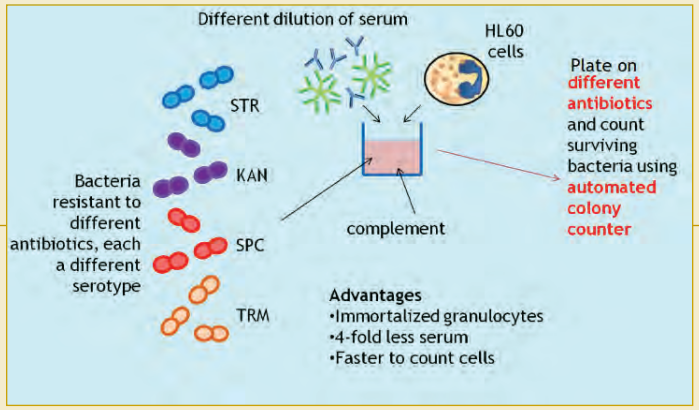
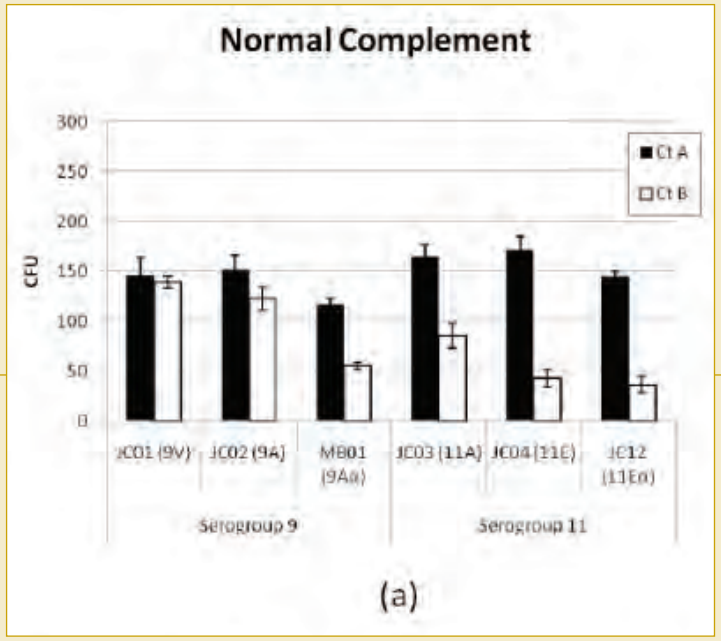


Figure 3. MOPA. The Pneumovax23 vaccine elicits antibodies to respond to the bacteria's capsule polysaccharide (2). The multiplexed opsophagocytic killing assay measures the capacity of antibodies against S. pneumoniae *and has been used to develop an effective vaccine. HL60 cells, which acted as phagocytes, and rabbit complement were used to create an environment similar to the natural environment in the host. A detailed protocol of the MOPA can be found at a www.vaccine.uab.edu (2).*



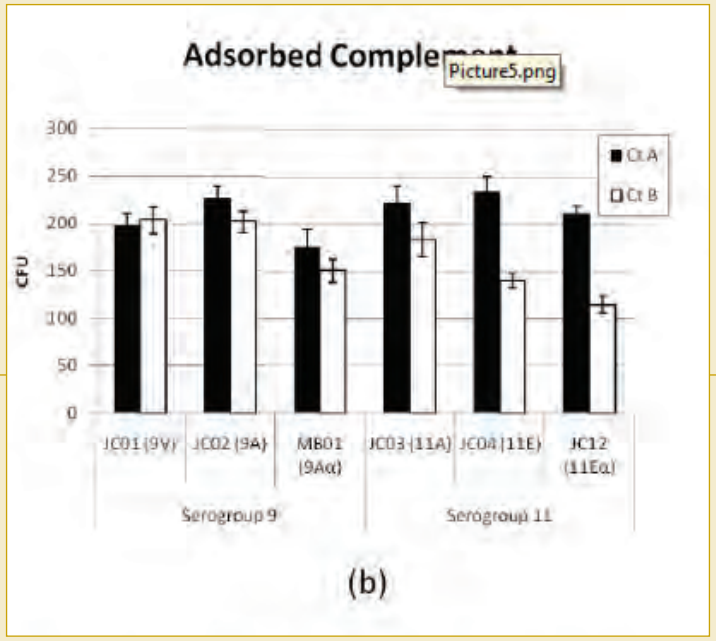


*Figure 4. Raw Data of the Normal and Adsorbed Complements. Control A (Ct A) includes the bacteria of interest, heat-inactivated complement, and HL60 cells. Control B (Ct B) is comprised of the same components with activated instead of heat-inactivated complement. The NSK was calculated using the following formula: (1-Ct B cfu/Ct A cfu) * 100. These graphs show that the NSK can be reduced by adsorbing the complement.*

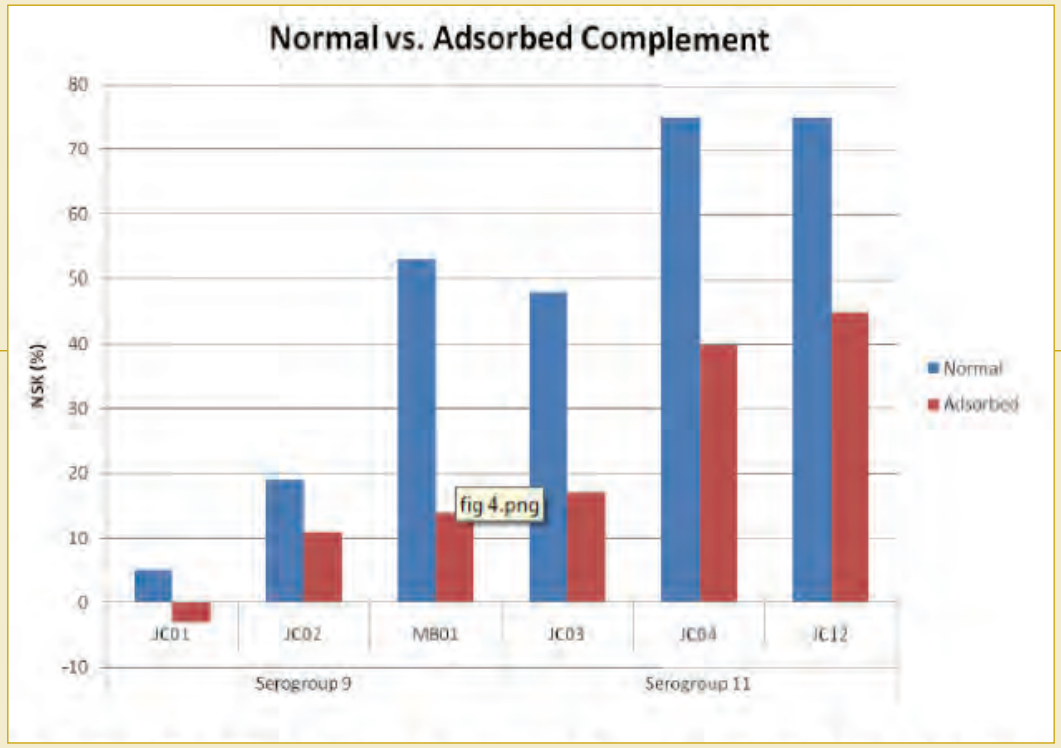


Figure 5. Non-Specific Killing Percentages Using Normal and Adsorbed Complement. Each strain was tested with normal and adsorbed complement. Adsorbed complement significantly reduced non-specific killing in all six serotypes.

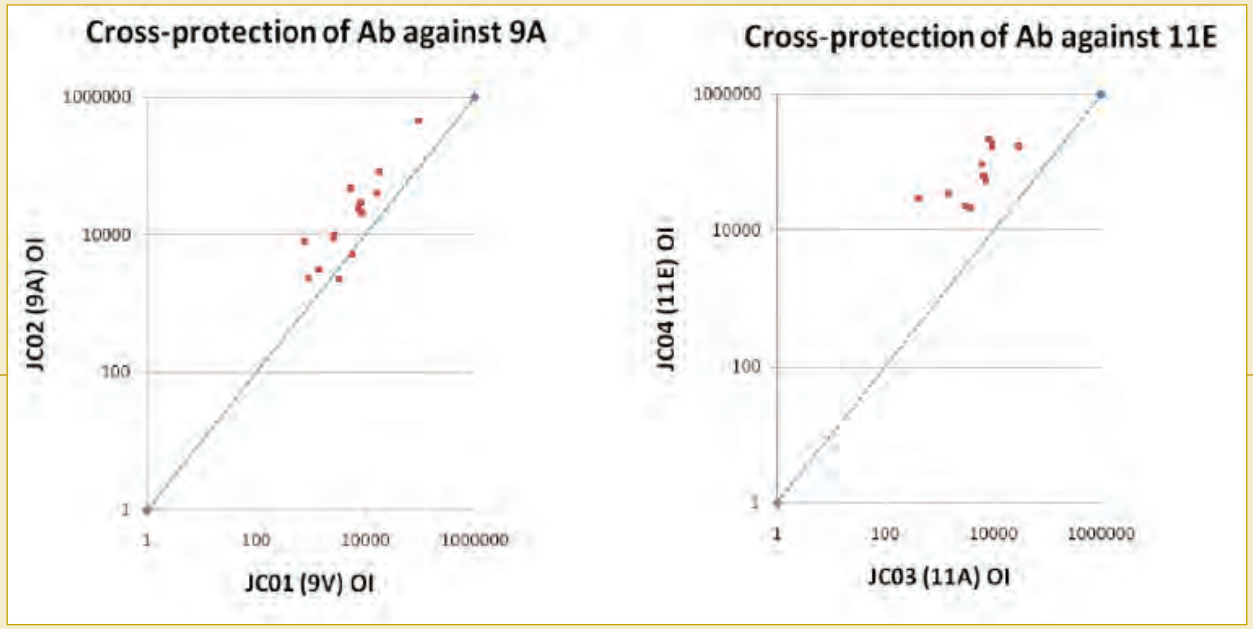


Figure 6. Cross-protection of Antibodies (Ab) against Serotypes 9A and 11E. (a) Sera displayed preferential killing of JC02. (b) Sera displayed preferential killing of JC04. The opsonizaiton (OI) index is the amount of diluted serum needed to kill 50% of the bacteria (2).

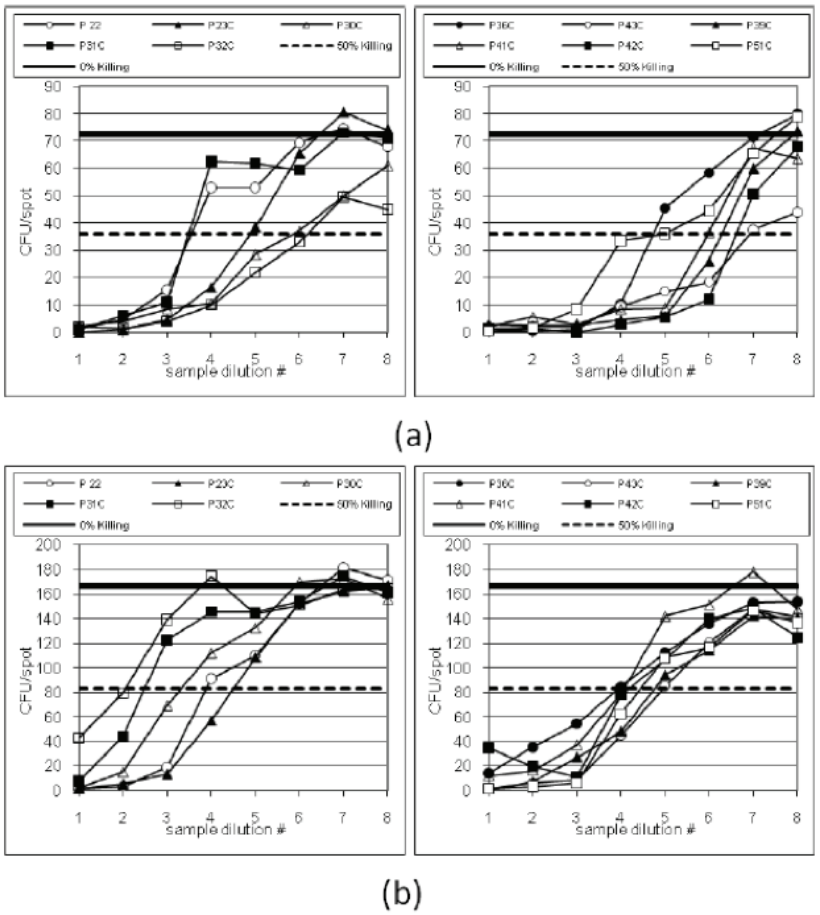


Figure 7. Examples of MOPA Data. These graphs display data from the MOPA for 9A (a) and 11A (b). The OI was determined from the data using a program called "Opsotiter 3" (2).

Acknowledgements
We would like to thank members of the Nahm lab for their assistance and support.

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short report

Drugs of Abuse on Coated Paper and Polymer Currency

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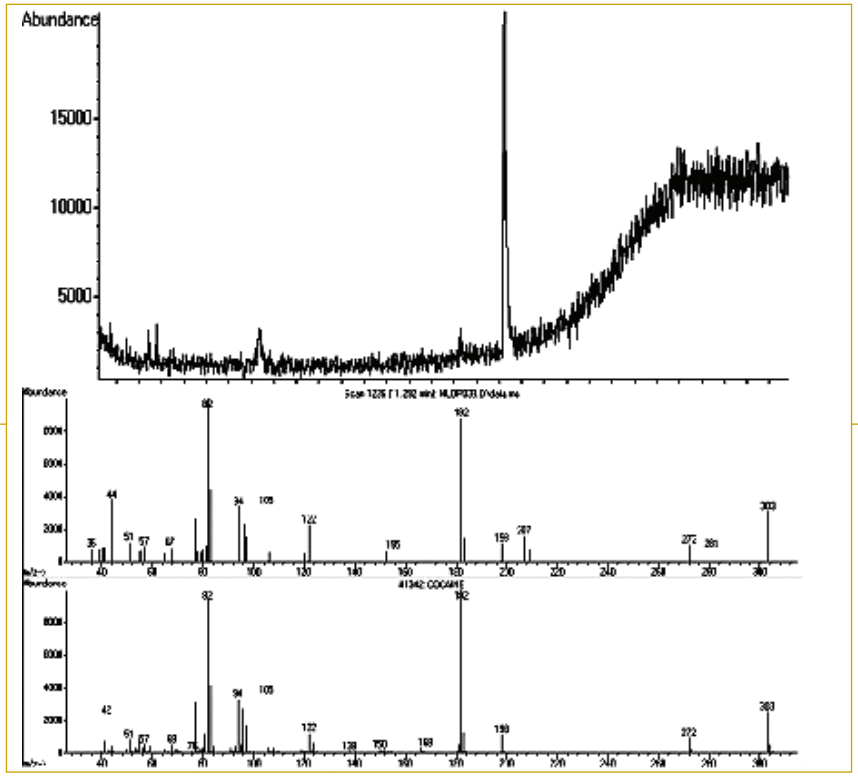
Abstract
Previous research has shown that cocaine contamination of bank notes is an international phenomenon. There are several hypothesized mechanisms by which cocaine is attracted to currency, based upon factors such as the fibers, pigments, and composition of the paper. Modern bank notes are made of several different substrates including polymers and coated papers. Acid-base extraction and GC-mass spectroscopy were used to extract and detect cocaine and other substances from Mexican 20 peso MXN (polymer) and Canadian $5 CAN (coated paper) bank notes taken from circulation.

Eighty percent of both polymer Mexican and coated paper Canadian bills tested positive for cocaine in agreement with results from testing paper US $1 bank notes. The results indicate that the bank note substrate is not the primary factor in determining cocaine attraction for currency. Dibutyl phthalate, a plasticizer, and (1-hydroxycyclohexyl)phenyl-methanone, a photoinitiator, were detected on all of the Canadian bills. Other interesting results for the Canadian bills include the presence of acetaminophen on 30%, caffeine on 35%, and nicotine on 80%.

Introduction
Stanford Angelos of the Chicago Office of the Drug Enforcement Administration (DEA) conducted one of the earliest studies of cocaine contamination on currency in the mid 1980s. He found that a third of randomly selected US bank notes were contaminated with cocaine and that the source of the contamination may have been currency sorting machines in use at Federal Reserve Banks. In 1989, the cocaine contamination of bank notes obtained from the Bank of Canada in Regina, Saskatchewan was compared to case samples seized from suspected drug dealers. The case sample contamination was 50 to 1000 times higher than the background contamination of 10 ng/note. Since then, cocaine contamination has been found on bank notes worldwide. The tendency for cocaine to adhere to bank notes has been attributed to the affinity of the cocaine crystals for the cotton fibers used in paper money, the inks used in the printing process, and the oils and grease residues left behind during handling.

