

Temple University

Annual Progress Report: 2009 Formula Grant

Reporting Period

July 1, 2013 – December 31, 2013

Formula Grant Overview

Temple University received \$2,375,033 in formula funds for the grant award period January 1, 2010 through December 31, 2013. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

The Anabolic Role of Cannabinoid Receptors in Bone - The goal of the planned project is to define the anabolic role of cannabinoid receptors in bone. Recent studies have demonstrated the presence of endocannabinoids and their G protein-coupled cannabinoid receptors, CB1 and CB2, in the skeleton. Although it has become clear that this system is functional in bone, the precise mechanisms of action are only beginning to emerge. The planned studies will generate new information regarding the anabolic effects of cannabinoid receptors and mechanisms of action on osteoblast differentiation and function. Once we understand how cannabinoid receptors function to promote bone formation, this information will be helpful in developing new therapeutic strategies to selectively enhance bone formation in patients with clinically significant bone loss.

Duration of Project

7/1/2010 – 6/30/2011

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

Research Project 2: Project Title and Purpose

Effects of IL-19 on Endothelial Cell Activation and Angiogenesis - Cardiovascular disease is the number one killer of Americans. Vascular diseases such as atherosclerosis and hypertension are inflammatory in nature. Very little has been reported on the potential protective effects of anti-inflammatory cytokines on development of vascular disease. We have novel preliminary data which shows that the naturally occurring anti-inflammatory compound, Interleukin-19 (IL-19) can have stimulatory effects on endothelial cells grown in culture. The purpose of this project is to determine if IL-19 can have pro-angiogenic effect in vivo.

Duration of Project

7/1/2010 – 6/30/2011

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

Research Project 3: Project Title and Purpose

A Targeted Drug Delivery System for Preventing Cardiac Remodeling after MI - Cardiac remodeling after myocardial infarction increases the stress on the surviving myocardium and predisposes viable myocardial cells to premature death leading to heart failure. Presence of an appropriate microvasculature is a prerequisite for preventing cardiac remodeling and improving cell survival resulting in maximal regeneration of a functional tissue after myocardial infarction. The goal of this project is to use targeted delivery of pro-angiogenic agents to enhance the morphology and function of post-infarct neovasculature, prior to scar formation, and to establish the optimal time post-myocardial infarction when pro-angiogenic interventional strategies could prevent cardiac remodeling and congestive heart failure.

Duration of Project

7/1/2010 – 6/30/2011

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

Research Project 4: Project Title and Purpose

Mechanism(s) of Vascular Damage in Obesity - Cardiovascular disease accounts for much of the morbidity and mortality ultimately suffered by patients with diabetes mellitus. Adiponectin is a protein from fat cells that circulates in the bloodstream and has been shown to protect the blood vessels in diabetes. Interestingly, adiponectin opposes the adverse effects on blood vessels of several factors including glucose that is known to be elevated in Type 1 and/or Type 2 diabetes. In this project, we seek to identify the cellular mechanisms by which adiponectin exerts its salutary effects in the endothelial cells that line the blood vessels. These results will advance our understanding of the role of adiponectin in vascular protection in diabetes and in obesity with insulin resistance, conditions with a high risk of life-threatening cardiovascular complications.

Duration of Project

9/1/2012 – 8/31/2013

Project Overview

The goal of this project is to define integrative mechanisms by which adiponectin preserves endothelial function in obesity-associated diabetes. The PI reported in the Journal of Clinical Investigation in 2007 that adiponectin deficient mice exhibit a distinct inflammatory phenotype in both micro- and macrovascular beds with endothelial dysfunction characterized by impaired endothelial nitric oxide (eNO) generation and increased leukocyte-endothelium interactions due to increased expression of the endothelial cell adhesion molecules (eCAMs), E-selectin and VCAM-1. This inflammatory phenotype was reversed by Ad-replenishing therapy, which was shown to act via enhancing eNO bioavailability, a well-known regulator of eCAMs expression. In metabolic disorders such as obesity and diabetes, loss of physiological eNO levels is in part attributed to eNOS uncoupling by reactive oxygen species (ROS) and it is linked to endothelial dysfunction with inflammatory leukocyte-endothelium interactions. Very recent studies have also suggested that adiponectin reduces accumulation of myeloperoxidase (MPO) in inflamed tissues. MPO is a powerful oxidant released by activated leukocytes that is able to bind to the vascular endothelium where it drastically reduces eNO bioavailability by catalytic consumption. We hypothesize that the action of adiponectin to enhance eNO bioavailability and attenuate leukocyte-endothelium interaction in the vasculature largely occurs both via direct suppression of endothelial ROS generation and prevention of leukocyte-derived MPO binding to the endothelium, two novel integrated mechanisms of the vascular protective effects of adiponectin in obesity and diabetes. Using a genetic mouse model of obesity-associated type II diabetes along with selected knockout mouse technology this project will test the following hypotheses according to these Specific Aims: 1) To study the effect of adiponectin on eNO/ROS balance and endothelial function in vivo; 2) To study the role of leukocyte-derived MPO in the endothelial protective action of adiponectin.

Summary: The results of these studies will provide important information on the intercellular mechanisms of the anti-inflammatory action of adiponectin in Type II diabetes. They will also provide new insight into the integrative mechanisms by which the endothelium and circulating leukocytes synergize to increase oxidative stress in the obese, diabetic vascular endothelium.

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Other Participating Researchers

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Expected Research Outcomes and Benefits

We expect to uncover the mechanisms by which adiponectin protects vascular function. In particular we anticipate finding that reduction of oxidative stress in the mitochondria is one of the key mechanism of the vascular protective action of adiponectin. We will also define the mechanisms by which the various circulating forms of adiponectin exert vasculoprotective effects, with a focus on endothelial cell function in vivo using mouse models as well as cultured endothelial cells in vitro. Overall, this project will uncover novel mechanisms of vascular damage in obesity and diabetes and it will provide a framework for developing new therapeutic strategies to avert vascular and organ damage in obese, insulin resistant humans.

The developed world is currently experiencing an epidemic of cardiovascular disease that is caused by an ever-increasing prevalence of obesity with insulin resistance due to excess caloric intake and sedentary lifestyles. A key contributor to this increased vascular risk is endothelial dysfunction, a major component of the initiation of the complex process of atherogenesis. By studying the effects of adiponectin isoforms in vascular endothelial cells, we will contribute to our understanding of the mechanisms by which adiponectin exerts its salutary effects on vascular impairment induced by several major agonists that accompany obesity and diabetes. A greater understanding of these signaling pathways may ultimately lead to new targets for the prevention or treatment of the early vascular injury that accompanies obesity and diabetes.

Summary of Research Completed

In the last two months of the project Mrs. Zhao (Master student) with the help of Mr. Preston Predoctoral Fellow worked on refining methods for isolation and transfection of adipocytes. Once they were able to culture adipocytes, they studied adiponectin expression after incubation with either myeloperoxidase (MPO) and/or its byproduct hypochlorous acid. In addition they established colonies of MPO deficient mice that are now needed to continue this very promising line of research. Finally, they were able to establish an ELISA assay to measure adiponectin levels in the culture media of adipocytes. Overall in this last segment of research we were able to acquire methodology to continue our studies and to secure additional funding in the area of obesity and related complications.

Research Project 5: Project Title and Purpose

Targeted Multidrug Delivery System to Overcome Chemoresistance in Breast Cancer – In this project, the Principal Investigators propose to develop a novel multidrug delivery system for the treatment of breast cancer. This novel treatment strategy would provide a significantly more efficacious platform for breast cancer treatment than current treatments.