The objective of this study was to use acid/base extraction to determine if the bank note substrate is the primary factor in the affinity of cocaine for currency. The contamination of paper bank notes, such as the US dollar, is well established. The notes tested were Canadian coated paper $5 bills and Mexican polymer 20 peso bills. At the time of these experiments, the 20 peso note was the only Mexican polymer bill in circulation. The remaining denominations were still paper. In addition, the results of testing Mexican polymer currency for the presence of cocaine is being reported for the first time.

Materials and Methods
Twenty circulated bills of each denomination ($5 CAN and 20 peso MXN) were obtained. The serial number, print year, and condition of each bill was recorded. Bills were crumpled, exposing as much of each face as possible, and placed in a 20mL vial. Then, 10mL of 0.1M HCl was added to the vials. Samples were placed on an orbital shaker overnight. The aqueous extract of each sample was analyzed using acid-base extraction. First, 0.5M NaOH was added dropwise until the pH of the solution was greater than 12. After 1mL of chloroform was added to each vial, they were agitated and allowed to sit for 30 minutes. This organic layer was then



MS results for cocaine on a 20 peso MXN bill.

Figure 1: This is consistent with previous experiments.

removed and injected into a Gas Chromatography/Mass Spectrometer (GC-MS) for analysis. In some cases, it was necessary to centrifuge the vial contents due to a large amount of precipitate in the solution.

Results and Discussion

MXN pesos:

Eighty percent of MXN pesos tested were found to be positive for cocaine. GC retention times were within ± 0.05 minutes of the standard, and the MS peaks of 82, 182, and 303 also matched the control and the NIST library record for cocaine. The retention time and mass spectra give a positive identification of cocaine.

CAN Dollars:

Analysis of Canadian $5 CAN bills resulted in the positive identification of cocaine on 80% of bills tested. In addition, several other peaks (Fig. 2) were analyzed and the mass spectra were indicative of nicotine on 80% of the bills, caffeine on 35%, and acetaminophen on 30%. Components of the coating on paper bills were also found on all the CAN samples. These were identified as dibutyl phthalate, a plasticizer, and (1-hydroxycyclohexyl)phenyl-methanone (HPM), a photoinitiator. Positive controls were not used to confirm the identification of nicotine, caffeine, acetaminophen, dibutyl phthalate, or HPM, but presumptive identifications were made by mass spectral analysis.

	MS Peaks: m/z	Retention time: min
Cocaine	82, 182, 303	8.515
Nicotine	84, 133, 161	6.420
Caffeine	194, 109, 55	9.078
Acetaminophen	109, 151, 80	5.622
Dibutyl phthalate	149, 76, 104	7.226
HPM	99,81,105	6.658

Table 1: Primary Mass Spectra Peaks and Retention Times

Another area of interest is the potential damage done to a bill by the acidic extraction. Microscopic observation showed only minor damage to the Mexican and Canadian bills, but it appeared that there was significant damage to a security element of the Canadian bills (Fig. 3).

The results of this study suggest that binding affinity of cocaine to currency is not related to bank note composition or coating. Further analysis of the Canadian bank notes demonstrated that it is also possible for substances other than narcotics to bind to the same substrates. However, it is necessary that further studies be carried out involving bank notes from more countries, using larger sample sizes. Additional studies must also be conducted to determine cocaine's affinity for binding to different substrates, as well as to understand what factors influence this binding.

Figure 2: GC analysis and product distribution of $5 CAN bills

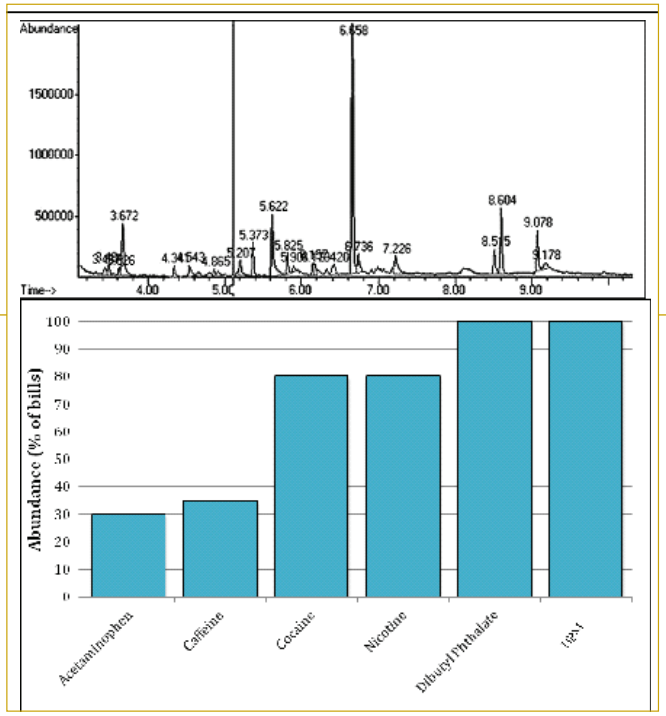
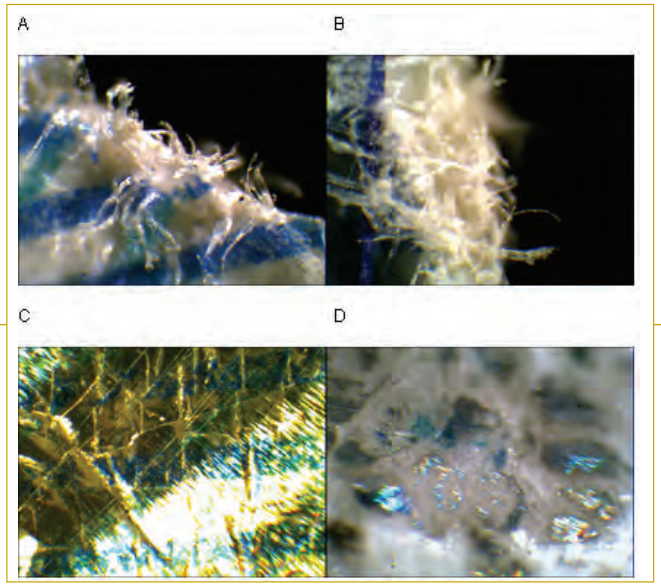


Figure 3: Microscopic observation of $5 CAN bill before and after acid-base extraction. (A-B) A torn corner before and after extraction. (C-D) A reflective security element before and after extraction.



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Characterization of calmodulin and Fas interactions in Fas-mediated death inducing signaling complex

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Abstract

Fas-mediated signaling pathway is an important mechanism for apoptosis in a variety of cells, including cholangiocarcinoma and other cancer cells. The formation of death inducing signaling complex (DISC) is a critical step for Fas-mediated signaling. Recent experimental studies showed that calmodulin (CAM) binds to Fas and regulates Fas-mediated (DISC) formation and the binding of (CAM) to Fas is inhibited by the (CAM) antagonist, trifluoperazine (TFP). The present study sought to characterize (CAM)/Fas binding affinity and the effects of Fas mutations and the effects of (CAM) antagonist, trifluoperazine (TFP), on (CAM)/Fas binding affinity. The results will be valuable to further elucidate the role of (CAM) in Fas-mediated death inducing signaling and provide a molecular mechanism for drug design of apoptosis mediators via (CAM) antagonism in the future. Isothermal Titration Calorimetry (ITC) binding experiments were used to quantify (CAM)-Fas binding. Recombinant (CAM) and Fas DD proteins were expressed in Escherichia coli cells and purified. Purified (CAM) and Fas were gel-filtered prior to ITC experiments. We have quantified the wildtype (CAM)/Fas binding with ITC experiments. We will further quantify the binding of (CAM)/Fas V254N mutant and the binding of (CAM)/Fas C-terminal deletion mutant to determine the effects of Fas mutations on (CAM)/Fas binding. The effects of (CAM) antagonist, TFP, on (CAM)/Fas binding will be also determined with ITC experiments. Results from these studies will facilitate the identification of novel strategies and drugs capable of effectively regulating Fas-mediated apoptosis in cancer cells.

Introduction

(CAM)

Calmodulin is a cytoplasmic protein with a high affinity for calcium ions (Ca^{2+}). (CAM) functions as an intracellular mediator of biological pathways and requires a certain concentration of intracellular calcium for signal transduction to occur. Higher expression of Ca^{2+} bound (CAM) has been associated with cancer [1, 2]; furthermore, the Ca^{2+}-(CAM) plays a key role in the propogation cholangiocarcinoma cells via inhibition of the Fas-Mediated apoptotic pathway [3]. Previous studies have shown that (CAM) binds to the Fas on its cytoplasmic death domain (DD) [10], specifically on residues 231-254 of the Fas [11]. Also it has been recently demonstrated that (CAM) is recruited into the (DISC) formation [2]. Computational modeling and simulations inspected the binding thermodynamics and conformation of the (CAM)-Fas complex, and provided evidence for the structural role of (CAM) and Fas binding in Fas induced (DISC) formation and ultimately Fas mediated apoptosis [5].

Fas

The Fas Receptor (Fas), alternatively known as APO-1/CD95, is a type-1 membrane protein categorized into tumor necrosis factor family [1]. The Fas is a key component in the Fas regulated apoptotic signaling pathway that regulates apoptosis in a variety of Fas expressing cells. Activation of the Fas occurs via the binding of the Fas ligand protein (FasL) or Fas agonistic antibodies [1].

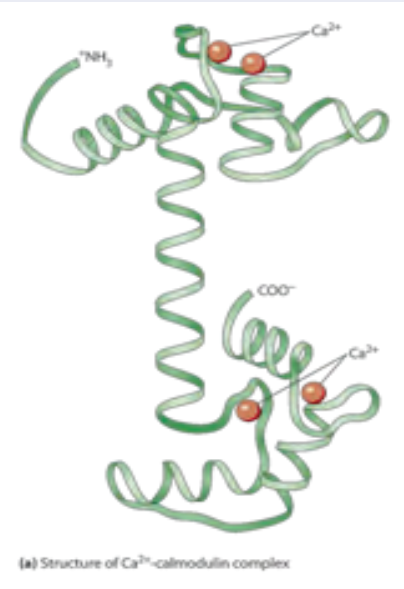


Figure 1: A structure of Calmodulin binding with four Ca2+ ions.

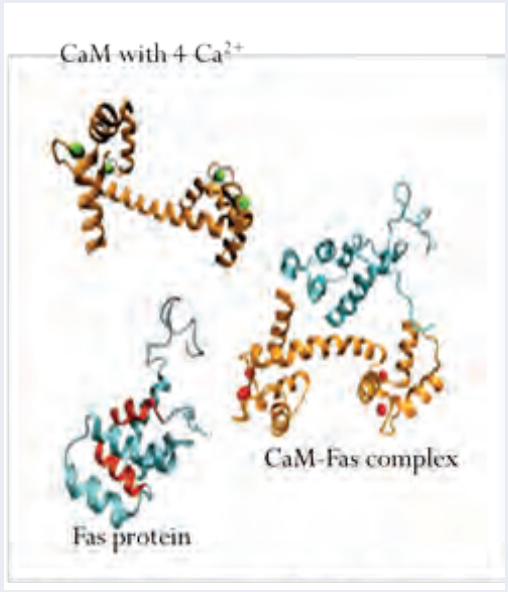


Figure 2: Molecular structures of CaM, Fas, and CaM-Fas complex generated via Visual Molecular Dynamic (VMD)

Upon activation, the Fas conveys the ligand signal by recruiting and activating the Fas-associated death domain protein (FADD), which induces the formation of the death-inducing signaling complex (DISC) composed of certain effector caspases and FLICE-like inhibitory protein (FLIP). These effector caspases activate pro-apoptotic proteins downstream in the pathway leading to apoptosis of the cell [2, 6]. Atypical expression or mutation of the Fas in cell types is associated with the propagation of a variety of diseases including lymph proliferative syndrome [5], and several types of cancers: breast cancer, hepatocellular carcinoma, and glioma [2].

Breast Cancer

A 2009 publication by the American Cancer Society ranked cancer as the second leading cause of mortality in the United States [7]. 15 percent of these cancer deaths were in females who had developed breast cancer [7]. These statistics make breast cancer treatment imperative to the improvement of patient survival rates. In April of 1997, the Federal Drug Administration approved the use of trifluoperazine (TFP), an organic phenothiazine, for cancer treatment [8]. The antipsychotic drug has been shown to inhibit the function of protein regulators in the Fas-associated death domain (FADD), and in vitro studies have revealed that continuous exposure to TFP inhibits the growth of breast cancer cells [9,10,11].

(CAM) and breast cancer

The primary protein that TFP affects is calmodulin (CAM), a small (Mr≈16700, ca.148 amino acids) acidic protein consisting of four calcium (Ca^{2+}) binding loops surrounded by α-helix structures [9]. When calcium levels reach a certain point, the three-dimensional structure of (CAM) opens up, allowing the protein to preferentially bind other molecules and activate cell signaling pathways [12]. One crucial point in Fas-mediated apoptosis is (CAM) binding to Fas ligand (FasL). Data from proton NMR studies and micro calorimetric titrations suggest that TFP induces structural changes to the Ca^{2+} bound (CAM) upon binding which could further affect (CAM) binding to other proteins [4, 7]. Affinity chromatography has been used to find the dissociation constant, Kd, of (CAM)/TFP binding to be between 4.5 and 5.8 [8]. Another group of researchers have found Kd to be 4.73 using back-scattering interferometry (BSI) [9]. Although different methods of experimentation have demonstrated that TFP affects (CAM)-Fas binding, no quantitative data characterizing the full binding kinetics of (CAM)-Fas/TFP binding has been reported.

Fas and breast cancer

In 1996, the National Cancer Institute (NCI) discovered that several breast cancer lines from original MCF-7 line are resistant to Fas-mediated apoptosis. Further research proved that Fas and its apoptotic pathway are down-regulated in breast cancer patients [10]. Current hypothesis suggest that tumor progression may be aided by decreased Fas sensitivity; therefore, modulation of Fas expression may be an approach to induce apoptosis in breast cancer cell [11].

Aims of the current study

This study aims to characterize (CAM)/Fas binding affinity and the effect of Fas mutations and the effect of (CAM) antagonist, trifluoperazine (TFP), on (CAM)/Fas binding affinity. It was hypothesized that (CAM) antagonist, TFP, would bind to (CAM) alone, and inhibit (CAM) binding to Fas in a TFP-concentration dependent manner. To test this hypothesis, this study will characterize (CAM)/Fas binding and the effect of TFP at different concentrations on (CAM)-Fas binding, in vitro, with Isothermal Titration Calorimetry (ITC) experiments.

The results of this study will lay the basis to clarify the cell signaling pathway by which TFP acts to inhibit malignant tumor growth. These findings will have a direct impact on cancer research, and specifically breast cancer mainly because knowledge about the pathway will allow modifications with other enzymes and in such create strong cell signals that will help pinpoint the exactly location of a cancer cell and thereby eradicate it using drug therapy. Understanding the molecular mechanisms of how TFP influences protein interactions will enable scientists to optimize the compound and design the new drug candidates so that the drug can act more effectual and side effects can be minimized. Future studies will characterize the effect of TFP on (CAM)-Fas binding in regulating the formation of the death-inducing signaling complex (DISC) to modulate apoptosis in breast cancer cells.

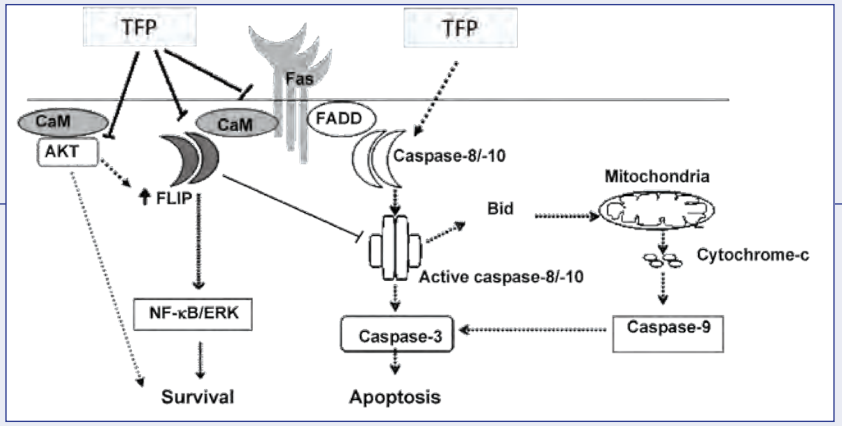


Figure 3: Hypothesized mechanism involved in CaM-Fas-TFP apoptotic pathway. This pathway can be used for survival as well as cell death.