Duration of Project

12/1/2010 – 12/31/2011

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

Research Project 6: Project Title and Purpose

Nanoconjugates for Targeted Treatment of Acute Lung Injury – The development of the acute respiratory distress syndrome (ARDS) following infection or injury is a major public health problem and one of the leading causes of death in Intensive Care Units (ICU). ARDS is characterized by excessive neutrophil infiltration of the lungs and neutrophil-mediated lung tissue damage. To date, there are no specific pharmacologic therapies available that protect the lung from neutrophil-mediated damage. We identified the enzyme delta-Protein Kinase C (δ -PKC) as a critical regulator of the inflammatory response. The goal of this project is to develop a specific δ -PKC peptide inhibitor into a viable therapeutic by the use of nanocarriers to transport the inhibitor directly to the lung. If successful, this approach could significantly decrease the morbidity and mortality associated with sepsis, burns, hemorrhagic shock and trauma.

Duration of Project

12/1/2010 – 6/30/2012

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

Research Project 7: Project Title and Purpose

Role of Dorsostriatal Glutamatergic Signaling in the Regulation of Cocaine-induced Synaptic and Behavioral Plasticity – Chronic drug use leading to drug addiction is associated with impairments in reinforcement seeking behaviors. The pathological mechanisms associated with these maladaptive behaviors are not known. The primary goal of this project is to understand the role of dorsostriatal glutamatergic signaling in the regulation of drug (cocaine)-induced synaptic plasticity and behavioral sensitization. To accomplish this goal, we will utilize multidisciplinary approaches in neurosciences to gain an integrative perspective on the neurobiological basis of compulsive cocaine-seeking behaviors. Results from this project will provide important preclinical data on potential targets for treatment of cocaine addiction.

Duration of Project

1/1/2011 – 12/31/2012

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

Research Project 8: Project Title and Purpose

Genetic Variations in Inflammation-Related Genes in Patients with Chronic Pelvic Pain Syndrome – The etiology and pathogenesis of Chronic Prostatitis/Chronic Pelvic Pain Syndrome (CP/CPPS) are unknown. The purpose of this study is to test the hypothesis that genetic polymorphisms of candidate genes COMT, CGH1, IL10, TNF, or HMGB1 contribute to the development of CP/CPPS. We will also capture data on past medical history to assess whether specific medical conditions such as cardiovascular disease, hematologic disease and psychiatric conditions are associated with specific polymorphisms, and if these conditions define specific subgroups of patients with CP/CPPS. We will measure RNA and correlate with specific polymorphisms to confirm the mechanism of action.

Duration of Project

12/1/2011 – 12/31/2013

Project Overview

The etiology and pathogenesis of Chronic Prostatitis/Chronic Pelvic Pain Syndrome (CP/CPPS) are unknown. Several candidate genes involved in chronic inflammation and/or chronic pain conditions (COMT, CGH1, IL10, TNF, and HMGB1) have been found to be genetically polymorphic. Our preliminary data indicates that the incidence of several of these polymorphisms are greater in patients with CP/CPPS than controls, either in the entire group of CP/CPPS patients, or correlating with specific aspects of past medical history. Therefore, in the current project, we plan to test the hypothesis that genetic polymorphisms of candidate genes COMT, CGH1, IL10, TNF, or HMGB1 contribute to the development of CP/CPPS. We will also capture data on past medical history to assess whether specific medical conditions such as cardiovascular disease, hematologic disease and psychiatric conditions are associated with specific polymorphisms, and if these conditions define specific subgroups of patients with CP/CPPS.

Specific Aim 1: Test the hypothesis that genetic polymorphisms in candidate genes correlate with the incidence of CP/CPPS. This will be tested in a group of patients with CP/CPPS compared to a group of asymptomatic controls.

Specific Aim 2: Test the hypothesis that genetic polymorphisms in the promoter regions of candidate genes also modulate their expression, resulting in alterations in the level of mRNA.

Patients with CP/CPPS will be recruited from the PI's practice at Temple University. A group of age matched asymptomatic controls will also be recruited. Samples including saliva and blood will be drawn for obtaining DNA and RNA. Symptoms will be assessed using the NIH-Chronic Prostatitis Symptom index and a past medical history questionnaire developed for an NIH study of CP/CPPS. Inclusion, exclusion and deferral criteria will be the same as the NIH Prostatitis cohort study.

We plan to correlate the symptoms with the polymorphisms found, comparing patients with and without CP/CPPS. Specific phenotypes of patients with CP/CPPS, i.e., those with specific items of past medical history, will be correlated with the frequency of polymorphisms. We will measure RNA and correlate with the polymorphism abnormalities to confirm that the polymorphisms are indeed causing alterations in RNA produced and to confirm the mechanism of action.

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Expected Research Outcomes and Benefits

The relation of genotypes with corresponding phenotypes will help to prospectively define the functional significance of the SNPs and define the relationship between genetic polymorphisms and CP/CPPS. Findings include the possibility that some SNPs are different in all patients with CP/CPPS compared to controls, whereas others may only be found more frequently in CP/CPPS patients with certain medical problems, such as cardiovascular disease. To establish the mechanism, alterations in RNA need to be confirmed in patients who have the polymorphisms identified.

If proved true, these findings will help elucidate the molecular mechanisms leading to pelvic pain and define possible treatment strategies for this syndrome. CP/CPPS is a common condition, with very poor quality of life, and for which no standard therapy has been identified. The goal and significance of this project is to identify a mechanism of action that contributes to the symptoms of CP/CPPS and can be exploited in treatment. For example, if patients with CP/CPPS are found to be IL-10 deficient, then administering IL-10 becomes a potential therapy, as has been done in other inflammatory and pain syndromes. Differences in SNPs for the other genes are also potential therapeutic targets. It is possible that the identified SNPs could be used as markers. For example, if IL-10 correlates with both the presence of CP/CPPS and cardiovascular disease, then men with CP/CPPS who have IL-10 gene polymorphisms should

also be screened for cardiovascular disease.

Summary of Research Completed

In the current funding period we have done a quality check on the RNA obtained and measured levels of MRNA for TNF-a, the SNP that has been found to be significantly associated with CPPS compared to controls with SNPs rs1800629 in the *TNF* gene having an odds ratio of 3.691, (95% CI 1.153-11.808, p=0.0278).

MATERIALS AND METHODS

Previously collected samples and the ladder were stored at (-20°C). The RNA was analyzed with the Agilent 2100 bioanalyzer using the Agilent RNA 6000 Nano Kit provided from Agilent Technologies (Waldbronn, Germany). The assay quantitative accuracy and reproducibility of quantitation are reported as being 20 % coefficient of variation (CV) for ladder and 10 % CV for sample respectively. The reagents and reagent mixes were refrigerated at 4°C. Dye and dye mixtures were protected from light. All reagents were allowed to equilibrate to the room temperature for 30 minutes before use. Samples were kept on ice during the experiment.

RNA analyzation:

Initially quality control was performed on the available samples of RNA by capillary gel electrophoresis. 550 µL of RNA gel matrix (red) was pipetted in to into a spin filter, and centrifuged at 1500 g ± 20 % for 10 min at room temperature. 65 µL of filtered gel was aliquotted into 0.5 mL RNase-free microcentrifuge tubes. RNA dye concentrate (blue) was equilibrated to room temperature for 30 min. The RNA dye concentrate (blue) was vortexed for 10 s, and then we added 1 µL of dye into a 65 µL aliquot of filtered gel; this solution was vortexed, and then centrifuged at 13000 g for 10 min at room temperature. Nine µL of the gel-dye mix was loaded into wells containing an RNA chip on the chip priming station. As a marker, 5 µL of RNA marker (green) was put in each well. One 1 µL of prepared ladder and 1 µL of sample were added to the well. The chip was then vortexed for 1 min at 2400 rpm. Each RNA chip contains an interconnected set of microchannels that is used for separation of nucleic acid fragments based on their size as they are driven through it electrophoretically.

Following the confirmation of RNA quality, samples with adequate quality RNA were quantified by qRT-PCR with TaqMan gene expression kit using GAPDH endogenous control. TNF-a RNA label was normalized by the relative standard curve method using sample 8014 for normalization.