Materials and Methods

Computational molecular simulation and biochemical techniques reported that (CAM) binds to residues 231-254 on Fas [11]. A recombinant protein His-Sumo-Fas 191-335 along with a recombinant His-Sumo-(CAM) protein will be used in ITC experiments. The molecular weight of 6XHis-Sumo-Fas (30621.71g/mol) and 6XHis-Sumo-Fas (30429.07 g/mol) were calculated using ApEA plasmid Editora Tcl/Tk script written by M. Wayne Davis Copyright 2003-2004.

Bacterial cell culture

Kanamycin and chloramphenicol-resistant Rosetta Escherichia coli cells line plasmids were transformed with the full human (CAM) protein sequence (pET28-His-SUMO-(CAM)) and the FADD-binding portion of Fas protein (pET28-His-SUMO-Fas 191-335-1). Separate 1 L bacterial cultures of 6XHis-SUMO-(CAM) and 6XHis-SUMO-Fas were inoculated with 20 mL starter cultures respectively, 1M kanamycin sulfate, and 0.3M chloramphenicol. Cultures were grown in suspension at 37°C. Once an optical density measured at 600nm reached approximately 0.6 units, 1mM isopropyl α-D-1-thioglactopyranoside (IPTG) was injected into cultures to begin overexpression of target protein. Induction lasted 3.5 hrs at 37°C after which cultures were incubated at 4°C overnight and spun down into cell pellets at 4200 rpm for 25min the following day.

Bacterial cell lysis, purification, and storage

Lysis buffer composed of 20mM Tris base, 10mM imidazole, and 300mM NaCl was adjusted to pH 8.0 with HCl and cell pellets were resuspended in 80 mL buffer per liter cultured. Cell suspensions were French pressed once at 1000 psi, 5000 psi, 10000 psi, and three times at 15000 psi. Cell lysate was centrifuged 30min at 13200 rpm. Supernatant containing soluble proteins was then filtered and purified the same day. Proteins carrying the 6XHis-SUMO tag were purified using nickel-nitriloacetic acid (Ni^{2+}-NTA) affinity chromatography and eluted into pH 8.0 buffer composed of 20mM Tris-HCl, 250mM imidazole, and 300mM NaCl. Elution purity was further increased by gel filtration, then quantified using a Pierce Bicinchoninic acid (BCA) assay, and concentrated. Concentrated protein samples stored for more than 1wk were flash frozen at -80°C and quickly thawed with a 20°C water bath when needed; however, samples used for ITC experiments within 1 week were kept at 4°C.

Protein tag cleavage

Elution samples used for without-tag experimental runs were dialyzed into pre-cleavage buffer following purification. Pre-cleavage buffer was composed of 20mM Tris-HCl, 10% glycerol, pH 8.0 and dialysis was performed at 4°C for 24 hrs. Buffer was changed at least twice to effectively remove imidazole. SUMO protease was added at 1unit/10μg target protein and incubated at 37°C for 1 hr in 20mM Tris-HCl, 1mM Tris(2-carboxyethyl)phosphine (TCEP), pH 8.0 (cleavage buffer). Protein was then re-purified on Ni^{2+}-NTA columns to remove tag pieces, subjected to gel filtration, and quantified using BCA.

Other methods and materials

Twelve percent sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to verify 1) protein expression after culture, 2) sufficient bacterial cell lysis after French press, and 3) protein purity after purification. Dihydrochloride trifluoperazine was purchased from Fisher Scientific. A MicroCal VP-ITC calorimeter was used in conjunction with MicroCal Origin data analysis software to perform ITC experiments.

ITC determines binding thermodynamics and kinetics

ITC plays an important role in finalizing the characterization behind the (CAM)-Fas binding by providing quantitative data in regards to thermodynamics and kinetics of the interactions. Furthermore, ITC has become a perfect choice in determining the binding energies of protein-protein and protein-ligand interactions. ITC is also capable of measuring the magnitude of the two thermodynamic components that contribute to binding affinity, enthalpy and entropy. The result of ITC is an Isothermal plot of Kcal/mole (injectant) vs molar ratio of injectant to macromolecule in the cell.

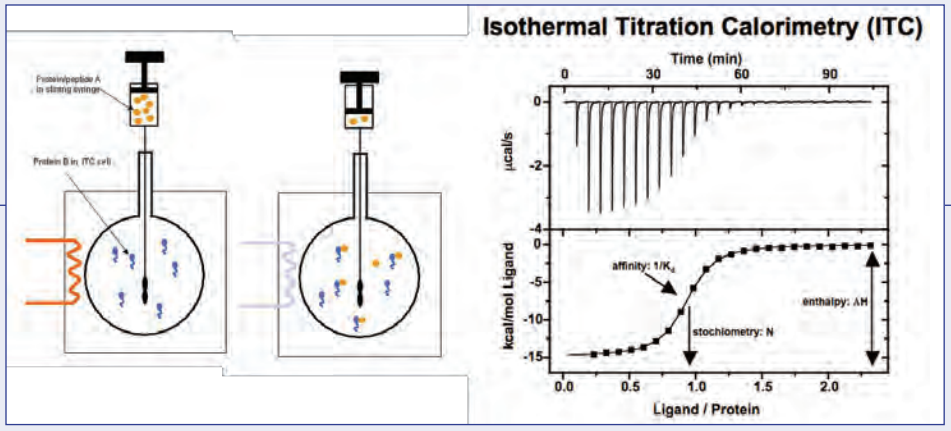


Figure 4: Isometric Calorimetry method and result format. Injection into ITC machine (left), data delivered from a standard ITC run right.

ITC Experimental controls

1. Controlled for the effect of the 6xHis-SUMO tag.

Protease was used to cleave the 6xHis-SUMO tag at the SUMO recognition site from 5 mg of (CAM) and 5 mg of Fas protein. These untagged samples underwent the same experimental conditions as tagged samples for the (CAM)/Fas and (CAM)/TFP binding reactions so that any possible effect of the tag on protein binding may be observed.

2. Establish positive and negative control for experimental cases:

a. Case #1: (CAM)/Fas binding
b. Case #2: (CAM)/TFP binding
c. Case #3: (CAM)-TFP/Fas binding

Positive Control: Because calcium binding has been shown to promote the active form of (CAM) protein, interactions between Ca^{2+}-saturated (CAM) and ligand in the absence of any drugs were used as the positive control in each of the three cases [7]. Protein interaction between (CAM) and ligand (Fas or TFP) in the absence of calcium and any drugs were used as the positive control. A solution of 100mM Ca^{2+} and 5mM (CAM) was used for this scenario.

Negative Control: The common chelating agent, ethylene glycol tetraacetic acid (EGTA), was used to strip calcium molecules from (CAM). A calcium-devoid scenario served as the negative control in all three cases. Calcium-devoid protein interaction served as the negative control in all three cases. A solution of 400mM EGTA and 5mM (CAM) was used for this scenario. These controls are important since calcium has been shown to increase the enthalpy of (CAM) reactions during ITC experiments [11].

3. Control for heat of dilution.

The ligand was inserted into the sample cell full of buffer without the presence of the macromolecule. The heat of dilution was found from this reaction. The heat of dilution is normally small and negligible, but it was checked anyways as a precaution. If heat of dilution is found to be signiticant, it is because the ligand dimerized or aggregated with itself. If it is found to be significant, it is because the ligand has dimerized or aggregated with itself. The heat of dilution should be subtracted from the heat of reaction found in experiments when ligand is injected into sample cell with macromolecule present.

4. Controlled for complex binding.

The backward and forward reaction kinetics were determined by simply reversing the ligand/macromolecule roles during ITC runs. For instance, in Case #1, (CAM) was put in the reference cell and Fas was injected into the cell as the ligand. Once the binding kinetics for that experiment took place, Fas was put in the reference cell and (CAM) was injected into the cell as the ligand. Unless complex binding is taking place, the backward and forward reaction kinetics should be the same.

ITC sample preparation

1. purified samples of (CAM) and Fas protein were separately eluted and then dialyzed against buffer consisting of(CAM) and Fas protein and then dialyze them against buffer consisting of 20mM Tris base, 1M NaCl, and 250mM imidazole. Samples of TFP were dissolved into the same buffer. Once all solutes were dissolved, each buffer solution was checked for a pH of 7 and subsequently equilibrated to pH 7 if needed.

2. The sample cell was adjusted to the desired temperature of 30°C to allow it to equilibrate while samples of macromolecule and ligand were individually stirred at medium speed and placed in the ThermoVac for 5-10 mins at 30°C.

3. Once bubbles were no longer visible in the samples, the knob on the ThermoVac (bleeder) was twisted to allow gas pressure to equilibrate and release the cap.

4. After cleaning reference cell and flushing the syringe with DI H_2O, the ligand buffer was positioned in the holder beneath the syringe on the outer ITC machine, and "Open Fill Port" was selected on the ITC software.

5. The syringe was filled with ligand, avoiding formation of air bubbles. "Close Fill Port" was selected.

6. The reference cell was slowly loaded with the macromolecule, ensuring that the cell was full and no bubbles had formed. The syringe was slowsly twisted to dislodge any bubbles that may have formed.

7. The run times parameters were set, "Run" was pressed, and the ITC machine was allowed to establish a baseline ΔH° and run.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Pre-induction as well as Post-induction samples were taking via SDS-PAGE.

ITC runtime parameters

Table 1 shows a list of runtime experimental and injection parameters to be held constant at all times.

Table 1: ITC constants

Experimental Parameters		Injection Parameters	
Total # injections	10	Volume (µL)	8
Cell temperature (°C)	30	Duration (sec)	13.7
Reference power (µCal/sec)	15	Spacing (sec)	150
Initial delay (sec)	200	Filter period (sec)	2
Stirring speed	260		

Purification

The (CAM) and Fas proteins were purified using Ni^{2+}-NTA (nickel nitriloacetic acid) affinity chromatography. In the first step, all proteins that were not 6xHis-tagged flowed through column while proteins with the tag bound to resin. In the second step, the bound proteins were washed to eliminate inclusion body remnants and other unwanted materials. Finally, elution exposed that bound proteins are unbound from resin with high concentrations of imidazole and collected for dialysis into appropriate ITC buffer.

Preliminary Results

Recent ITC trials were ran on (CAM)-Fas and (CAM)-TFP binding. Although validation of the binding mechanism is still being studied, the results of the ITC trials that were run on (CAM)-Fas and (CAM)-TFP binding validate that validates that 1) there is determinant binding between (CAM) and Fas, and 2) there are multiple binding points in the (CAM)-TFP complex, as seen in figures 8 and 9 respectively.

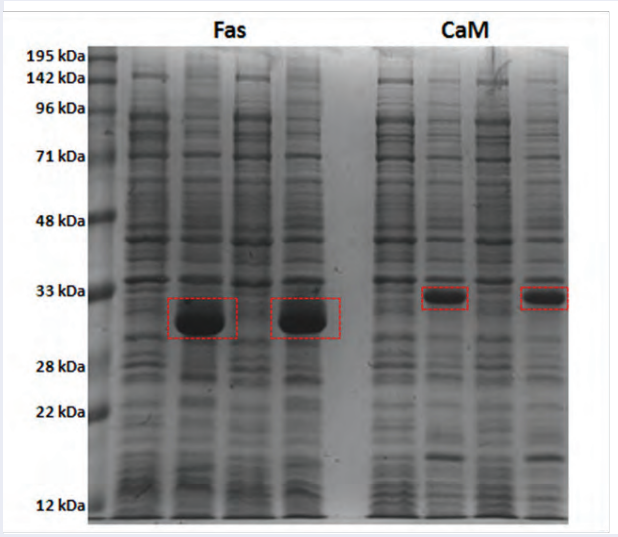


Figure 5: SDS-PAGE results of samples from 1L bacterial cultures of Fas and CaM protein, pre and post induction (2.1.11).

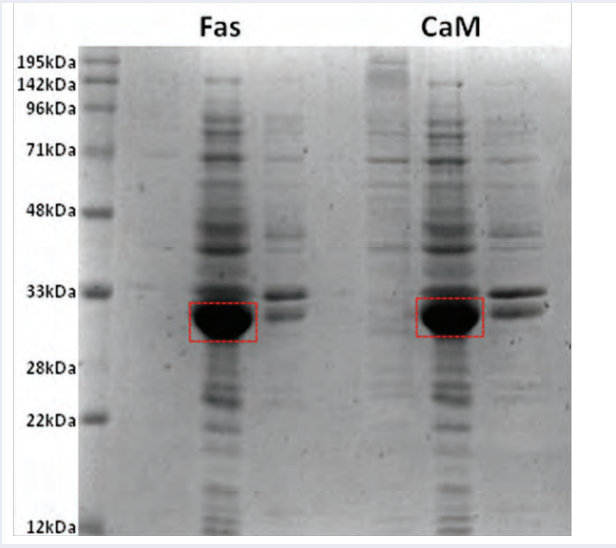


Figure 6: SDS-PAGE results of samples from 1L bacterial cultures of Fas and CaM protein, pre and post cell lysis.

Discussion

While still preliminary, the results from this ITC study will contribute to the current literature on (CAM)/Fas binding and the effect of TFP on (CAM)/Fas binding by shedding light onto the following parameters: 1) the number of binding sites for ligand to macromolecule, n, 2)the change in heat capacity, ΔCp, 3) the standard change of enthalpy, ΔH°, 4) the standard change of free energy, ΔG°, 4) the standard change in entropy, ΔS°, and 5) the number of binding sites for ligand to macromolecule, n. Because most biological reactions are exothermic and follow the rules of thermodynamics, a negative change in enthalpy is expected along with a positive change in entropy in each of the binding cases.

Possible sources of error

If the stoichiometric relationship between the (CAM)-Fas macromolecule and TFP ligand is not indicative of at least a 1:1 binding ratio (i.e., no binding occurs), then four possible sources of error should be considered: 1) All the ligand binding sites on (CAM) are not identical and independent, 2) the expressed protein is not thoroughly pure, 3) the calculated protein concentrations are not correct, and 4) all of the protein used was not properly folded and active.

Other potential sources of error stem from the use of bacteria to express human genetic material encoding for the proteins, Fas and (CAM), discussed in this study. It must be noted that human expression of these proteins may differ from bacterial expression resulting in alterations in protein conformation, configuration, and function. No true or quantitative testing of protein purity, activity, or functionality was performed. It should also be noted that only the FADD-binding portion of Fas protein (res191-335) was used to complete this study; therefore, the binding effects of (CAM) and full, trimerized Fas protein have yet to be quantified and could differ from the effects found in the present study.

Conclusion

The goal of this research was to characterize (CAM)/Fas binding affinity and the effect of Fas mutations and the effect of (CAM) antagonist, trifluoperazine (TFP), on (CAM)/Fas binding affinity. This project shows an in vitro representation of how well the proteins bind and dissociate under a range of given conditions from a thermodynamic perspective. The results from this study could help to understand Fas and (CAM) binding in Fas-mediated death inducing signaling complex formation and potentially useful for developing the novel strategy or novel drug candidates for the chemotherapy of breast cancer. The next step of the research will be to perform biological studies that focused on the cellular level to determine how the protein binding dynamics shown in this study affect breast cancer cells.

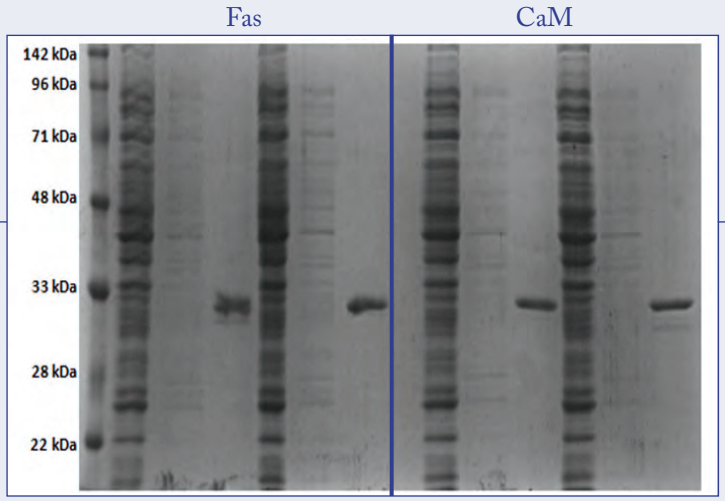
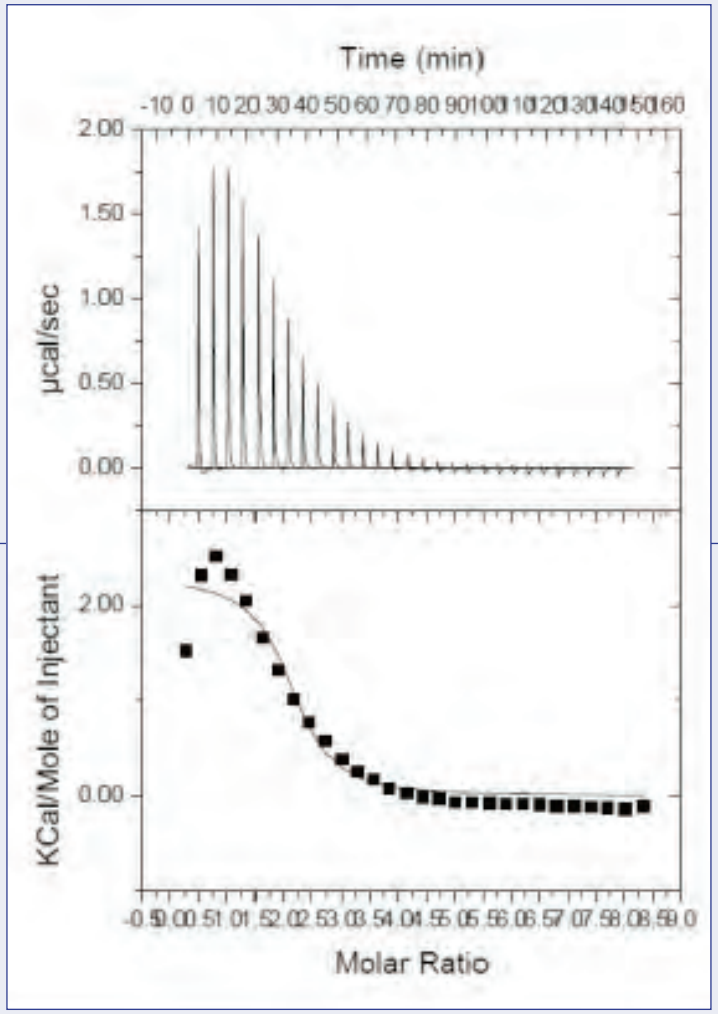


Figure 7: SDS-PAGE results of steps of affinity chromatogrphy protein purification for Fas and CaM (2.10.11).

n=2 for each protein purification's 3 basic purification steps shown above.



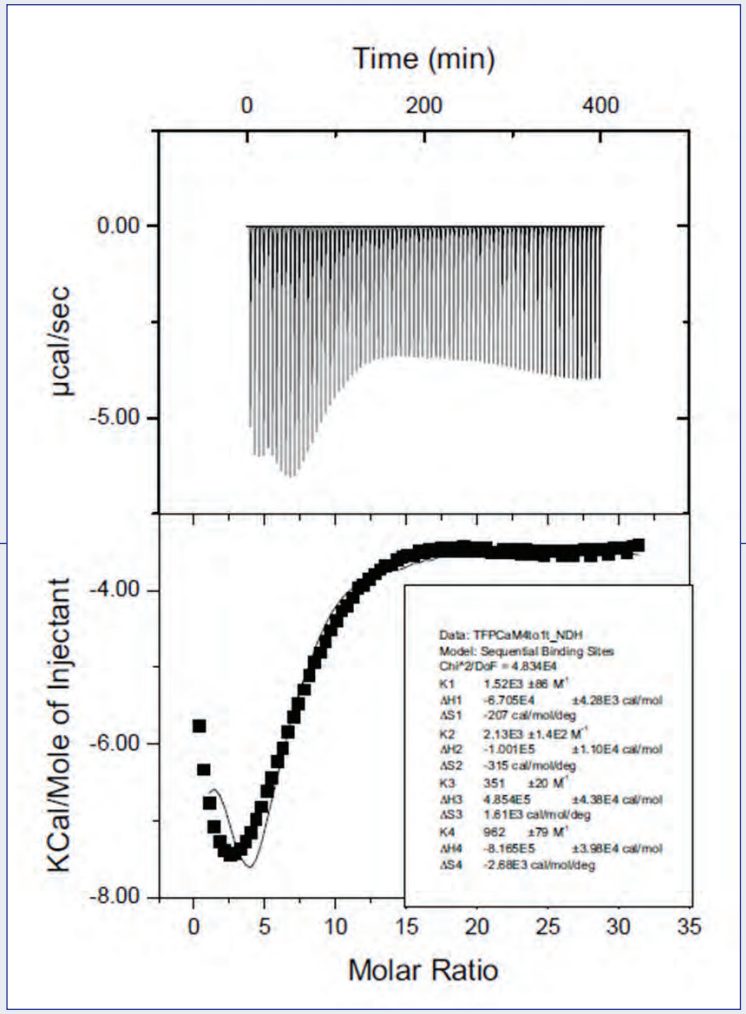
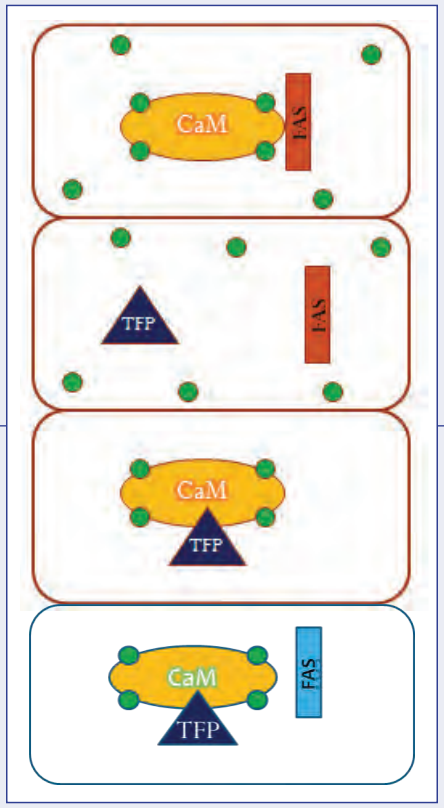


Figure 8: CaM-Fas ITC binding result.

Figure 10: CaM-Fas-TFP binding mechanism. Ca2+ binds to CaM to stimulate CaM-Fas complex (first from top), TFP does not bind directly with Fas (second from top), Ca2+ bind to CaM to stimulate CaM-TFP complex (third from top), but TFP inhibits CaM from binding to Fas.



Acknowledgements

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Structure Based Design of Inhibitors of *Trypanosoma cruzi* DHFR as Potential Therapeutic Agents for Chagas' Disease

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Abstract

Dihydrofolate reductase (DHFR) enzyme of the parasite *Trypanosoma cruzi* is a potential target for developing drugs for the treatment of Chagas' disease. We report here the rational design, synthesis, characterization and biological evaluation of four potent inhibitors of this parasitic enzyme. Inhibitory activity of each compound is determined against *T. cruzi* as well as against human DHFR for comparison. Potent nanomolar inhibitors of *T. cruzi* DHFR have been synthesized. Moderate increase in Selectivity Index (SI) has been achieved for some of these inhibitors.

Introduction

Chagas' disease is caused by the protozoan parasite *Trypanosoma cruzi*, which is primarily transmitted by an insect vector. This disease is also transmitted via blood transfusion, organ transplant and genetically from mother to child. The disease affects millions of people in Latin America where the vector and the parasite are endemic.[1] There are two stages for this disease: acute and chronic. There is no effective drug for the treatment of the chronic stage of this disease. Only two drugs are available that to treat the acute stage (Nifurtimox and Benznidazole). However, these two drugs result in toxic side effects. With more than 100 million people in 20 countries at risk and no effective treatments available, there is an immediate need for new drugs to treat this disease. Therefore, validating potential drug targets and identifying novel drug candidates to treat Chagas' disease are needs of considerable global importance. [2-5]

Since the dihydrofolate reductase (*Tc*DHFR) activity of *T. cruzi* is essential for the life of parasite, it represents a potential target for rational drug design. DHFR has a proven track record as a drug target in cancer chemotherapy. More importantly, DHFR inhibitors are successfully used in the treatment of bacterial and parasitic infections.[2-5] Senkovich et al showed that trimetrexate (TMQ, Fig.1) inhibits *Tc*DHFR and kills the growth of *T. cruzi* amastigote and trypomastigote in culture.[6] However, TMQ is a potent inhibitor of human DHFR and is therefore toxic to the cell. It is important to develop inhibitors that are selective to *Tc*DHFR. Even though reports of such selective inhibitors for *Tc*DHFR are limited,[7] recent drug discovery efforts that integrate structural biology with computational and medicinal chemistry reiterate the promise of this enzyme as a novel target for developing drugs against Chagas' disease.[8-10]

We have used structure based drug design strategies to facilitate the rational design of a selective inhibitor of the *Tc*DHFR. As part of this study we previously characterized the structure of the biologically relevant bifunctional form of the parasitic enzyme, dihydrofolate reductase-thymidylate synthase (*Tc*DHFR-TS), and determined the structure of the enzyme with TMQ bound to the DHFR active site.[11] The potent activity of TMQ against the parasite combined with previous studies indicating antiparasitic activity of 2,4-diaminoquinazoline antifolates emphasizes the importance of further structure-activity studies on this class of compounds. So, our initial SAR studies focused on making TMQ analogs as inhibitors of *Tc*DHFR. We have synthesized and evaluated several analogs of TMQ, resulting in the identification of several potent inhibitors of *Tc*DHFR. One such potent inhibitor of *Tc*DHFR is compound 1 (Fig. 1).

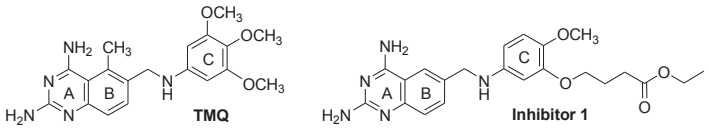


Figure 1: Structures of TMQ and Inhibitor 1

To aid in the SAR studies and further improve the activity and selectivity of inhibitor 1, we have determined the X-ray crystal structure of the enzyme/inhibitor complex using inhibitor 1.[10] The bifunctional *Tc*DHFR-TS enzyme was used in crystallization. The structure of this complex was refined to a resolution of 2.5Å (fig. 2).[10]

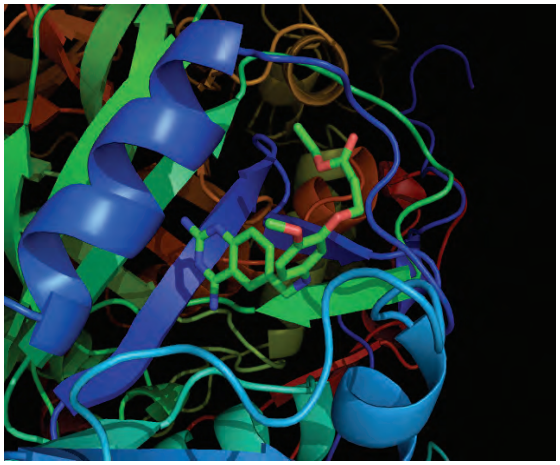


Figure 2: X-ray crystal structure of inhibitor 1 bound to TcDHFR-TS.

Results and Discussion

Analysis of the crystal structure of the enzyme/inhibitor 1 complex clearly shows how inhibitor 1 is bound in the active site. This also has given us insight into the nearby amino acid residues in the active site of *Tc*DHFR around the molecule and the space in to which the structure of the molecule can be expanded to improve binding and selectivity. A comparison of this crystal structure with the crystal structure of *h*DHFR revealed the presence of five amino acid residues (Gly20, Asp21, Phe31, Gln35 and Asn64) around the inhibitor in the active site that are unique to *Tc*DHFR as compared to *h*DHFR. A list of those residues along with corresponding residues in *h*DHFR is given in Table 1 and shown in Fig.3.

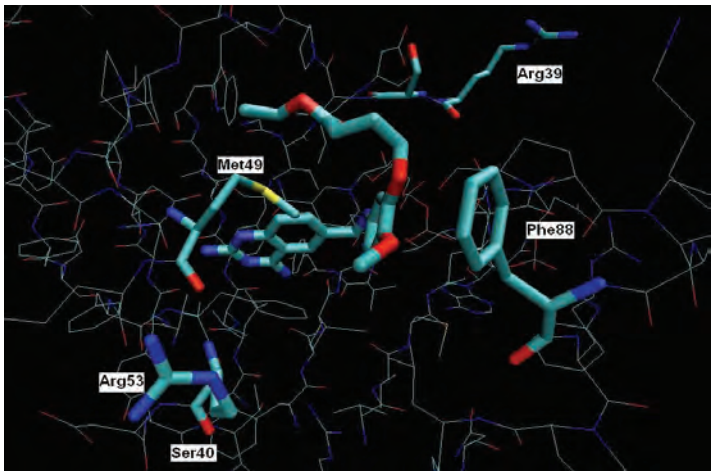


Figure 3: Inhibitor 1 bound in TcDHFR active site. Differences in residues in the DHFR active sites of trypanosomal and human DHFR are labeled.

We hypothesize that the structural modifications of the lead inhibitor 1 targeting these five specific residues in the *Tc*DHFR active site will lead to improvement in selectivity of the inhibitor towards *Tc*DHFR. Specifically, in this manuscript we targeted the residue Phe88, which is near the ethyl butanoate side chain present on the phenyl ring C of inhibitor 1. This side chain is pointed towards the solvent space in the crystal structure and does not have any important interaction with the enzyme in the active site. The side chain substituent is in close proximity to Phe88 on ring C. It is well known that the phenyl ring on phenyl alanine has the ability to stack with the phenyl rings of the inhibitors. Based on this rationale, we propose that the substitution of ethyl butanoate side chain on the ring C oxygen atom with other groups containing phenyl rings might result in stacking interactions with Phe88 leading to better selectivity for *Tc*DHFR. With this objective the following four target compounds (2a-d, Fig. 4) are proposed for synthesis and biological evaluation.

In order to position the phenyl group on the inhibitor at an appropriate distance from Phe88 to maximize the stacking interaction, we proposed to vary the length of attachment of the phenyl group to the oxygen atom on ring C with one carbon (2a), two carbon (2b) and three carbon (2c) linkers. In addition, a target compound with two phenyl groups (2d) has also been proposed. The goal in this case is to maximize the chance of stacking interaction with Phe88. All four target compounds were synthesized and evaluated for their inhibitory activity against *Tc*DHFR and *h*DHFR.

Synthesis of target compounds (2a-d) is outlined in Scheme 1. Treatment of commercially available 2-methoxy-5-nitrophenol (3) with various alkyl halides (R-Br) in the presence of K_2CO_3 in anhydrous DMF afforded the compounds 4a-d in 60-88 % yields. Reduction of nitro groups present in compounds 4a-d using either Pd/C and H_2 (compounds 4b-d) or Sn/HCl (compound 4a) resulted in the formation of amino compounds 5a-d in 70-100 % yield. We had to use Sn/HCl for the reduction of compound 4a as the attempted reduction of this compound using Pd/C and H_2 resulted in the debenzylation of the compound. Reductive amination of 2-fluoro-5-formylbenzonitrile (6) using anilines 5a-d in the presence of $NaCNBH_3$ and $ZnCl_2$ in anhydrous MeOH afforded the compounds 7a-d in 50-76 % yield. Refluxing of compounds 7a-d with guanidine carbonate in N,N-Dimethyl acetamide resulted in the formation of target compounds 2a-d in rather lower yields of 15-62 %.

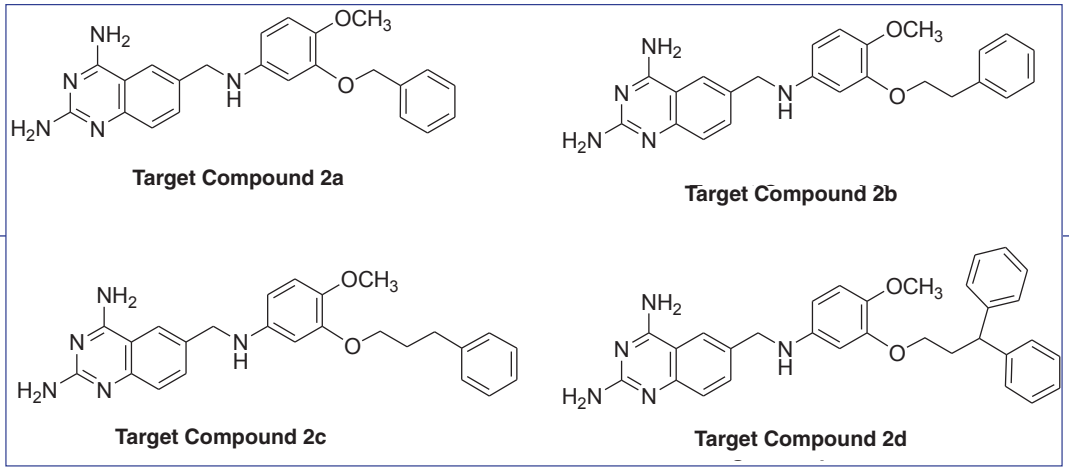
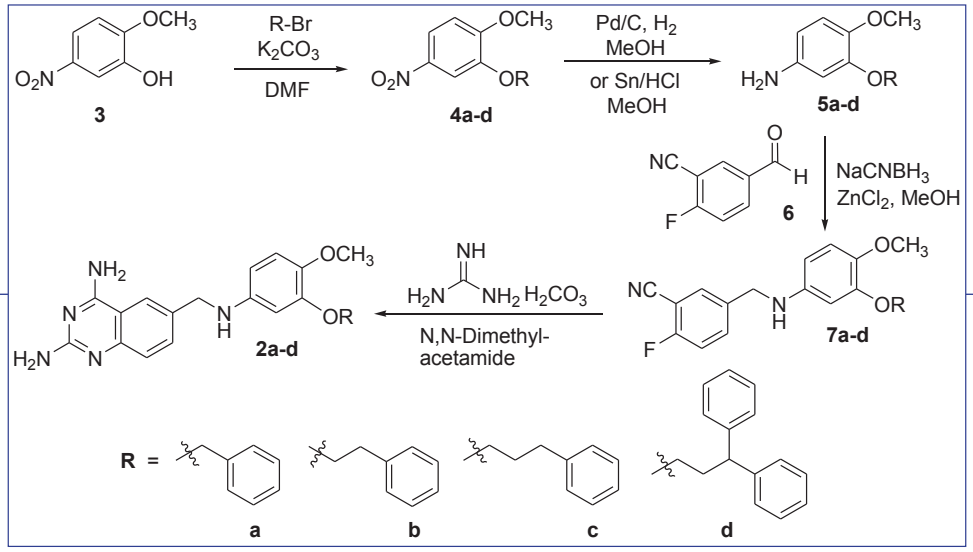


Figure 4: Target compounds 2a-d.