PCR methods: samples were prepared using the following components: RNase-free water (variable); 10X TaqMan RT Buffer (volume/tube: 1.0 mL; final concentration: 1X); 25mM MgCl₂ (volume/tube: 2.2 mL; final concentration: 5.5 mM); deoxyNTPs Mixture 2.5 mL (volume/tube: 2.0 mL; final concentration: 500 mM per dNTP); Random Hexamers 50 mM (volume/tube: 0.5 mL; final concentration: 2.5 mM); RNase inhibitor 20 U/L (volume/tube: 0.2 mL; final concentration: 0.4 U/mM); MultiScribe Reverse Trascriptase 50 U/mL (volume/tube: 0.25 mL; final concentration: 1.25 U/mL); total volume/tube 6.15 mL.

Thermal cycling parameters for reverse transcriptase reactions: Incubation for 10 min at 25°C; Reverse Transcription for 30 min at 48°C; Reverse Transcription Inactivation for 5 min at 95°C.

RESULTS:

From 40 samples included in the study, 22 had good RNA quality and the rest (18) had poor RNA quality, which precluded their use. Values are listed as relative to patient 8014, used as a standard for the curve.

The mean of the CPPS patients is 0.57 relative to the index compared to 0.21 for the controls, or between 2-3 times the amount of TNF-α mRNA on average per patient. By a 2 tailed unpaired T test this is statistically significant (p= 0.016) despite the small number of patients. The correlation of level of TNF-α mRNA in a given patient correlates better with the pain score (question 4 on the NIH-CPSI) r= 0.46 than with the total NIH-CPSI score, r= 0.05.

cases TNF mRNA	
8013B	0.889826
8019B	1.368918
8014B	1
8032	0.802611
8011B	0.728576
8038	0.708071
8015B	0.679806
8016B	0.45333
8033	0.411638
8043	0.369423
8017B	0.277099
8007	0.122031
8041	0.016502
8044	0.016502
8039	0.795692
mean	0.576002
st dev	0.383996

controls TNF mRNA	
8012B	0.551366
8037	0.476575
8042	0.363632
8010B	0.097319
8027	0.016502
8034	0.016502
8036	0.016502
mean	0.219771
stdev	0.236484

Research Project 9: Project Title and Purpose

Characterization of the Effects of Hypertension on Adult Brain Neurogenesis – Our underlying hypothesis is that hypertension impairs neurogenesis by decreasing the number of neural stem cells (NCS) in the subventricular zone (SVZ) and the dentate gyrus leading to changes in intrinsic properties and functional characteristics. Changes in intrinsic properties that affect subsequent neural progeny turn-over and diminished growth factor support to the neuronal

environment. No studies to date have directly evaluated the effect of hypertension on the neurogenic response. Besides the well-known susceptibility of hypertensive individuals to stroke there is also strong correlation between hypertension and mild cognitive impairment. The NSC dysfunction in hypertension being studied here would shed light on explaining the occurrence of both mild cognitive dysfunction and poor stroke recovery when hypertension is present.

Duration of Project

12/1/2010 – 6/30/2012

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

Research Project 10: Project Title and Purpose

PDMD: A Complex II-Regulated Form of Mitochondrial Dysfunction in Exhausted Cells – Functional impairment of mitochondria contributes to many human diseases including myocardial infarction, stroke, cancer and aging. However, the underlying mechanisms of mitochondrial dysfunction are not well understood. Our preliminary studies elucidate a mode of mitochondrial depolarization that is independent of cyclophilin D, Bcl-2 family proteins, extra mitochondrial energy, or high amplitude swelling. We termed this mode of mitochondrial dysfunction proton-dependent mitochondrial depolarization (PDMD), which prominently manifests in ischemic cells. The evolutionarily conserved PDMD is dependent on dissipation of the mitochondrial potential and can be reversed by the mitochondrial substrate. This route of reversible mitochondrial impairment provides a potential target for rejuvenation of exhausted cells in acute injury.

Duration of Project

12/1/2010 – 12/31/2011

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

Research Project 11: Project Title and Purpose

Role of the B55 α Protein Phosphatase 2A Holoenzyme in Modulating the Phosphorylation of p107 and Related Proteins – Members of the retinoblastoma family of growth suppressor proteins are inactivated via phosphorylation by cyclin dependent kinases (CDKs) in mid-G1 through mitosis. Hyperphosphorylation of these proteins inactivates them as repressors of E2F-

dependent transcription and prevents interaction with other proteins. Pocket protein phosphorylation is challenged through the cell cycle by protein phosphatase 2A (PP2A), and we have recently identified a trimeric PP2A holoenzyme containing the regulatory B55 α subunit that dephosphorylates p107 and p130. The purpose of this work is to characterize this complex in reactivation of pocket proteins in response to anti-mitogenic signals, as tumor cells are often refractory to anti-mitogenic signaling.

Duration of Project

12/1/2010 – 8/31/2011

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

Research Project 12: Project Title and Purpose

Are There Sex Differences in Kappa Opioid Receptor-Mediated Pharmacology? – Activation of the kappa opioid receptor (KOPR), one of the three types of opioid receptors (mu, delta and kappa), produces effects such as analgesia, sedation, dysphoria, water diuresis, antipruritic effects and attenuation of cocaine craving in addicts. Clinical studies have suggested sex differences in KOPR-induced analgesia. We have demonstrated that the selective KOPR agonist U50,488H produces higher levels of antinociception and sedation-like behavior in male than female guinea pigs. The underlying mechanisms are unclear. In the proposed studies, we will examine whether sex differences exist in KOPR distribution and signaling and alteration of neuronal circuitry activities by KOPR activation. We will investigate in vivo sex differences by measuring 1) receptor autoradiography, 2) G-protein coupling using [³⁵S]GTP γ S autoradiography and 3) immunohistochemical labeling of p44/42 MAP kinase activation induced by systemic KOPR agonist, U50,488H using intact male and female guinea pigs across the estrous cycle. The guinea pig will be used as a model since its KOPR levels and distribution in brain resemble those of the human, unlike those of the rat and mouse.

Duration of Project

12/1/2010 – 6/30/2011

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

Research Project 13: Project Title and Purpose

Enhancing Stem Cell Engraftment and New Myocyte Formation in the Damaged Heart – The purpose of this study is to develop and test novel approaches to enhance the survival and engraftment of cardiac stem cells in the injured heart. We have characterized a population of resident cardiac stem cells in the normal heart and have shown that these cells have the capacity to differentiate into new cardiac myocytes. In the proposed research we will modify the properties of these stem cells so that they have enhanced survival in the hostile environment of the injured heart. We will also modify the stem cells so that they have an enhanced ability to electrically couple to cardiac myocytes. The purpose of the research is to test the idea that enhancing coupling increases survival and induces differentiation.

Duration of Project

9/1/2011 – 8/31/2012

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

Research Project 14: Project Title and Purpose

Neural Effects of Acute Traumatic Brain Injury – The purpose of this research is to investigate how brain neural activity is altered in response to traumatic brain injury. Whereas other researchers have investigated both short and long term neural behavior in brain injury patients, there has been no systematic study of what changes occur in the moments immediately following the insult or how these changes vary as a function of the severity of the injury. That knowledge is a crucial missing link in understanding the mechanisms of brain injury; a deeper understanding will lead to enhanced strategies for prevention, detection, and treatment.

Duration of Project

12/1/2010 – 12/31/2012

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

Research Project 15: Project Title and Purpose

NIBP/NF κ B Signaling in Neurogenesis – Neurodevelopmental disorders and cognitive/mood dysfunction are major public health issues. Impairments in embryonic and adult neurogenesis are

implicated in such diseases. The molecular mechanisms that control various stages of neurogenesis remain poorly understood. We have identified a novel protein NIBP that is required for NFκB activation and neuronal differentiation as well as trans-Golgi networking. NIBP has been also implicated in human mental retardation and autism. The goal of this project is to understand the underlying cause-effects and mechanisms of NIBP/NFκB signaling in neurodevelopmental disorders. We will test the central hypothesis is that NIBP acts as a novel proneuronal protein to guide neuronal lineage differentiation through NFκB signaling during neurogenesis.

Duration of Project

9/20/2011 – 6/30/2013

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

Research Project 16: Project Title and Purpose

Relationships of Histochemistry to Muscle Activity in the Child with Cerebral Palsy – Cerebral palsy (CP) is characterized by altered muscle activity and decreasing function with age. Altered muscle physiology has also been reported in CP; however, the relationship between muscle activity and muscle physiology is not known. If relationships can be identified, it may possible to predict which clinical interventions will be appropriate for the child with CP. The significance of this study is that an understanding of the histochemical mechanisms behind observable changes in muscle activity will be determined and aid in the development of an assessment method that will allow clinicians to determine which child with CP will respond best to a given clinical intervention. This will reduce the number of ineffective treatments that a child with CP may undergo, and reduce the costs of care and risks to the child.

Duration of Project

9/20/2011 – 6/30/2012

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

Research Project 17: Project Title and Purpose

Stem Cell Mediated Repair of the Ischemic Heart – The purpose of the research is to determine if

cKit+ bone marrow derived stem cells, when injected into the border of the infarct border zone, can enhance endogenous repair.

Duration of Project

9/20/2011 – 12/31/2013

Project Overview

The central theme of the proposed project is to develop novel therapies for the post myocardial remodeling that improve the structure and function of the heart. The major goal of our project is to test the idea that either a population of bone marrow derived stem cell that are thought to be cardiovascular precursor cells or gene therapy to enhance cardiac regeneration will enhance the repair of the heart after a myocardial infarction (MI).

The Swine MI model: We have developed a facility for induction of myocardial infarction and evaluation of the subsequent disease progression in large mammals. We have all the equipment needed for an induction and evaluation of MI in pigs. These large animal models are essential for preclinical testing of novel cell and gene therapy products and novel delivery systems. The data generated in our large animal laboratory are essential for commercializing any novel therapeutics or devices or for applications requiring FDA approval before testing begins in humans.

Cell and Gene Therapy Development: Cell therapy: We have developed an approach to isolate and expand cKit+ stem cells from the bone marrow of the pig. These cells are labeled with GFP (so we can follow their fate in-vivo). We are also able to introduce genes that we feel will enhance the survival and engraftment of these cells into damaged hearts. We believe that novel cell therapies be able to regenerate heart tissue after injury. Gene Therapy: We will also take advantage of the newly developed AAV core and a novel production technology to produce the quantities of AAV vectors we will require for experiments in large animal models.

The post MI project: MI is induced by balloon occlusion of the left anterior descending artery for 60 minutes, followed by reperfusion. This results in a large infarction and depressed heart function. 24 hours after MI, either cKit+ stem cells or AAV-mediated gene transfer of bARK-CT (to normalize adrenergic signaling defects) or a novel caveolin targeted Ca channel blocker, termed Cav-Rem1-265, will be injected into the heart. Animals will be followed for two months. Cardiac structure and function will be measured. At sacrifice we will determine if either of the two therapies have been effective in reducing infarct size, enhancing regeneration and improving cardiac function. It is our goal to identify novel therapies that can be used to more effectively treat patients with ischemic heart disease.

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Expected Research Outcomes and Benefits

Myocardial Infarction results from an interruption of blood flow to a portion of the heart that causes the death of cardiac tissue reliant on constant perfusion. MI reduces functional myocardial mass which causes an abrupt decline in cardiac pump function. A host of reflex responses occur to maintain blood pressure and cardiac output, including activation of sympathetic and neuroendocrine systems. These responses increase the Ca²⁺ dependent contractile function of surviving myocytes which helps maintain cardiac pump function. Over time, myocytes within the infarcted zone die, the surviving myocytes hypertrophy, there is some new myocyte formation, and the ventricle dilates. These changes provide a period of compensated pump function. However, the post MI heart usually has progressive ventricular dilation with ever worsening cardiac pump function (ejection fraction and dP/dt), culminating in a syndrome called Congestive Heart Failure (CHF). The goal of the proposed research is to identify and test novel therapies to reduce the damage caused by an MI and to enhance the repair of the heart.

We will test novel cell and gene therapies for post MI myocardial dysfunction. In one study we will test if a specific cell found in the bone marrow, a cKit⁺ cell, has the ability to enhance revascularization following MI. In two separate studies we will examine novel gene therapies, delivered through the coronary arteries. These therapies will test if altering signaling through adrenergic or calcium pathways can repair the damaged heart. We will test if these therapies improve heart function, which would improve the quality and duration of life for patients suffering from ischemic heart disease.

Summary of Research Completed

Ischemia reperfusion-induced MI was performed in all twelve minipigs to characterize the dysfunction over a three month period. Animals were then evaluated for a 12 week period post MI. To evaluate pathological manifestation and treatment, we performed serial transthoracic echocardiography at 5 time points throughout the duration of the study (Baseline, 4, 8, 12 weeks post MI). NOGA electromechanical mapping was also utilized for assessment of function.

METHODS

Ischemia-reperfusion induced Myocardial Infarction

The right femoral artery was cannulated with a 6-French percutaneous sheath and femoral venous access was also acquired with a 5-French percutaneous sheath for administration of any necessary pharmacological intervention. To prevent thrombosis throughout the duration of the procedure, heparin was administered with a loading dose of 100U/kg and a maintenance dose of 40U/kg every hour. A 5-French pigtail catheter was placed in the left ventricle and a ventriculography was performed. A 6-French guide catheter was used to cannulate the left coronary artery to perform arteriography (figure 1). Next, a 0.014-in angioplasty guide wire was

place in the left anterior descending coronary artery (LAD), then a 3.0mm x 12mm Quantum Maverick, monorail, balloon (Boston Scientific, M.A. USA) was placed in the LAD just distal to the first diagonal branch, all placements were performed under fluoroscopic guidance and recorded. The balloon was then inflated to 8.0 atmospheres for a period of 90 minutes and cessation of flow distal to the balloon was confirmed with another angiography (figure 1). ST-segment elevation was also used to assess onset on infarction (figure 1). After deflation of the balloon, the guide wire and balloon were removed and reperfusion of the LAD was confirmed by angiography (figure 1). Next, the guide catheter was removed and the animal was given 30 minutes of reperfusion prior to any subsequent treatment being performed.

Transthoracic Echocardiography

Transthoracic echocardiography was performed at baseline, 4, 8 and 12 weeks post MI with the Zonare z. one ultra Ultrasound system on all animals. Images were acquired in the 2D-mode in long- and short- axis; while m-mode images were taken in the short axis. All measurements including end-diastolic dimensions, end-systolic dimensions, end-diastolic volume (EDV), end-systolic volume (ESV), ejection fraction (EF) and fractional shortening (FS) were calculated by the American Society of Echocardiography guidelines.

NOGA Electromechanical Mapping and Intramyocardial Injections

Electromechanical assessment was performed pre- and post-MI to confirm the ischemia-reperfusion MI induction. This catheter based technique acquires 80-100 discrete points by contact with the endocardial surface. We're able to assess the changes in conductance and motion of the left ventricular myocardium. This technique also allows us to directly locate our injection sites, for accurate delivery of therapy (figure 2).