Scheme 1: Synthesis of target compounds 2a–d.

All four final compounds along with TMQ and inhibitor 1 were evaluated for their inhibitory activity against *Tc*DHFR and hDHFR. Recombinant bifunctional enzyme (*Tc*DHFR–TS) was used throughout this study. Inhibitory activity of the reported compounds was measured in a Spectrophotometric assay using the bifunctional enzyme as described previously.[6, 10] Inhibitory activities against *Tc*DHFR and hDHFR are summarized in Table 2. All compounds were found to be potent inhibitors of *Tc*DHFR with IC50 values in nanomolar range. Compounds also showed inhibition of *h*DHFR with the selectivity indices ranging from 2.4 to 17.8. Compound 2a showed a moderate increase in SI (17.8) as compared to TMQ (SI = 3.9) and our earlier lead compound 1 (SI = 2.4). The SI for other derivatives ranged from 2.8 to 4.7.

Conclusions

We have rationally designed and synthesized four inhibitors of *Tc*DHFR. New synthetic methods were developed for making these target compounds. All new compounds and the intermediates were characterized by ^{1}H-NMR, ^{13}C-NMR and mass spectrometry. The synthesized molecules are potent inhibitors of *Tc*DHFR activity. However, these compounds also showed inhibitory activity against the human enzyme. One of the introduced chemical modifications (benzyl group, 2a) resulted in a moderate increase in SI (17.8) as compared to original lead compound (SI = 2.4). Further work to improve the SI of the current lead compound is in progress.

Experimental

General Methods for Synthesis: Solvent evaporations were carried out *in vacuo* with rotary evaporator. Thin layer chromatography (TLC) was performed on silica gel plates with fluorescent indicator (Whatmann, silica gel, UV254, 25 µm plates). Spots were visualized by UV light (254 and 365 nm). Purification by column and flash chromatography was carried out using 'BAKER' silica gel (40 µm) in the solvent systems indicated. The amount (weight) of silica gel for column chromatography was in the range of 50-100 times the amount (weight) of the crude compounds

*h*DHFR	*Tc*DHFR
Gly20	Arg39
Asp21	Ser40
Phe31	Met49
Gln35	Arg53
Asn64	Phe88

Table 1: Differences in residues in the DHFR active sites of trypanosomal and human DHFR

Compound	IC50 (nM)		Selectivity Index
	*Tc*DHFR	*h*DHFR	
TMQ	20.4	80.9	3.9
1	20.5	50.3	2.4
2a	14.7	262.3	17.8
2b	80.7	375.3	4.7
2c	80.8	344.6	4.3
2d	37.5	106.6	2.8

Table 2: Inhibitory activity of TMQ, inhibitor 1 and compounds 2a-d against TcDHFR and hDHFR

being separated. Proton nuclear magnetic resonance (^{1}H-NMR) and carbon nuclear magnetic resonance (^{13}C-NMR) spectra were recorded on a Bruker Avance DPX-300 spectrometer using TMS as internal standard. The values of chemical shifts (δ) are given in ppm and coupling constants (J) in Hz. Mass spectra were recorded on Micromass Platform LCC instrument. Anhydrous solvents used for reactions were purchased in Sure-Seal™ bottles from Aldrich Chemical Company. Other reagents were purchased from Aldrich or Fisher chemical companies and used as received.

General procedure for the preparation of 4a-d:
To a solution of 2-methoxy-5-nitrophenol, 3 (6 mmol) in anhydrous DMF (10 mL), K_2CO_3 (18 mmol) was added and stirred at room temperature for 15 minutes. Alkyl bromide (6 mmol) was added drop-wise to the reaction mixture and stirred at room temperature for 3 hours. After the completion of the reaction (as indicated by TLC, NH_3 saturated $CHCl_3$), the reaction mixture was diluted with water (100 mL) and extracted with EtOAc (3 × 50 mL) and brine (1 × 50 mL). The EtOAc layer was then dried over Na_2SO_4. After the removal of the drying agent, the solvent was concentrated to obtain the compounds 4a-d.

1-((2-methoxy-5-nitrophenoxy)methyl)benzene (4a):
(88%); mp. 94°C; ^{1}H NMR ($CDCl_3$) δ 3.98 (s, 3H), 5.20 (s, 2H), 6.93 (d, 1H, J = 9.0 Hz), 7.34-7.49 (m, 5H), 7.81 (d, 1H, J = 2.4 Hz), 7.92 (dd, 1H, J_1 = 2.6 Hz, J_2 = 8.8 Hz); ^{13}C NMR ($CDCl_3$) δ 56.4, 71.2, 108.6, 110.2, 127.6, 128.4, 128.7, 135.7, 141.3, 141.9, and 155.1; MS (ES+): m/z = 260 [M+H].

1-(2-(2-methoxy-5-nitrophenoxy)ethyl)benzene (4b):
(60%); mp. 97°C; ^{1}H NMR ($CDCl_3$) δ 3.20 (t, 2H, J = 7.3 Hz), 3.97 (s, 3H), 4.28 (t, 2H, J = 7.3 Hz), 6.91 (d, 1H, J = 9.0 Hz), 7.24-7.37 (m, 5H), 7.92 (d, 1H, J = 2.7 Hz), 7.91(dd, 1H, J_1 = 2.7 Hz, J_2 = 9.0 Hz); ^{13}C NMR ($CDCl_3$) δ 35.5, 56.5, 70.1, 107.9, 110.2, 117.9, 126.8, 128.6, 129.1, 137.4, 141.4, 148.1, 154.9; MS (ES+): m/z = 274 [M+H].

1-(3-(2-methoxy-5-nitrophenoxy)propyl)benzene (4c):
(71%); mp 55°C; ^{1}H NMR ($CDCl_3$) δ 2.18-2.25 (m, 2H), 2.84 (t, 2H, J = 7.5 Hz), 3.97 (s, 3H), 4.08 (t, 2H, J = 6.5 Hz), 6.91 (d, 1H, J = 8.8 Hz), 7.19-7.32 (m, 5H), 7.70 (d, 1H, J = 2.7 Hz), 7.90 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.7 Hz); ^{13}C NMR ($CDCl_3$) δ 30.7, 32.4, 56.8, 68.7, 108.1, 110.4, 118.2, 126.5, 128.9, 128.9, 141.4, 141.8, 148.7, 155.3; MS (ES+): m/z = 288 [M+H].

3-(2-methoxy-5-nitrophenoxy)-1,1-diphenylpropane (4d):
(95%); mp 104°C; ^{1}H NMR ($CDCl_3$) δ 2.60-2.66 (m, 2H), 3.97(s, 3H), 4.01 (t, 2H, J = 6.7 Hz), 4.24 (t, 1H, J = 7.8 Hz), 6.89 (d, 1H, J = 8.9 Hz), 7.18-7.32 (m, 5H), 7.59 (d, 1H, J = 2.5 Hz), 7.88(dd, 1H, J_1 = 2.5 Hz, J_2 = 8.9 Hz); ^{13}C NMR ($CDCl_3$) δ 34.9, 47.6, 56.8, 67.9, 108.1, 110.4, 118.2, 126.9, 128.2, 129.1, 141.7, 144.3, 148.5, 155.3; MS (ES+): m/z = 364 [M+H]

3-(benzyloxy)-4-methoxybenzenamine (5a):
To a stirred solution of compound **4a** (603 mg, 2.33 mmol) in anhydrous MeOH (20 mL), Sn (733 mg, 3.1 mmol) was added, followed by concentrated HCl (8 mL). The reaction mixture was refluxed for 15 minutes. After the completion of the reaction (as indicated by TLC, 50% EtOAc in hexanes), the solvent was removed *in vaccuo*. The residue obtained was then dissolved in $CHCl_3$ (200 mL) and washed with 1M $NaHCO_3$ (2 × 150 mL) and brine (1 × 100 mL). The organic layer was dried over Na_2SO_4.

The drying agent was filtered off and the solvent was removed *in vaccuo* to afford the compound **5a** (480 mg, 70%); ^{1}H-NMR (CDCl3) δ 3.37 (bs, 2H), 3.80 (s, 3H), 5.09 (s, 2H), 6.22 (dd, 1H, J_1 = 2.4 Hz, J_2 = 8.4 Hz), 6.30 (d, 1H, J = 2.7 Hz), 6.72 (d, 1H, J = 8.4 Hz), 7.24-7.43 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 57.1, 71.0, 103.3, 107.3, 114.2, 127.3, 127.8, 128.6, 137.3, 140.7, 142.9, and 149.3; MS (ES+): m/z = 230 [M+H].

General procedure for the preparation of compounds 5b-d:
To a solution of compound **4b-d** (200 mg, 0.83 mmol) in EtOAc (25 mL), 10% Pd/C was added (88 mg, 0.083 mmol). The reaction was stirred at room temperature under H_2 atmosphere (from a balloon) for 12 hours. After the completion of the reaction (as indicated by TLC, 50% EtOAc in hexanes), the reaction mixture was filtered through celite. Celite was washed with EtOAc and the combined EtOAc filtrate was concentrated *in vaccuo* to obtain the compounds **5b-d**.

4-methoxy-3-(phenethyloxy)benzenamine (5b):
(76%); ^{1}H NMR ($CDCl_3$) δ 3.10 (t, 2H, J = 7.5 Hz), 3.35 (bs, 2H), 3.71 (s, 3H), 4.11 (t, 2H, J = 7.5 Hz), 6.15 (d, 1H, J = 8.4 Hz), 6.22 (s, 1H), 6.67 (s, 1H, J = 8.4 Hz), 7.18-7.30 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 36.3, 57.5, 70.1, 102.8, 107.3, 114.8, 127.0, 129.0, 129.6, 138.6, 141.5, 142.8, and 149.7.

3-(3-phenylpropoxy)-4-methoxybenzenamine (5c):
(100%); ^{1}H NMR ($CDCl_3$) δ 2.14-2.24 (m, 2H), 2.86 (t, 2H, J = 7.5 Hz), 3.45 (bs, 2H), 3.84 (s, 3H), 3.99 (t, 2H, J = 6.6 Hz), 6.21-6.28 (m, 2H), 6.76 (d, 1H, J = 8.4 Hz), 7.21-7.36 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 30.7, 32.1, 57.1, 67.9, 102.5, 106.8, 114.3, 125.9, 128.4, 128.5, 140.8, 141.6, 142.7, 149.6; MS (ES+): m/z = 257 [M+H].

3-(3,3-diphenylpropoxy)-4-methoxybenzenamine (5d):
(100%); ^{1}H NMR ($CDCl_3$) δ 2.52-2.60 (m, 2H), 3.34 (bs, 2H), 3.75 (s, 3H), 3.85 (t, 2H, J = 6.7 Hz), 4.19 (t, 1H, J = 7.8 Hz), 6.07 (s, 1H), 6.15 (d, 1H, J = 8.4 Hz), 7.12-7.25 (m, 10H), ; ^{13}C NMR (CDCl3) δ 34.8, 47.4, 57.1, 66.9, 102.4, 106.8, 114.2, 126.4, 128.0, 128.6, 140.8, 142.6, 144.4, and 149.4.

General procedure for the preparation of 7a-d:
To a solution of 2-fluoro-5-formylbenzonitrile, **6** (1.31 mmol) in anhydrous MeOH (5 mL), compound **5a-d** (1.31 mmol) was added and stirred at room temperature for 15 minutes. A solution of $NaCNBH_3$ (1.44 mmol) and $ZnCl_2$ (0.65 mmol) in MeOH (5 mL) was added dropwise to the reaction mixture and stirred at room temperature for 12 hours. After the completion of the reaction (as indicated by TLC, 30% EtOAc in hexanes), the solvent was removed *in vaccuo*. The residue obtained was dissolved in EtOAc (60 mL), washed with water (2 × 30 mL) then brine (1 × 30 mL) and dried over Na_2SO_4. The drying agent was filtered off and the solvent was removed *in vaccuo* to obtain the crude product, which was purified by flash chromatography on silica gel (12 × 1 in) using $CHCl_3$ as eluent.

5-((3-(benzyloxy)-4-methoxyphenylamino)methyl)-2-fluorobenzonitrile (7a):
(76%); ^{1}H NMR ($CDCl_3$) δ 3.81 (s, 3H), 4.21 (s, 2H), 5.08 (s, 2H), 6.09 (dd, 1H, J_1 = 2.6 Hz, J_2 = 8.5 Hz), 6.75 (d, 1H, J = 8.5 Hz), 7.13 (t, 1H, J = 8.5 Hz), 7.28-7.37 (m, 5H), 7.49-7.55 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 47.5, 57.0, 71.0, 101.4, 101.6 (C–F coupling), 104.8, 114.0, 114.2, 116.4, 116.7, 127.0, 127.8, 128.5, 131.8, 133.6 and 133.7 (C–F coupling), 136.9 and 137.0 (C–F coupling), 137.1, 142.0 and 142.5 (C–F coupling), 149.3, 160.4 and 163.9 (C–F coupling); MS (ES+): m/z = 363 [M+H].

5-((4-methoxy-3-(phenethyloxy)phenylamino)methyl)-2-fluorobenzonitrile (7b):
(71%); ^{1}H NMR ($CDCl_3$) δ 3.12 (t, 2H, J = 7.5 Hz), 3.81 (s, 3H), 4.13 (t, 2H, J = 7.5 Hz), 4.26 (s, 2H), 6.06 (dd, 1H, J_1 = 2.7 Hz, J_2 = 8.5 Hz), 6.20 (d, 1H, J = 2.7 Hz), 6.72 (d, 1H, J = 8.5 Hz), 7.15 (t, 1H, J = 8.5 Hz), 7.21-7.32 (m, 5H), 7.55-7.60 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 35.8, 47.6, 57.1, 69.9, 101.1, 101.4 and 101.6 (C–F coupling), 104.4, 114.0, 114.5, 116.4, 116.7, 126.5, 128.5, 129.0,131.9, 133.7 and 133.8 (C–F coupling), 137.0 and 137.1 (C–F coupling), 138.1, 142.2 and 142.6 (C–F coupling), 149.6, 160.5 and 164.0 (C–F coupling).

5-((3-(3-phenylpropoxy)-4-methoxyphenylamino)methyl)-2-fluorobenzonitrile (7c):
(50%); ^{1}H NMR ($CDCl_3$) δ 2.12-2.20 (m, 2H), 2.82 (t, 2H, J = 7.5 Hz), 3.81 (s, 3H), 3.96 (t, 2H, J = 6.4 Hz), 4.29 (s, 2H), 6.07 (dd, 1H, J_1 = 2.4 Hz, J_2 = 8.4 Hz), 6.19 (d, 1H, J = 2.4 Hz), 6.75 (d, 1H, J = 8.4 Hz), 7.15-7.33 (m, 6H), 7.57-7.62 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 30.7, 32.1, 47.7, 57.1, 68.0, 101.1, 101.5 and 101.7 (C–F coupling), 104.1, 114.0, 114.4, 116.5, 116.7, 126.0, 128.5, 128.6, 132.0, 133.8 and 133.9 (C–F coupling), 137.1 (C–F coupling), 141.5, 142.2 and 142.7(C–F coupling), 149.8, 160.6 and 164.0 (C–F coupling).