Tissue Processing & Gross Morphometric Analysis

Cardiectomy was performed under general anesthesia 12 weeks post MI. The heart was weighed; then the bottom third was sliced in cross-section. This portion was cut into two short-axis slices; the basal slice was then sectioned and frozen immediately in liquid nitrogen, then stored at -80°C for future protein analysis; the apex was submersion fixed in 10% formalin for histological analysis. The intact two-thirds of the heart were fixed via gravity perfusion with 2-liters of 10% formalin. Once fixed, this portion was sectioned into 3 short-axis slices. Each slice (including slice for protein analysis) was measured for mean thickness prior to further processing. The basal surface of each slice was photographed using a Nikon DS-F11 camera. The infarct area was identified by the scar, while the viable area was healthy tissue and both were measured using NIH Image J software. The infarct volume was calculated by multiplying the infarct area of each slice by the average thickness, this calculation was also performed for the viable area. The percent infarct volume of each heart was determined by adding the volume of all slices and dividing by the total infarct area.

RESULTS

Gross and Histological Analysis Post MI

Upon cardiectomy, the infarcted region is distinguished from other cardiac tissue through coloration and anatomical location (pale white/anterior-apical) (Figure 3). This gross visual observation confirms the location of the infarct to be distal of the occlusion via balloon angioplasty (observed on fluoroscopy). To assess hypertrophy we weighed the hearts of control

and post MI animals and looked at the heart-to-body weight ratio (HW/BW ratio). There was a significant increase in the HW/BW ratio at 12 weeks post MI (Figure 3). After fixation, we then sliced the heart into cross-section to observe the scar on the anterior, septal, and apical regions. To quantify the infarct, we assessed the infarct volume as a percent of the total ventricle volume at 12 weeks post MI to be $17.29 \pm .057\%$ (Figure 3).

After tissue processing for histological analysis we performed H&E staining to investigate changes in cellular morphometry. In Figure 4, we see representative H&E images using bright-field microscopy of three distinct zones which are created due to the ischemia-reperfusion injury (Infarct Zone [I.Z.], Border Zone [B.Z.] and the Remote Zone [R.Z.]). To investigate hypertrophy at the cellular level we measured myocyte-cross sectional area from H&E stained tissue sections of the control and MI groups. The mean LV myocyte cross-section was significantly increase at 12 weeks post MI vs. control Figure 3.

Cardiac Function Analysis

Cardiac structure and function was evaluated using echocardiography before and 4, 8, and 12 weeks post MI (Figure 5). During the 12 weeks after MI there was a progressive depression of pump function (Ejection Fraction [EF] $66.52\% \pm 1.16$ [baseline] to $32.66\% \pm 1.02$ [12 weeks after MI] $p < 0.0001$) and dilation of the LV volumes (End-diastolic volume [EDV] was $40.38 \text{ ml} \pm 3.01$ [baseline] to $75.73 \text{ ml} \pm 3.18$ [12 weeks post MI] $p = 0.0123$ and End-systolic volume [ESV] was $15.14 \text{ ml} \pm 2.32$ to $50.75 \text{ ml} \pm 1.86$ $p = 0.0004$). This was confirmed with our ADI hemodynamic instrument, which allows us to visualize the pressure-volume relationship as it changes over the three month time period. There was a shift in the relationship indicating increased volumes, confirming our echocardiography measurements, and decreases in the developed pressure during systole without stem cell treatment. Within the stem cell treatment group we saw less change from baseline indicating the preservation of chamber size post MI. Inhibiting the dilation of the chamber indicates that the cell therapy is slowing the progression of the pathological state towards dilated cardiomyopathy and CHF. Cell treatment significantly increased EF (from $32.66\% \pm 1.02$ to $41.43\% \pm 2.87$ $p = 0.0282$). Cell therapy also significantly reduced EDV and ESV (from $75.73 \text{ ml} \pm 3.18$ to $63.58 \text{ ml} \pm 4.06$ $p = 0.0494$, and from $50.75 \text{ ml} \pm 1.86$ to $37.45 \text{ ml} \pm 3.52$ $p = 0.0156$ respectively). Having the capability to confirm our echocardiography measurements to the hemodynamics assures us that in fact our treatment strategy is having a beneficial effect on the heart post MI. This effect is through inhibition of progressive expansion of the infarct zone upon reperfusion and the dilation of the chamber over the long-term. These data show that acute cell therapy treatment reduces infarct size preserves cardiac function post MI.

Figure 1.

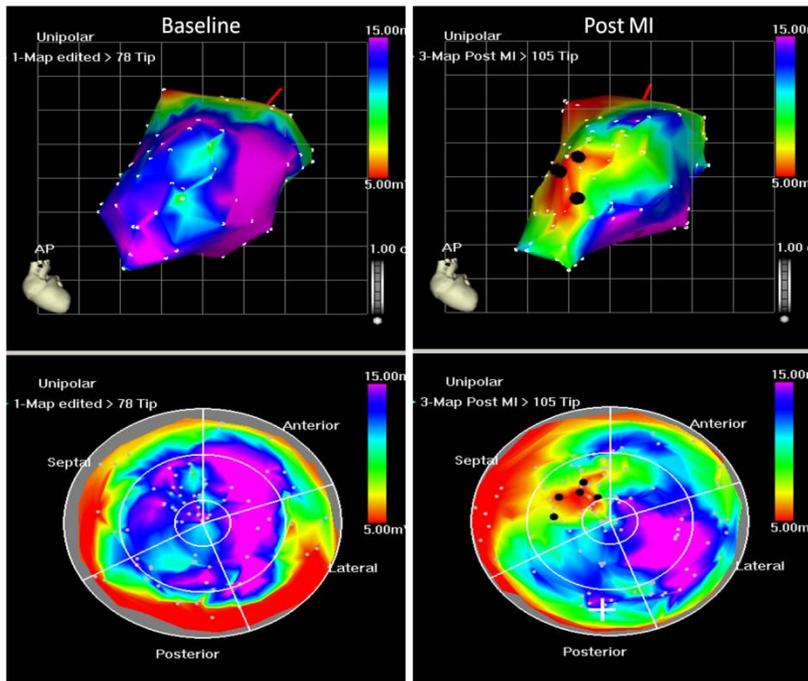
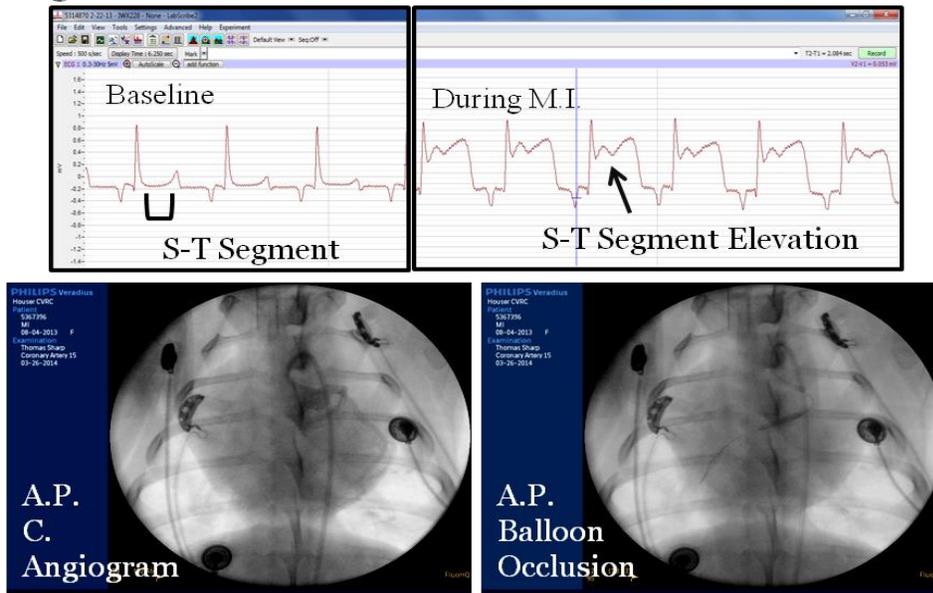


Figure 2.

Figure 3.

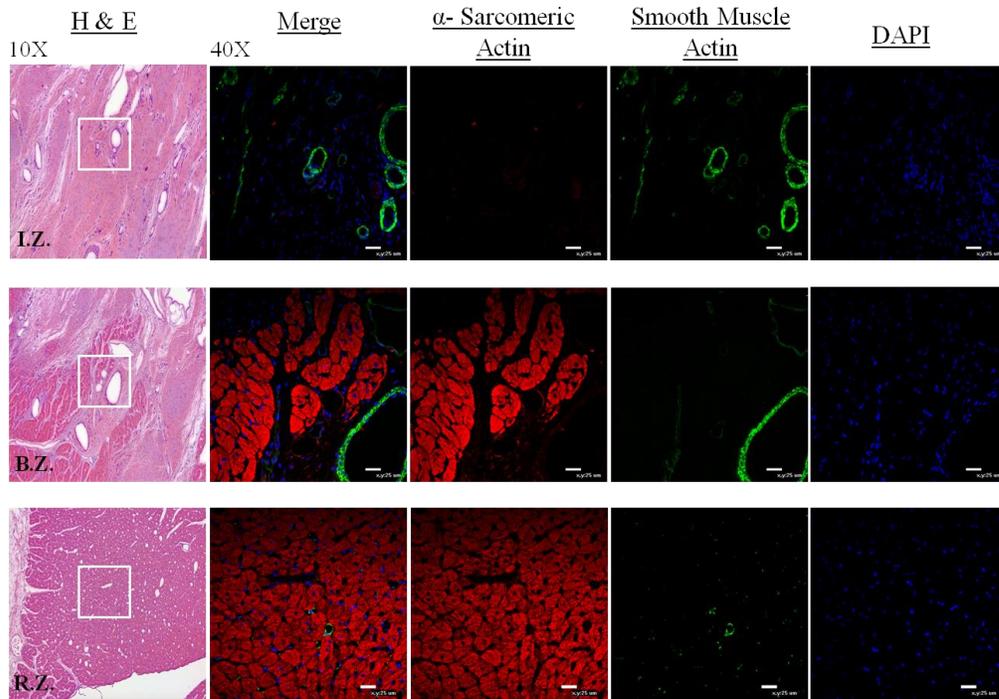
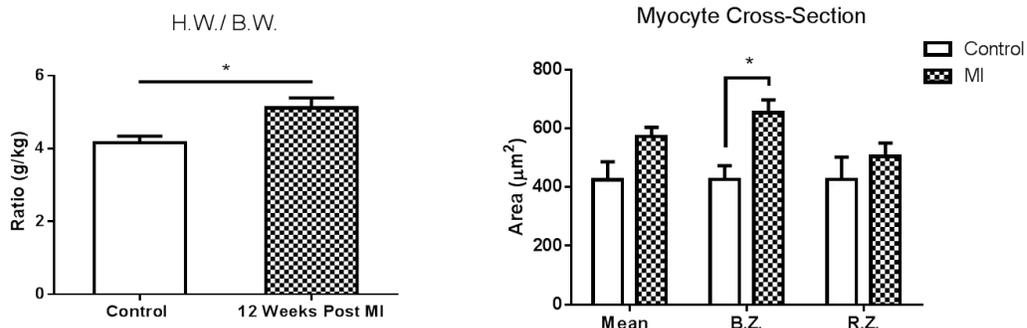
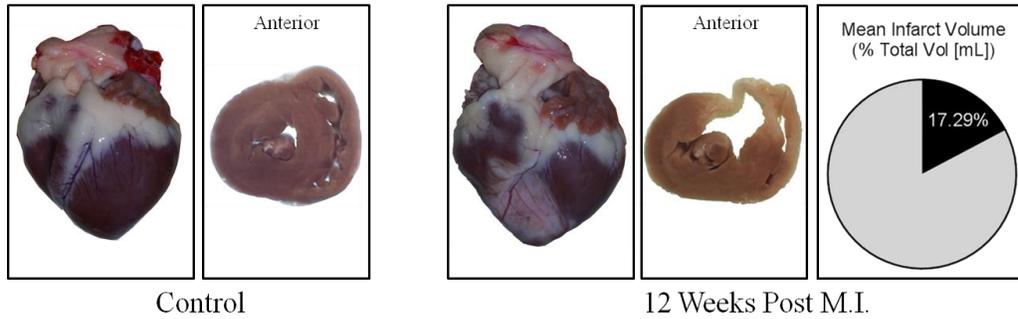
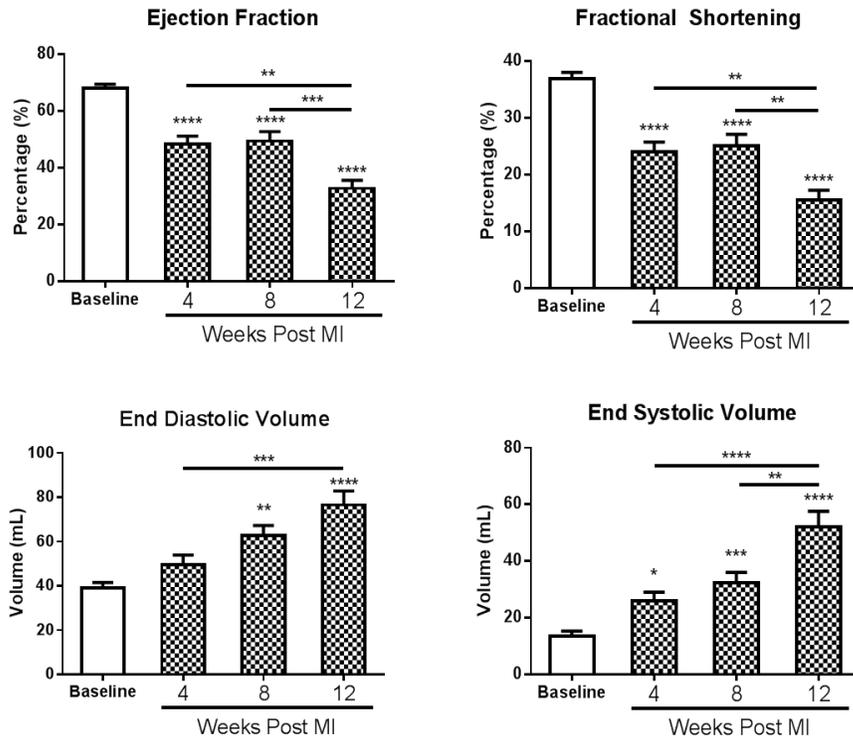


Figure 4. Representative bright field and confocal imaging of heart tissues from our preliminary MI study in swine. Immunohistochemistry was performed for structural proteins to identify viable vs. non-viable tissue in the three distinct zones (infarct zone [IZ], border zone [BZ] and remote zone [RZ]).

Figure 5.



Research Project 18: Project Title and Purpose

Enhancing Effective Healthcare Decision Making with the Use of Brain Imaging Tools – The purpose of this project is to enhance decision making in healthcare by identifying and suppressing self-positivity bias. Self-positivity bias is a major problem in healthcare by preventing people from pursuing healthy lifestyles, obtaining proper health care, and adhering to health recommendations. Self-positivity bias is especially serious among older patients, minorities, and the poor. Using functional Magnetic Resonance Imaging (fMRI) tools, we seek to identify the neural correlates of self-positivity bias to understand its neural origins and accordingly design customized decision aids that overcome it for specific diseases. In doing so, we aspire to help people engage in effective healthcare decision-making, pursue a healthier lifestyle, seek preventive medical care, and adhere to medical advice by healthcare providers to prevent diseases.