5-((3-(3,3-diphenylpropoxy)-4-methoxyphenylamino)methyl)-2-fluorobenzonitrile (7d):
(62%); ^{1}H NMR ($CDCl_3$) δ 2.61 (q, 2H, J = 7.0 Hz), 3.83 (s, 3H), 3.92 (t, 2H, J = 7.0 Hz), 4.24 (s, 2H), 4.27 (t, 1H, J = 7.0 Hz), 6.07-6.12 (m, 2H), 6.77 (d, 1H, J = 8.4 Hz), 7.14 (t, 1H, J = 8.5 Hz), 7.20-7.36(m,10H), 7.53-7.60 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 34.6, 47.4, 47.5, 57.1, 67.1, 101.0, 101.3 and 101.5 (C–F coupling), 104.1, 114.1, 114.3, 116.4, 116.7, 126.4, 128.0, 128.6, 131.9, 133.8 and 134.0 (C–F coupling), 137.1 and 137.2 (C–F coupling), 142.2 and 142.5(C–F coupling), 144.4, 149.6, 160.5 and 163.9 (C–F coupling).

General procedure for the preparation of 2a-d:
To a solution of compound **7a-d** (0.67 mmol) in anhydrous N,N-dimethyl acetamide (DMA) (3 mL), guanidine carbonate (0.6 mmol) was added and the reaction mixture was stirred at 140°C for 5 hours. TLC examination (10 % MeOH in NH_3 saturated CH_2C_{l2}) showed that the reaction was complete. Solvent was completely removed and the crude product was purified by flash chromatography on silica gel (12 × 1 in) using 10 % MeOH in

NH_3 saturated CH_2Cl_2 to obtain pure compounds **2a-d**.

6-((3-(benzyloxy)-4-methoxyphenylamino)methyl) quinazoline-2,4-diamine (2a):
(31 %); ^{1}H NMR (DMSO-d_6) δ 3.62 (s, 3H), 4.21 (d, 2H, J = 5.5 Hz), 4.97 (s, 2H), 5.72 (t, 1H, J = 5.5 Hz), 5.91(s, 2H), 6.11 (dd, 1H, J_1 = 2.4 Hz, J_2 = 8.5 Hz), 6.70 (d, 1H, J = 8.5 Hz), 7.13-7.49 (m, 9H), 7.98 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 48.6, 57.8, 70.8, 101.7, 104.9, 110.8, 116.0, 123.2, 125.2, 128.4, 128.5, 129.2, 132.6, 133.2, 138.3, 141.6, 144.9, 149.8, 152.6, 161.5, and 163.2; MS (ES+): m/z = 402 [M+H].

6-((4-methoxy-3-(phenethyloxy)phenylamino)methyl) quinazoline-2,4-diamine (2b):
(62 %); mp. 176oC; ^{1}H NMR (DMSO-d_6) δ 2.97 (t, 2H, J = 6.0 Hz), 3.56 (s, 3H), 4.05 (t, 2H, J = 6.0 Hz), 4.15 (d, 2H, J = 6.0 Hz), 5.66 (t, 1H, J = 6.0 Hz), 5.91(s, 2H), 6.09 (dd, 1H, J_1 = 3.0 Hz, J_2 = 9.0 Hz), 6.35 (d, 1H, J = 3.0 Hz), 6.67 (d, 1H, J = 9.0 Hz), 7.13-7.31 (m, 8H), 7.49 (d, 1H, J = 3.0 Hz), 7.96 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 35.5, 48.1, 57.4, 69.2, 100.7, 103.9, 110.4, 115.7, 122.8, 124.6, 126.7, 128.7, 129.4, 132.2, 133.8, 138.9, 140.9, 144.5, 149.5, 151.8, 160.9, and 162.8; MS (ES+): m/z = 416 [M+H].

6-((3-(3-phenylpropoxy)-4-methoxyphenylamino)methyl) quinazoline-2,4-diamine (2c):
(15%); mp. 150oC; ^{1}H NMR ($CDCl_3$) δ 2.11-2.15 (m, 2H), 2.78 (t, 2H, J = 7.5 Hz), 3.80 (s, 3H), 3.95 (t, 2H, J = 7.5 Hz), 4.31(s, 2H), 4.8 (bs, 2H), 5.45 (bs, 2H), 6.17 (d, 1H, J = 9.0 Hz), 6.23 (d, 1H, J = 2.0 Hz), 6.76 (d, 1H, J = 9.0 Hz), 7.18-7.58 (m, 8H); MS (ES+): m/z = 430 [M+H].

6-((3-(3,3-diphenylpropoxy)-4-methoxyphenylamino)methyl) quinazoline-2,4-diamine (2d):
(20 %); mp 216oC; ^{1}H NMR ($CDCl_3$) δ 2.57 (q, 2H, J = 7.2 Hz), 3.80 (s, 3H), 3.89 (t, 2H, J = 7.2 Hz), 4.19 (t, 1H, J = 7.2 Hz), 4.27 (s, 2H), 5.12 (bs, 2H), 5.48 (bs, 2H), 6.11-6.17 (m, 2H), 6.74 (d, 1H, J = 8.5 Hz), 7.14-7.30 (m, 9H), 7.46 (d, 1H, J = 8.5 Hz), 7.54-7.59 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 34.7, 47.3, 49.0, 57.1, 67.0, 101.0, 104.1, 110.1, 114.3, 120.1, 125.9, 126.3, 127.9, 128.5, 133.2, 133.3, 142.4, 142.9, 144.3, 149.5, 151.7, 159.8, and 162.2; MS (ES+): m/z = 506 [M+H].

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research paper

The Effects of Diet on Crystallin Protein Levels in the Zebrafish Lens

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Abstract

Crystallins are the major class of proteins within the lens, constituting over 90% of the total soluble protein. There are three major classes of crystallins: α, β, and γ. Defects in crystallin levels and structures have been linked to lens cataract disease. The zebrafish (*Danio rerio*) is rapidly emerging as a model for the study of ocular conditions. However, there has been little research on the role diet may have upon lens composition and function in the zebrafish and other research organisms. The goal of this study was to identify and relatively quantify some of the most abundant, and perhaps most important, crystallin proteins from the lenses of zebrafish that had been maintained on five diets of different protein sources: wheat gluten, soy protein isolate, fish protein hydrolysate, casein vita-free, and a mixture of all four. The overall proteome of the zebrafish lens was analyzed using mass spectrometry to identify the proteins present in the lens. From these data, specific crystallins were selected for comparison across the diet groups. For each crystallin, a proteotypic peptide was chosen to use for relative quantitation. The lenses of five zebrafish from each of the five diet groups were then analyzed for crystallin levels using a multiple reaction monitoring (MRM) mass spectrometric method. It was found that the lenses of zebrafish on the diet containing soy protein had significantly greater levels of α- and β-crystallins as compared to fish on the wheat protein and casein diets, while γ-crystallin levels remained approximately the same across all groups. The implications of this study could lead to new research on the correlation between diet and lens composition as well as lens-related diseases such as cataracts.

Introduction

The lens is an avascular organ that focuses light coming into the eye onto the retina. As the lens develops, epithelial cells on the anterior lens surface differentiate into fiber cells that form tightly packed concentric layers.[1,2,3] In order for the lens to be transparent, mature fiber cells are programmed to eliminate all internal organelles, which would otherwise scatter light. Without organelles, mature lens cells lack the necessary machinery for protein synthesis or maintenance and are left only with the proteins synthesized when they were undifferentiated.[1] The zebrafish lens is fully functional by 72 hours post fertilization. Epithelial cell proliferation followed by fiber cell differentiation, as with most other vertebrates, continues throughout the life of the zebrafish, making it reasonable that diet could be a factor affecting the protein composition of the new cells that are forming.[2]

After the loss of its cellular organelles, the lens still maintains a high concentration of proteins in order to refract and focus incoming light onto the retina.[1,4] Over 90% of these proteins are crystallins, which are divided into three distinct classes (α, β, and γ).[5] Often comprising over 40% of total lens protein, the α-crystallins are small heat-shock proteins (sHSP) that act as chaperones and are important in maintaining the solubility of unfolded proteins within the lens.[1,5,6,7] By constraining proteins in large complexes that involve αA- and αB-crystallins (20 kDa subunits that are 57% homologous)[1,5], the crystallins maintain the structure and solubility of other proteins. When α-crystallin chaperone activity is compromised, a decrease in solubility causes aggregation of many proteins, which has been associated with cataract formation.[1,5,6,7] Post-translational modification of proteins can change the internal environment of the lens after fiber cells have differentiated and organelles are no longer present. In the mammalian lens, C-terminal truncation of αA-crystallin has led to a 50% decrease in the protein's activity as a chaperone.[6] In humans, levels of full-length αA-crystallin are reduced as age progresses.[8] In rodents, the 1-53 αA-crystallin truncation product has been found localized to the nuclear region of the lens, where the most opaque region of small heat-shock proteins (sHSP) that act as chaperones and are important in maintaining the solubility of unfolded proteins within the lens.[1,5,6,7] By constraining proteins in large complexes that involve αA- and αB-crystallins (20 kDa subunits that are 57% homologous)[1,5], the crystallins maintain the structure and solubility of other proteins. When α-crystallin chaperone activity is compromised, a decrease in solubility causes aggregation of many proteins, which has been associated with cataract formation.[1,5,6,7] Post-translational modification of proteins can change the internal environment of the lens after fiber cells have differentiated and organelles are no longer present. In the mammalian lens, C-terminal truncation of αA-crystallin has led to a 50% decrease in the protein's activity as a chaperone.[6] In humans, levels of full-length αA-crystallin are reduced as age progresses.[8] In rodents, the 1-53 αA-crystallin truncation product has been found localized to the nuclear region of the lens, where the most opaque region of many cataractous lenses is found.[1] In contrast, in zebrafish cloche mutants that express a cataract phenotype, overexpression of αA-crystallin has led to a 64% increase in lens transparency.[9] These data show that α-crystallins are clearly integral to lens structure and function, which implies that normal levels of unmodified α-crystallins are crucial for a properly functioning lens.

The β- and γ-crystallins serve primarily as structural proteins, contributing to the refractive properties of the lens. They share the same core tertiary structure and are often referred to as members of the βγ-family. It has been proposed that these crystallins protect lens epithelial cells from stress.[4] Insolubilization of β-crystallins after proteolysis by the protease calpain II has been associated with lens maturation and cataract formation in rodent lenses.[10] The chaperone properties of α-crystallins aid in retaining β- and γ-crystallins in solution and preventing aggregation.[4] Crystallins have important structural and functional roles in the lens, and crystallin deficiencies and patterns of degradation are linked to increased lens opacity and cataract disease.

The zebrafish is an increasingly popular model for the study of physiological pathways, including those related to lens biochemistry and ocular diseases.[11,12] They are small and more economically sustainable than larger animal species, and their generational span is short (3-4 months). Additionally, a single mating pair can produce 100+ offspring each week, making them an ideal organism for providing large amounts of data.[3] The formation of the zebrafish lens follows typical vertebrate morphology. Multiple zebrafish mutants have been identified that model human ocular disorders, including but not limited to glaucoma, coloboma, retinitis pigmentosa, and cataractogenesis. The physiology and developmental processes of the zebrafish eye, as well as the species' heavy reliance on visual orientation, make it an ideal model for the study of human ocular disorders.[11,12] Zebrafish eyes are regularly exposed to light, giving them a degree of similarity to human eyes that most rodent models, which are predominantly nocturnal, naturally lack. Regarding studies of the crystallins in particular, characteristics of mammalian and zebrafish α-crystallins are similar. Zebrafish αA-crystallin has 71.7% percent homology and 90.8% similarity to human αA-crystallin, although αB-crystallin has a lower homology (58%).[13,14] If the zebrafish is to continue to be used for ocular studies, factors that could affect lens composition and experimental outcome need to be taken into consideration. One of the most obvious but often least controlled of these is diet.

Besides its impact on the future of zebrafish eye research, the experiment was conducted for its potential contribution to the future of treatment and prevention of human ocular diseases, the most common of which is cataracts. Cataracts are the leading cause of human blindness worldwide. Surgical intervention is presently the only method of cataract treatment.[15] Lifestyle intervention such as dietary control may be an early step to reduce the incidence or slow the progression of cataracts at an early stage. In addition to the effects of this phenomenon on quality of life, the high incidence of cataract surgery represents a significant economic cost to the nation, especially to the Medicare program.[16] The Federal government spends an estimated $3.4 billion annually treating cataracts through Medicare. In America, cataracts affect nearly 20.5 million people in the over-40 population, representing 1 in 6 within this age group.[15] Even a small breakthrough in cataract treatment that presents an alternative to surgery would save hundreds of millions of dollars per year in healthcare costs. While cataract surgery is readily accessible and prevalent in the U.S., many countries around the world do not have access to the facilities and capabilities necessary for the operation. Thus, a better understanding of cataract disease and the potential alternatives to surgery could result in widespread economic and individual benefits. This study looks at the effects of diet on crystallin levels in the lens of the zebrafish, an area that could lead to new approaches in the development of a novel preventative method for cataract treatment.

Materials and Methods

Wild-type adult zebrafish on a standard lab diet of fish protein were provided by Stephen Watts, PhD, UAB Department of Biology. The zebrafish on specific diets used in this experiment were part of a study conducted by Daniel Smith, PhD, UAB Department of Nutrition Sciences. All fish were fed a standard background mixture supplemented with one of five protein sources: fish protein hydrolysate, casein vita-free, soy protein isolate, wheat gluten, or a mixture of these four. Protein sources were isonitrogenous and constituted approximately 48% of the overall diet by weight. Lipid content was 24-25%, and carbohydrates constituted roughly 19%. Inorganic compounds and fiber accounted for the remaining makeup of the diet. The fish began the diets at 4-6 weeks of age, and tissues were collected at 4 months of age. All animal research was performed at the University of Alabama at Birmingham under approved protocols by the Institutional Animal Care and Use Committee and within the guidelines set forth by UAB IACUC.

Part 1: Total Lens Proteomic Analysis

Lens Extraction and Processing

Fish were euthanized by immersion in ice-cold water. Eyes were removed and stored at -80°C until used. Frozen intact eyes were thawed, and lenses were dissected with the aid of a dissecting microscope. Lenses were washed with deionized water to remove any contaminants. Both lenses from each animal were combined and homogenized into the same fraction. Two water-soluble extractions were performed by homogenization in 50 µL of 50 mM Tris-HCl (pH 7.4) followed by centrifugation at 18,000 x g and 4°C for 15 minutes. For the third extraction, the pellet was homogenized in 50 µL of 50 mM Tris-HCl - 6 M urea and re-centrifuged. A BCA protein assay was used to determine the concentrations of the extracts. Water-soluble and water-insoluble/urea-soluble proteins were resolved by SDS-polyacramide gel electrophoresis (PAGE) on a 10.5-14% (w/v) acrylamide Criterion Tris-HCl gel (Bio-Rad). The gel was fractionated into 12 bands from the water-soluble lane and 13 bands from the water-insoluble/urea-soluble lane. The gel image and bands that were excised are shown in Figure 1.

Gel fractions were de-stained overnight in 50 mM ammonium bicarbonate (ABC)/50% acetonitrile (ACN). Samples were then reduced by treatment with 20 mM dithiothreitol (DTT) in 100 mM ABC for 1 hour at 37°C. The DTT was removed and the

samples were then alkylated by treatment with 55 mM iodoacetamide (IA) in a dark environment for 1 hour. The IA was removed, and samples were de-hydrated with 100% ACN. Trypsin was added to samples at a 1:50 trypsin to protein ratio. Samples were incubated at room temperature for 10 minutes and then covered with 100 mM ABC. The digestion was allowed to continue for 48 hours at 37°C. After this time, peptides were extracted from gel pieces at room temperature using 50% ACN-5% formic acid (FA) for 1 hour. The supernatant was removed and saved, and the extraction was repeated a second time. Again the supernatant was saved, and the gel pieces were then dehydrated with 100% ACN. The supernatants were combined and then dried down in a Speed-Vac™. Samples were de-salted twice using C_{18} ZipTips (Millipore, Inc.). After de-salting, samples were dried down using a Speed-Vac™ and resuspended in 0.1% w/v FA containing 1 femtomol of trypsin-digested bovine serum albumin (BSA) as an internal standard. An aliquot (2 µL) of each digest was loaded onto a 2 cm x 75 µm i.d.PepMap100 C_{18} reverse-phase trap cartridge (Dionex, Sunnyvale) at 2 µL/min using an Eksigent autosampler.