Duration of Project

9/20/2011 – 12/31/2013

Project Overview

Effective healthcare depends on the extent to which patients adhere to messages for a healthy

lifestyle, follow their physicians' advice in the course of an illness, and seek preventive treatment. However, patients often ignore these messages and advice because they tend to underestimate the likelihood of a disease happening to them. This phenomenon is formally termed self-positivity bias, and it was shown to prevent patients from adhering to health communications and advice. This project uses fMRI tools to enhance effective decision-making in a healthcare context, particularly for older adults, minorities, and the poor, by designing and testing decision aids that correspond to the brain's functionality to help patients reduce self-positivity bias.

fMRI tools can help identify the neural correlates of self-positivity bias (which areas of the brain are activated when self-positivity bias is triggered). They measure the brain activity induced by self-positivity bias, when subjects are asked to estimate their own versus other people's probability of contracting a certain disease. Based on the neural correlates of self-positivity bias across common diseases (i.e., AIDS, STDs, cancer) and analysis of their underlying neural origins drawn from the neuroscience literature, we will design decision aids and interventions that seek to overcome the negative effects of self-positivity bias and motivate people to pursue a healthier lifestyle, seek preventive medical care, and adhere to medical advice by healthcare providers.

Depending on how self-positivity bias is mapped onto the brain for the set of common diseases and what brain areas are activated (i.e., cognitive versus emotional areas), we will design customized decision aids that specifically target self-positivity bias for the specific disease. These aids will be first evaluated on their potential to overcome self-positivity bias by suppressing brain activity. Then, we will test these decision aids on their potential to reduce self-positivity bias and encourage patients to behaviorally adhere to proper health practices. The studies will be conducted across different populations (older adults, minorities, and the poor) to assess the susceptibility to self-positivity bias, thus rendering them less likely to seek medical care and adhere to medical advice. Comparisons in the neural correlates across populations will be conducted across diseases, and the possibility to design customized decision aids and interventions across different populations will be evaluated based on the fMRI results. Accordingly, the behavioral field studies will use distinct decision aids for different populations based on the neural correlates of self-positivity bias in the focal populations.

Specific Aims: Specific Aim 1. The first objective is to identify the neural self-positivity bias perceptions across individuals in a behavioral study. Specific Aim 2. The second objective is to identify the neural correlates of self-positivity bias (which areas of the brain are activated when self-positivity bias is triggered) using fMRI tools, which allow the temporal and spatial measurement of brain activity. Specific Aim 3. The identification, analysis, and understanding of the neural origins of self-positivity bias across these common diseases will be used as a basis for designing customized decision aids and interventions for each disease that specifically target the neural correlates of self-positivity bias.

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Yanliu Huang, PhD – employed by Drexel University
Carolyn Yoon, PhD – employed by the University of Michigan, Ann Arbor, MI

Expected Research Outcomes and Benefits

The ultimate benefit of this research project is to enhance the quality of healthcare decisions of patients by identifying and overcoming a serious bias that prevents them from making effective healthcare decisions. Specifically, self-positivity bias has been touted as one of the primary reasons that patients engage in unhealthy lifestyles, do not seek proper medical care, and ignore important medical advice by physicians and healthcare providers. By identifying the neural correlates of self-positivity bias (which brain areas are activated) across diseases with fMRI tools, it is possible to design and test appropriate customized decision aids and interventions that would reduce self-positivity bias and encourage people to make effective healthcare decisions. We believe that proper decision aids that correspond to the brain's underlying functionality across common diseases will be more effective than behaviorally-induced interventions that are susceptible to introspection.

Special attention will be paid to differences in the degree and type of self-positivity bias among older adults, minorities, and the poor who are generally more susceptible to self-positivity bias and are more prone to diseases. Thus, a comprehensive set of customized decision aids will be designed based on the neural correlates of self-positivity bias across common diseases, and they will be tested in a field setting to assess their behavioral effectiveness to reduce self-positivity bias and encourage people to make effective healthcare decisions. The expected outcomes of this research will vary across constituents. For patients, the decision aids could help reduce self-positivity biases and encourage people to adhere to safe lifestyle practices, seek proper medical care, and follow physicians' advice. For physicians, decision aids could be used to more convincingly offer medical advice to different populations. For healthcare policy officials, decision aids could be used to encourage the public to pursue healthier lifestyles against specific diseases, encourage people to seek preventive treatment, and promote appropriate medical care.

Summary of Research Completed

For this part of the study (aim 1), we explored the nature of the self-positivity bias and its implications for the domain of health marketing. 154 participants were recruited using Amazon Mechanical Turk. 15 were excluded from the data analysis process after failing an attention

check placed in the experiment. Data from 139 participants were used in the data analyses. Of the participants, the average age was 37 (min: 20, max: 70, median: 33). For the experimental task used in the behavioral pilot study, participants were first asked to complete a demographics questionnaire. Next, participants had to provide information about the health profile of an average person in their peer group.

In the first block of the experiment, participants were presented with the same three diseases as used in the previous two parts of the project; STD, Cancer, and Drinking Problem. In each trial, subjects were presented with a disease and were asked either to estimate their likelihood of developing/contracting the disease or to estimate the likelihood that an average person in their peer group would develop/contract that disease. Between the first and second block, participants completed the State-Trait Anxiety Inventory (STAI) and The Rosenberg self-esteem scale (RSES). In a second block, participants were asked to make the same judgments about the diseases they had previously seen, and were shown the actual probabilities again. To ensure that participants were reading the instructions and paying attention, the attention check was embedded in the second block.

In a third block, participants were asked to answer four questions for the three diseases. First, to measure perceived controllability of each disease, participants were asked to judge whether or not a person could control whether or not they developed/contracted a specific disease. Second, participants were asked to judge how serious or dangerous a disease is. Third, participants had to indicate, relative to other diseases seen, the desirability of developing/contracting a specific disease. Finally, as a test of memory, participants were asked to recall the actual probability of developing/contracting each disease. Finally, at the end of the experiment participants had to complete a lifestyle survey as well as the Life Orientation Test-Revised (LOT-R) questionnaire.

Pre Phase. Participants are asked to make estimations for 3 diseases (STD, Lung Cancer, Drinking Problem). The actual probabilities for each disease, respectively, are: 1) 35% 2) 7% 3) 10%

Post Phase. After making judgments for all 3 diseases and being exposed to the average probability of each disease, participants are asked to make the same judgment again for the same 3 diseases for self and other.

The data were analyzed using SPSS. Preliminary analyses were carried out to see if the self-positivity bias was observed for each disease. If estimations for ‘self’ are significantly lower than estimations for ‘other,’ then it can be inferred that the self-positivity bias has been exhibited.

First, pre and post phase estimations for self and other were analyzed using the two analysis frameworks outlined above. ANOVAs were run in SPSS. From the preliminary analyses, participants exhibited the self-positivity bias for two of the three selected diseases (STD and Drinking Problem). Since participants did not show a significant self-positivity bias in estimations for lung cancer, this disease was excluded from the analyses (Figure C2a, and C2b). We also calculated if subjects overestimated (estimated higher than the actual percentage of contracting the disease) or underestimated (estimated lower than the actual percentage of contracting the disease).

Next, ANCOVAs were carried out to analyze the moderating effect of the covariates in the study (controllability, seriousness, desirability, memory, LOT-R, Figure C3). The only covariates that seemed to have any effect on pre and post measures were: desirability and memory (Figure C4).

From the analyses done using the “overestimation/underestimation” framework, the only covariate that had a significant effect was memory.

STD Results: For these results the actual probability of the average person contracting an STD was 36%. The results indicated that participants showed a self-positivity bias when making estimations for the STD category. Significance was seen in both the pre-condition and the post-condition.

Self-estimations were significantly lower than other estimations for both diseases with high controllability ratings and those with low controllability ratings. When taking a closer look at self-estimations, the results indicate that estimations were significantly lower for participants in the high controllability group, for both the pre and post conditions. In both the pre and post conditions, self-estimations were significantly lower than other estimations for both diseases with a high seriousness classification and those with a low seriousness classification.

In both the pre and post conditions, self-estimations were significantly lower than other estimations for participants who gave a high desirability classification than those who gave a low desirability classification.

Participants were categorized as high and low optimists based on LOT-R scores. In both pre and post conditions, self-estimations were significantly lower than other estimations for STD with a high optimism classification and those with a low optimism classification.