After washing the cartridge for 4 minutes with 0.1% formic acid in ddH_2O, the bound peptides were flushed onto a 15 cm x 75 µm i.d. PepMap100 C_{18} reverse-phase analytical column (Dionex) with a 40 min linear (5-50%) acetonitrile gradient in 0.1% formic acid at 300 nl/min using an Eksigent Nano1D+ LC. (Dublin, CA). The column was washed with 90% acetonitrile-0.1% formic acid for 15 minutes and then re-equilibrated with 5% acetonitrile-0.1% formic acid for 30 minutes. The Applied Biosystems 5600 TripleTof mass spectrometer (AB-Sciex, Toronto, Canada) was used to analyze the protein digest. The IonSpray voltage was 2300 V, and the declustering potential was 60 V. Ionspray and curtain gases were set at 10 psi and 20 psi, respectively. The interface heater temperature was 120°C. Eluted peptides were subjected to a time-of-flight survey scan from 400-1250 m/z to determine the top twenty most intense ions for MS/MS analysis. Product ion time-of-flight scans at 50 msec were carried out to obtain the tandem mass spectra of the selected parent ions over the range from m/z 400-2000. Spectra are centroided and de-isotoped by Analyst software, version TF (Applied Biosystems). A β-galactosidase trypsin digest was used to establish and confirm the mass accuracy of the mass spectrometer. In-house MASCOT database searches were carried out against the *Danio rerio* genome on the UniProt database. The mass tolerances for precursor scans and MS/MS scans were set at 0.05 Daltons. One missed cleavage for trypsin was allowed. A fixed modification of carbamidomethylation was set for cysteine residues, and a variable medication of oxidation was allowed for methionine residues. Proteins with at least one individual peptide MOWSE score of <40 were considered significant.

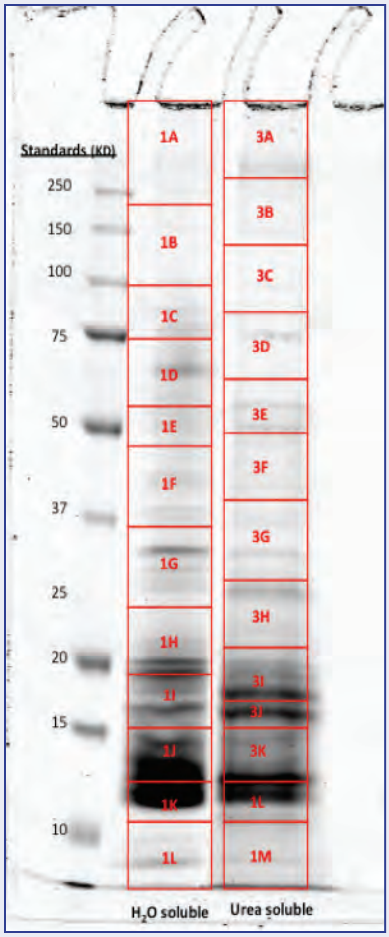

Figure 1: SDS-PAGE zebrafish lens homogenates. The first lane (Fractions 1A-1L) displays 7 µg of water-soluble proteins; the second lane (Fractions 3A-3M) displays 7 µg of urea-soluble proteins on a 10.5-14% Criterion Tris-HCl Gel. Darkest bands are in the 10-20 kDa range, corresponding with the masses of the major crystallins.

Peptide selection for LC-MRM-MS relative quantitation

The α-, β-, and γ-crystallins with the highest intensities from the overall proteome analysis were examined. Peptides were analyzed for relatively strong intensity, a comprehensive spectrum of y- and b- ions, and a mass range of 800-1600 Da (to have a 400-800 m/z $[M+H]^{+2}$ "doubly charged" mass). Selected peptides that did not contain missed cleavages from the enzymatic digest were subjected to BLAST (Basic Local Search Alignment Tool) searches to ensure that they were unique to the particular protein they were going to be used to quantify for the zebrafish. Peptides were also chosen on the basis of minimum post-translational modifications. A peptide meeting each of these criteria was found for 17 of the crystallins. These peptides were used as precursor ions, and for each crystallin, a fragment ion was chosen for use in relative quantitation. The crystallins, the peptide sequences and the ions used are shown in Table 1.

Part 2: Comparison of Crystallin Levels in Diet-Group Zebrafish

Lens Extraction and Processing

Lenses were dissected using the same methods described previously. Samples were randomized. Each pair of lenses was homogenized in 50 mM ABC with 8 M urea and centrifuged at 18,000 x g and 4°C for 15 minutes. A BCA protein assay was used to determine the concentration of the 25 extractions. A 20 µg portion of the total protein from each sample was loaded onto an 18-well criterion 12% Bis-Tris gel (Bio-Rad) for SDS-PAGE analysis. Instead of running the SDS-PAGE gel to completion, the samples were run only ~1 cm into the gel. The gel was then fixed in 30% methanol/10% acetic acid for 10 minutes, washed with water twice for 5 minutes each, and stained with Coomassie blue for 5 minutes. The bands were then excised and allowed to

Protein Name	Peptide Sequence	$[M+H]^{+1}$ Mass of parent ion	$[M+H]^{+2}$ Mass observed (m/z)	$[M+H]^{+1}$ Mass of ion used for relative quantitation
α-crystallin A chain	FTVYLDVK	985.53	492.77	736.424
α-crystallin B chain	FVINLDVK	946.55	474.28	701.4192
α-crystallin βB chain	ILFPIFFPR	1148.68	575.35	776.4454
βA1-2-crystallin	HSGDFQHWR	1168.52	585.26	1032.4646
βB3-crystallin	VSLYEFENFR	1302.62	652.32	1004.4472
β-crystallin A4	ENYLGR	750.37	376.19	232.1404
β-crystallin B2	LVIYEQENFQGR	1494.75	748.38	1170.5174
βA1c-crystallin	HSGDYQHWK	1156.51	386.5	688.2685
crystallin, βB1, like 2	HFNEYGAR	992.45	497.23	856.3948
crystallin, βB1, like 3	GYQYLFEFGSYK	1500.69	751.36	877.409
crystallin, γM2a	VVFYEDR	926.45	464.23	729.3202
crystallin, γM7	IIFYEDR	954.48	478.25	729.3202
crystallin, γMX	ITFYEEK	928.45	465.23	715.3297
crystallin, γS1	IIFFEDK	910.48	456.24	685.3192
γ-crystallin D	YSDWGALSSR	1140.52	571.27	590.3297
γ-crystallin N-A	IVFFEGK	838.46	420.23	627.3137
γ-crystallin N-B	GEYPEFQR	1024.46	513.24	676.3413

Table 1: Unique crystallin peptides and fragment ion masses. Zebrafish crystallins that were studied and the peptides and ions used for relative quantitation across the diet groups.

de-stain overnight in 50 mM ABC/50% ACN. Proteins were reduced and alkylated as described previously. Trypsin was added in a 1:50 trypsin to protein ratio, and samples incubated at room temperature for 10 minutes and then were covered with 100 mM ABC. The digest proceeded for 48 hours at 37°C. Peptides were extracted and dried down as described previously. Samples were then re-suspended in 0.1% FA mm. Samples were then de-salted twice using C_{18} ZipTips (Millipore, Inc.). After de-salting, samples were dried down using a Speed-Vac™ and resuspended in 0.1% w/v FA containing 1 femtomol BSA. Aliquots of the resuspended extract were resolved by nanoLC as described previously. Analysis was performed to relatively quantitate the peptides selected in Table 1, using a multiple reaction monitoring (nanoLC-MRM) mass spectrometric (MS/MS) method on a 5600 TripleTOF mass spectrometer (AB Sciex). Doubly charged ions corresponding to the m/z values of each of the selected peptides were filtered by the quadrupole for successive periods of 50 msec; these were subjected to collision-induced dissociation and the fragment ions analyzed by the TOF analyzer. The collected data were processed to obtain the total peak area of the most abundant fragment ion and compared to the BSA peptide for relative quantitation.

Results

From the analysis of the overall zebrafish lens proteome, a total of 306 different proteins were detected in the fractions. Of these, 120 proteins were detected in both fractions. There were a total of 73 crystallins identified, accounting for 24% of the total number of proteins. Of the 73 crystallins detected in the lens, 20 were chosen to compare levels across the diet groups using nanoLC-MRM-MS analysis – 17 of them were proteotypic (Table 1).

The experiment began with n=5 zebrafish for each diet group. Two samples from the casein diet group and one from the diet group containing all four protein sources were deleted from the final analysis due to technical errors in processing and handling. One sample from the soy diet group was omitted due to technical issues during mass spectral analysis, and another sample from the soy diet group was dropped during data processing due to adverse abnormalities that skewed the data to an unacceptable degree. After these adjustments, the number and sexes of the fish included in the data were as shown in Table 2.

Diet Group	Males	Females	Unknown Sex	Total Fish
Wheat	3	1	1	5
Soy	2	1	0	3
Fish	3	2	0	5
Casein	2	0	1	3
Mix	3	1	0	4

Table 2: Zebrafish numbers and sexes for each diet group. Characterization of fish included in the data.

Duncan's Multiple Range Test was used for statistical analysis.[24] Soy protein isolate increased levels of αA-, αB-, and αBb-crystallin in the zebrafish lens when compared to wheat protein and casein diets (Fig. 2). The soy diet also led to significantly increased levels of βB3- and βB2-crystallins in the zebrafish lens when compared to wheat protein and casein diets (Fig. 3). The diets containing fish protein hydrolysate and a mixture of all four protein sources followed a trend of similar, intermediate crystallin levels for the α-crystallins and βB3- and βB2-crystallins. Of the β-crystallins, βB3 and βB2 were the most abundant, followed by βB4 and βB1-like-2. There was no significant difference between levels of βA1-2, βA4, and βB1-like-2 crystallins across the diet groups. The β-crystallins βA1-2 and βB1-like-3 were not very abundant

within the lens (Fig. 3). The various diets did not significantly affect the amount of λ-crystallin in zebrafish lenses in any consistent manner. λM7-crystallin was the most abundant λ, followed by λM2a (Fig. 4).

Discussion

Soy protein had the most significant impact on the amount of crystallins in the lens. The lens homogenates from zebrafish that were fed the diet containing 48% soy protein displayed greater levels of αA, αB, αBb, βB2, and βB3 crystallins than those of fish that were fed wheat gluten and casein. In general, fish on the soy diet were larger in size than fish on the other four diets, and they displayed some malformations such as deformities of the operculum. Conversely, wheat protein diet fish were the smallest, likely due to lysine deficiency. However, because the same amount of protein from each lens was originally loaded onto the gel and because many of the γ-crystallin levels were similar across diets, the differences in α- and β-crystallins are believed to be significant.

Because the α-crystallins were affected by the diet differences, the overall chaperone function of the lens is likely to be compromised in diets for which α-crystallin levels were lower. Soy contains isoflavones such as genistein, which has been shown to prevent the formation of diabetic cataracts by inhibiting the enzyme aldose reductase.[17,18] The results of this experiment – that soy contributes to high levels of α-crystallins, which should keep proteins from aggregating – are consistent with some of the past research regarding soy and lens physiology.

Soy's contribution to the high levels of certain β-crystallins is also consistent with past findings. β-Crystallins are mainly structural and aid in refracting light, so high levels of β-crystallins should indicate a normal functioning lens, whereas low levels or modified insoluble forms of β-crystallins may be associated with aggregation and lens degeneration. It is reasonable that high levels of chaperone α-crystallins corresponded with high levels of certain β-crystallins. If there is credence to the proposal that β-crystallins function in protecting lens epithelial cells from stress, then decreased levels of these proteins may lead to ocular health issues relating to eye conditions that result from stress.

Interestingly, soy protein did not seem to have an effect on the levels of γ-crystallins, nor did any other diet. Whereas α- and β-crystallins showed distinct variations amongst the groups, no pattern was observed within the relative intensities of γ-crystallins. While γ-crystallins are primarily structural in purpose, they have been linked to cataracts. A mutant strain of zebrafish (cloche) is deficient in αA-crystallin, displays high levels of insoluble γ-crystallin, and exhibits cataracts. In these mutants overexpression of αA-crystallin decreased cataract opacity by keeping γ-crystallins soluble.[9] Further study is needed to determine if there is an unseen significant correlation between diet and γ-crystallin levels; however, this study would indicate that there is not.
These experiments are best viewed as preliminary work toward further research in this area. In the future, larger sample sizes will permit the data to be stratified on the basis of characteristics of the zebrafish such as gender, size, and other potentially relevant factors. A method of standardizing fish consumption may be developed, as it is difficult to determine how much a single fish eats and variability is observed within and across tanks for body size (length and weight). A protein from the lens homogenizations could be identified for use as a control for total amounts of protein identified by mass spectrometry: most likely a non-crystallin protein that should be the same across all lenses (e.g. α-tubulin). A Western blot could confirm that the control is present in equal amounts in different samples. Having such a control will allow normalization for slight variations in the samples that occur during mass spectrometry analyses; however, quantitation would still be relative. For absolute quantitation, a synthetic peptide could be generated that is identical to the proteotypic peptide used for a certain crystallin. This synthetic peptide would be analyzed at several known concentrations to generate a standard curve. Comparing intensities and areas under the peak of the peptides in lenses to this standard would allow for absolute quantitation of the crystallins.

Another issue to consider in future experiments is the forms of the proteins that are being quantified. With this method, C-truncations of the crystallins due to proteolysis were not identified. In the lens of ICR/f rats, 16 different αA-crystallin truncation products have been identified.[1] The peptide used for relative quantitation of αA-crystallin was FTVYLDVK, corresponding to amino acids 72-79 from a protein whose full-length form contains 173 amino acids. The full-length αA-crystallin (aa 1-173) would be detected with the same intensity as a C-terminally cleaved 1-100 truncation product. αA-crystallin loses 50% of its chaperone ability when just 16 amino acids are cleaved, so it is apparent that differences in crystallin form could play a prominent role in lens morphology.[6]

It would also be beneficial to look into the pathway by which these diets are influencing lens protein composition. Do the differences arise at the transcriptional level, the translational level, or post-synthesis? The soy diet may have contributed to higher α-crystallin levels by creating a more stable lens environment, preventing protein degradation, or increasing synthesis. One method that could look into this question would be to explore gene regulation techniques. It is suspected that proteolysis of α-crystallins is accomplished by means of calcium-dependent enzymes known as calpains, which are known to have proteolytic activity in rodent, human and bovine lenses.[18,19,20] If one or more calpains were identified and overexpressed or inhibited in fish on specific diets, the effects would give insight to the particular biochemical mechanisms affected by diet.

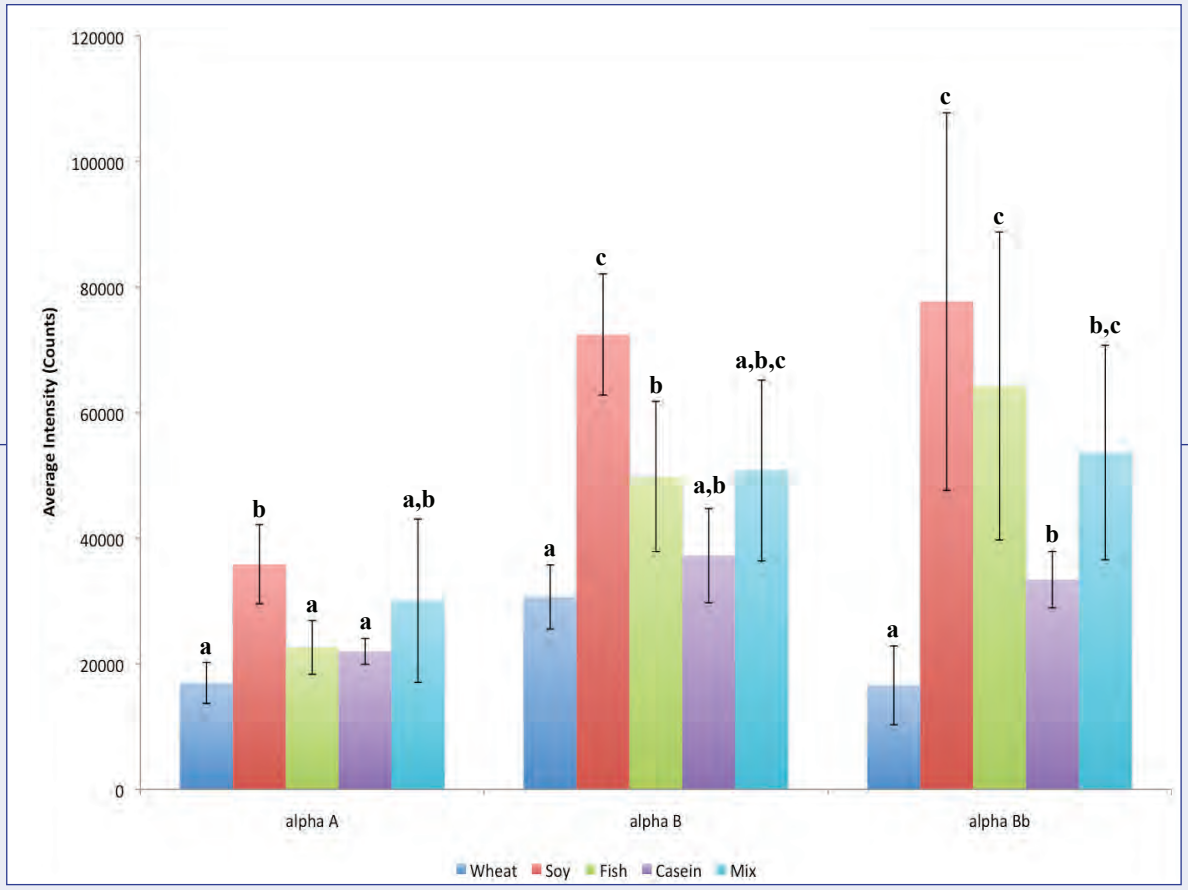

Figure 2: Relative quantitation of α-crystallin levels between diet groups, plotted with standard error of the mean and analyzed using Duncan's multiple range test. Differences between diet group intensities are compared within individual protein types, not across different proteins.

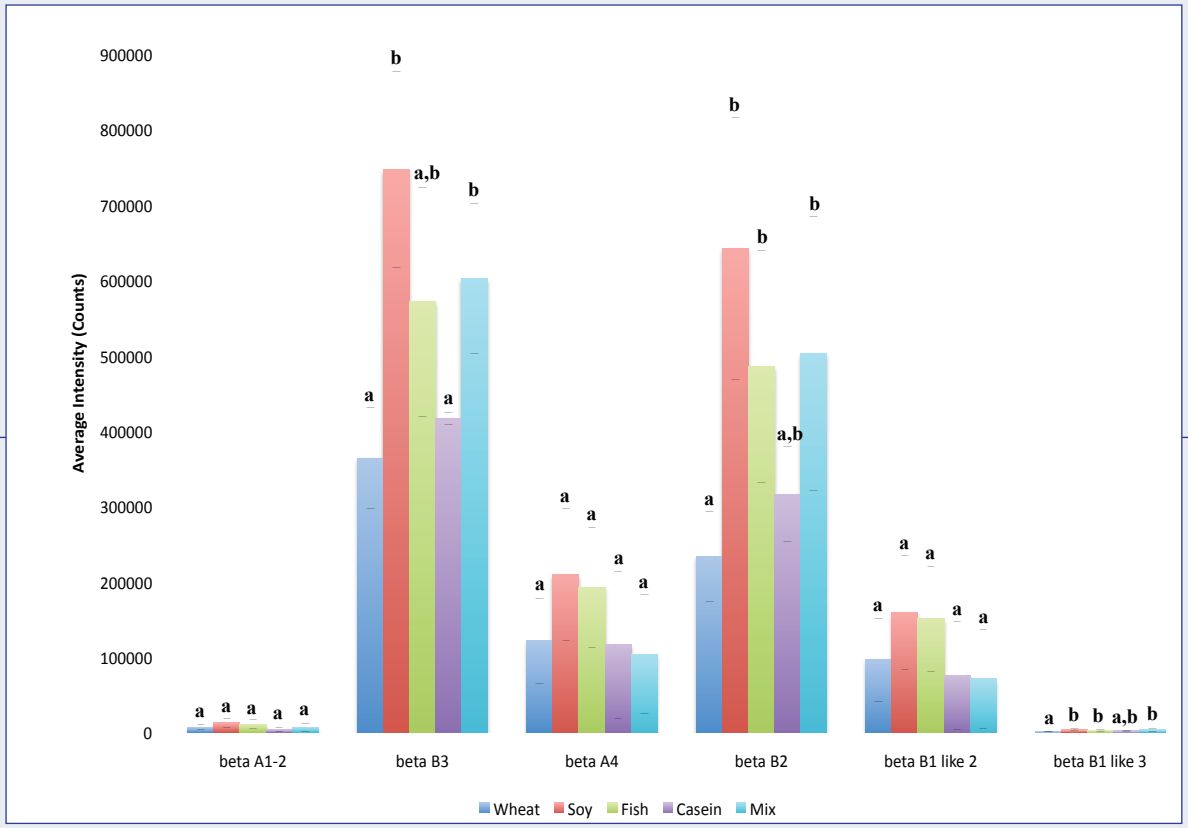

Figure 3: Relative quantitation of β-crystallin levels between diet groups, plotted with standard error of the mean and analyzed using Duncan's multiple range test. Differences between diet group intensities are compared within individual protein types, not across different proteins.

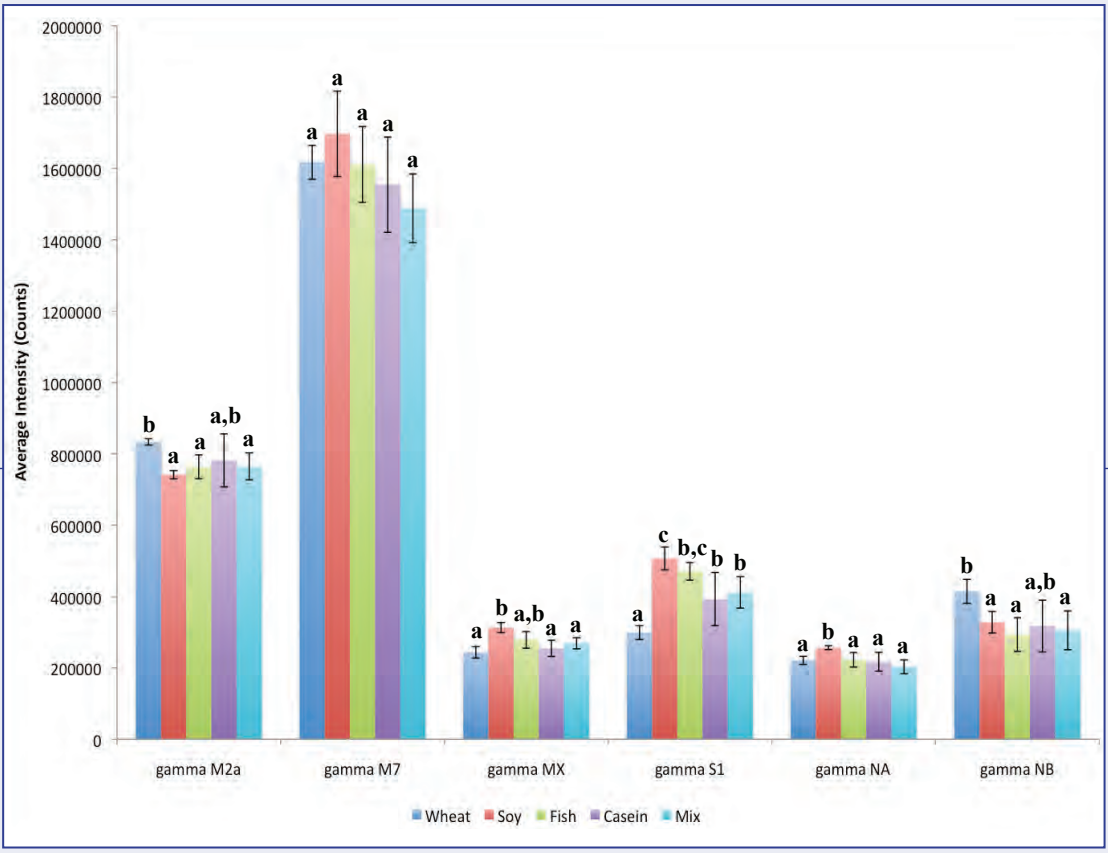

Figure 4: Relative quantitation of γ-crystallin levels between diet groups, plotted with standard error of the mean and analyzed using Duncan's multiple range test. Differences between diet group intensities are compared within individual protein types, not across different proteins.

The idea that diet and eye health are linked to one another is not new. Studies have suggested that women whose diets are in agreement with the diet recommended by the publication Dietary Guidelines for Americans have a smaller percentage of cataracts than those who have a lower Healthy Eating Index.[21] Another study saw a correlation between diet and vitamin intake and nuclear lens opacities in adult humans.[22] Animal studies have demonstrated that diets low in methionine and diets with restricted caloric intake lead to delays in cataract progression.[17,23] However, none of these examine how diet affects lens composition on the biochemical level.

Here it is demonstrated that different protein sources have direct impacts on the amount of major functional and structural proteins in the zebrafish lens. With further studies that examine the effect of diet on issues such as PTMs, truncated forms, and protein insolubilization in the lens, a more conclusive view of the extent to which diet regulates ocular health can be attained.

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Mallick Hossain wins 2011 Goldwater Scholarship

Rachael Rosales

As the 2011 Goldwater Scholar, Ethan Mallick Hossain has been recognized for his accomplishment in academics and research. The Barry M. Goldwater Scholarship and Excellence in Education Program provides scholarships to highly aspiring scientists, mathematicians, and engineers. In addition to a perfect academic record, which includes graduate level math courses, Mallick researches in the lab of Dr. Kevin Harris. His ultimate goal is to find a biomarker that will help doctors tailor chemotherapy treatments to different patients. Currently, Mallick and his colleagues are searching for a relationship between the cell-free DNA of patients with Acute Myeloid Leukemia (AML) and the effectiveness of the type of chemotherapy that they receive.

Mallick sought out a research experience in order to augment his education beyond the traditional classroom setting. He believes that his experiences have been a crucial part of his intellectual development.

"Rather than learning about topics that have already been discovered, a student researcher creates new knowledge," Mallick explains. "There is no solutions manual for research, and that is what makes it so exciting. The minute I stepped into the lab, I was enthralled by the energy of my colleagues. I knew this was something new and exciting to which I could contribute."

Mallick's phenomenal successes described above already identify him as one of the top students at the University of Alabama at Birmingham. However, these describe only a small subset of his achievements. His debate career has included two national championships. As Mr. UAB, he serves as the paragon of scholastic achievement, leadership, campus and community involvement, and UAB school spirit.

"Life is full of opportunities, and college is the perfect place to take advantage of all those opportunities because it is all about exposing yourself to different experiences," Mallick insists. "I've learned so much about different topics and about myself. At the end of the day, it's about never stopping my explorations!"

Mallick's explorations have ranged from forming mathematical proofs to learning how to salsa dance. When asked what advice he might offer to incoming freshmen, he offered, "Never count anything out. College is a time to explore your passions and you might just stumble onto something you would have never seen yourself doing."

2012 Inquiro Submission Guidelines

Any student participating in scientific research at UAB is invited to submit a research paper for consideration for publication in the 2012 issue of Inquiro. Papers will be subject to student and faculty review.

The deadline for submissions is June 1, 2012; however, students participating in summer research at UAB or another institution are encouraged to submit by August 31, 2012.

Initial submissions should follow these guidelines:

1) Research papers should follow the general format: Abstract, Introduction, Materials and Methods, Results, Discussion, Conclusion and References. Variation may occur as long as the general sections are clearly delineated.

2) All manuscripts should be written in third person perspective. The Materials and Methods and Results sections should be written in past tense.

3) 12 point font, double spaced, pages should be numbered with the author's name appearing in a header on every page (further formatting will be required upon acceptance).

4) Figures, tables, and graphs should be submitted in their original formats in the highest resolution possible as separate files. A .tiff file at 300 dpi is ideal.

5) All research papers should be submitted with the Inquiro Permission to Publish Form.

Staff also invites students to submit research narratives, interviews with faculty members, and science related editorials.

Short Reports: These reports are short papers derived from the text of science posters. Please convert the original poster to a Word document which includes all text, figures, tables, and images from the poster. As above, images should be submitted additionally as a separate file. The suggested length is 2,500 words.

Research Narratives/Other: If students would like to submit editorial or narrative pieces related to scientific research, they may certainly do so. The journal staff will review the article and consider it based on relevance and quality. The suggested length is 900 words.

Anyone who wishes to join the Inquiro staff should fill out the application on our website.

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Khushboo Jhala is a senior in the Early Medical Acceptance Program and University Honors Program at UAB. She is majoring in International Studies and minoring in Chemistry. She will begin medical school this summer and intends to use her global background experience to address policy changes in international medicine. Her research interests include analyzing stage transitions of various cancers by their clinical, radiologic, and histologic features.

Rachael Rosales

Rachael Rosales is a junior studying biology. She is a member of the University Honors Program (UHP) and the Early Medical School Acceptance Program (EMSAP). In addition to her scientific interest, Rachael has done research in the UAB Philosophy Department and participates in UAB Ethics Bowl. She plans on pursuing a career in the medical field after her undergraduate career.

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Miranda is a sophomore in the University Honors Program majoring in Chemistry and minoring in Mathematics and Economics. She works for the UAB student newspaper and is a Teaching Assistant in the UAB Chemistry Department. Her research focuses on lens physiology and ocular diseases. She aspires to attend medical school where she plans to further her interest in vision.

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Amiya Ahmed is a sophomore double majoring in Molecular Biology and Philosophy with a minor in Chemistry. He is a member of the University Honors Program and the Biology Scholars Program. Amiya works at the McWane center as a LabWorks! Facilitator and teaches science to middle school children. He also researches geriatrics and heart failure under the UAB Center for Aging. For fun, he enjoys playing basketball and playing piano and wishes to pursue a career as a physician-scientist.

Sadhvi Batra

Sadhvi Batra is a sophomore majoring in Neuroscience and Philosophy. She is part of the Science and Technology Honors Program and is currently working with Drs. Geldmacher, M.D., at UAB School of Medicine, and Sullivan, Ph.D. on a project that aims to investigate the qualities of "self" that are often overlooked by those living with Alzheimer's Disease. In the future, she hopes to pursue a career in medicine.

Atbin Doroodchi

Atbin is a senior Molecular Biology and Mathematics double major. He is a member of the Science and Technology Honors Program, Math Fast-Track program. Atbin currently works on his honors thesis in the Department of Neurology under the direction of Dr. Yuqing Li for which Atbin received 2010 Barry M. Goldwater Scholarship. Outside of school, he enjoys drawing and running.

Naveed Farrukh

Naveed Farrukh is a sophomore pursuing his Bachelors in Neuroscience and Master's in Public Health. He is also also part of the University Honors Program (UHP) and conducts research in the UAB Department of Neurobiology on growth of neural cells in the dentate gyrus region of the brain. In the future he hopes to get a medical degree, but craves the added rush of one day working on domestic health policy. In life, this kid doesn't sit inside waiting for storms to pass - he plays in the rain.

Timothy Fernandez

Timothy Fernandez is a junior double majoring in Biochemistry and Molecular Biology. He is in the University Honors Program and is a Chemistry Scholar. Currently, he is researching HIV-1 protein interactions with cellular constituents as well as protein interactions in the Fas-mediated apoptosis pathway. After graduation, he hopes to pursue an MD/PhD.

Pooja Gajare

Pooja Gajare is a sophomore Molecular Biology major in the University Honors Program and the Early Medical School Acceptance Program. She is currently doing research under the direction of Dr. Karolina Mukhtar. Her hobbies include running and baking. She plans on attending medical school after graduation.

Zac Ingram

Zachariah Ingram is a sophomore in the Science and Technology Honors program and is majoring in both mathematics and chemistry. He is involved in two research projects at UAB which correlate to his chosen majors. His dream is to bring science and understanding to the less fortunate across the globe.

Aditi Jani

Aditi Jani is a sophomore majoring in Molecular Biology and is in the University Honors Program and Early Medical Acceptance Program. She is interested in a career in academic medicine and has been doing research in the department of Pediatrics, division of Neonatology, with Dr. Brian Sims.

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Helen Lin is a senior Molecular Biology major and a Chemistry/Linguistics double minor. She is a member of the Early Medical School Acceptance Program and president of the Phi Sigma Biological Honor Society. Currently, she is researching the characteristics of membrane targeting of the protein Golgi-specific brefeldin-A sensitive binding factor 1 (GBF1), which is important in cellular trafficking. When she graduates she plans to attend Medical School at UAB.

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Kavita Nadendla is a senior double major in Biology and Neuroscience with a Chemistry minor. She is a member of the University Honors Program and Early Medical School Acceptance Program and serves as an officer for the Asian American Organization and a Laboratory Teaching Assistant for the undergraduate Anatomy course. Kavita is currently working on her Neuroscience Research Thesis with Dr. Rajesh Kana using fMRI to investigate autism. She will attend medical school in Fall 2012 upon completion of her undergraduate degree.

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Ashruta Patel is a senior Chemistry major and a Biology and Mathematics minor. She is currently conducting research related to schizophrenia in Dr. Rita Cowell's lab in the Department of Psychiatry and Behavioral Neurobiology. Her interests include volunteering, traveling, and reading. Ashruta plans on pursuing a degree in medicine and working at an institution where medicine is incorporated in a clinical and laboratory setting.

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Ramya is a second-year UAB student and aspires to attend medical school after her undergraduate years. She is a member of the EMSAP honors program and the Undergraduate Neuroscience program, and is an undergraduate research assistant in the Shelby Building. She is a native Californian and enjoys reading, dance, and watching movies.

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