Lung Cancer Results: For these results the actual probability of the average person contracting lung cancer used was 7%. There was no significant difference between self and other estimations in either the pre or post condition for Lung Cancer. For lung cancer, in the pre-condition, only participants in the high controllability group showed a self-positivity bias.

There was a significant difference in pre estimations for self, with estimations being lower for participants in the high controllability group than those in the low controllability group. There was a significant difference in pre estimations for self, with estimations being lower for participants in the high desirability group relative to those in the low desirability.

Participants were also categorized as high and low optimists based on LOT-R scores. In both the pre and post conditions, self-estimations were significantly lower than other estimations for lung cancer with a high optimism classification and those with a low optimism classification.

Drinking Problem Results: For the drinking problem results the actual probability of the average person having a drinking problem was 10%. The results indicated that participants showed a self-positivity bias when making estimations for the STD category. Significance was seen in both the pre-condition and the post-condition.

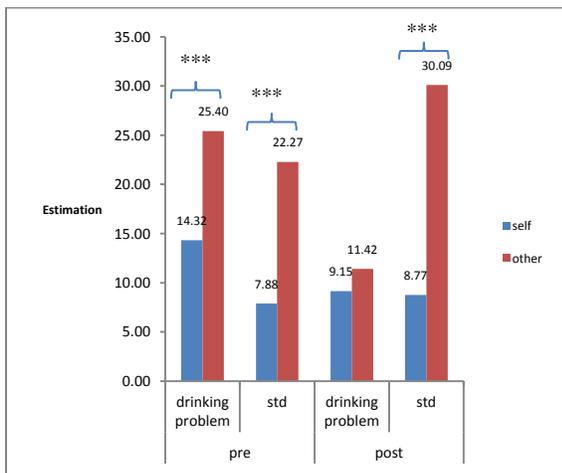
In the pre-condition, in both the high and low controllability splits, self-risk estimations were significantly lower than other-estimations. When looking at pre estimations for drinking problem, self-estimations were significantly lower than other-estimations for participants in both the high and low seriousness groups. For drinking problem, in both the pre- and post-conditions, self-estimations were significantly lower than other-estimations only for participants who categorized drinking problem as having low desirability. In the pre-condition, participants in the low and high optimism groups gave significantly lower self-estimations than other-estimations.

Discussion

In this part of the study, we were able to replicate previous findings, i.e., that people update for only overestimation but not for underestimation cases. Also, subjects updated estimations for others more than for themselves, especially for underestimation cases. We also found that covariates such as controllability, memory, seriousness, and desirability, LOT-R do not seem to be interacting with the updating process. These results apply to both STD and Drinking Problem, but not to Lung Cancer.

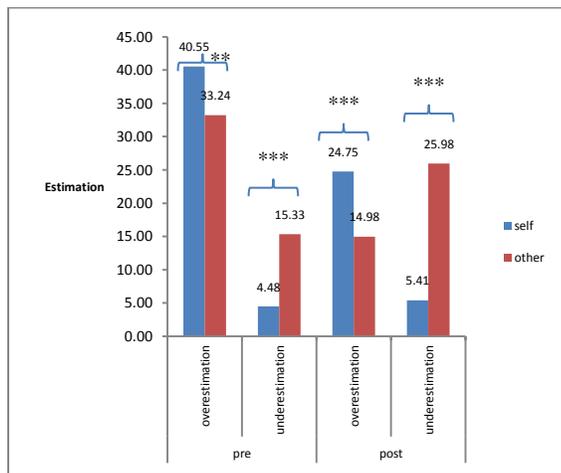
Comparing STD and Drinking Problem, it seems that people tend to underestimate the probability of STD contraction and overestimate the probability of developing a Drinking Problem. Thus, STD works like an “underestimation” case and Drinking Problem works like an "overestimation" case. After being presented with the actual probabilities, participants updated their estimations not enough for the "underestimation" case (STD) so the self-positivity bias was still observed in post measures for STD. Nonetheless, they updated their probabilities adequately (especially for others) for the "overestimation" case (drinking problem) so the self-positivity bias disappeared afterwards.

Figure C2a) “Disease” variable Analyses



- Pre-estimations for drinking problem and STD were significantly higher for ‘other’ than for ‘self.’
- In the post condition, other-estimations were significantly higher than self-estimations for STD.

Figure C2b) “Over/Underestimation” Split



- For “overestimations” in both pre and post, self-estimations were significantly higher than estimations for other.
- Conversely, in cases classified as “underestimations,” other estimations were significantly higher than those for self.

Figure C3. “Disease Variable” Analyses

Effect	F Value	Sig.
pre_post	0.04	0.851
pre_post*controllability	1.02	0.314
pre_post*seriousness	2.57	0.109
pre_post*desirability	7.08	0.008
pre_post*memory	3.92	0.048
pre_post*lotr	2.67	0.103
pre_post * Disease	14.38	0.000
pre_post * selfother	0.60	0.438
pre_post * Disease * selfother	42.43	0.000

Figure C4. “Overestimation/Underestimation” Analyses

Effect	F Value	Sig.
pre_post	.49	.484
pre_post*controllability	1.69	.195
pre_post*seriousness	.98	.322
pre_post*desirability	1.87	.172
pre_post*memory	5.87	.016
pre_post*lotr	1.88	.171
pre_post * overestimation/underestimation	239.06	.000
pre_post * selfother	7.06	.008
pre_post *goodbad*selfother	25.10	.000

Research Project 19: Project Title and Purpose

Temple PET/SPECT Imaging Tracer Initiative – Positron emission tomography (PET) and single photon emission computed tomography (SPECT) have become invaluable clinical and preclinical tools for imaging, diagnosis, treatment, basic research, drug discovery and drug development. Several PET and SPECT ligands are known, but novel research requires the development of novel radioligands. Temple University does not have an internal resource for generating novel PET and SPECT imaging radioligands. The proposed initiative will put in place a multidisciplinary center with the expertise and resources needed to design, develop and

produce novel PET and SPECT radioligands, and will provide a much needed platform to support collaborators' clinical, biological and drug discovery projects as well as a valuable training resource for scientists.

Duration of Project

9/20/2011 – 11/30/2013

Project Overview

The objective of the project is to establish a successful, self-sustaining multidisciplinary center with the expertise and resources needed to design, prepare and develop novel small molecule PET/SPECT imaging ligands. While the combined medicinal chemistry-PK/PD approach that will be used for the project is applicable to numerous therapeutic targets (and it is envisioned that the center will ultimately take on projects addressing numerous therapeutic targets), the inaugural project will target the identification of a novel PET/SPECT ligand for imaging 5-lipoxygenase (5-LO) in the CNS. Since the pharmacophore of most 5-LO inhibitors requires the presence of structural features that limit CNS penetration, a prodrug approach will be employed to design molecules that rapidly deliver high concentrations of the parent 5-LO inhibitor to the brain while being cleared quickly from the periphery. Suitable parent 5-LO inhibitors will be selected from the literature. Selection will be based on physicochemical properties (molecular weight, lipophilicity, polar surface area), potency for 5-LO and synthetic amenability. Prodrug candidates of the selected parent 5-LO inhibitors will be prepared and examined in vitro for their stability and conversion rates in plasma and brain. Compounds that display a potential therapeutic advantage over the parent 5-LO inhibitor will be examined in plasma and brain PK studies to confirm the therapeutic advantage as well as the suitability of the prodrugs as imaging candidates. For prodrugs that meet the criteria for advancement, an efficient synthesis will be designed in order to affect installation of the radioisotope late in the synthesis, preferably in the last step. With the preliminary data obtained from this inaugural study, funding will be sought to support the next phase of the project, the radiolabeling of the ligand prodrug and examination in small animal imaging studies.

Specific Aim 1: Identify appropriate 5-LO inhibitors or inhibitor scaffolds as starting points

Specific Aim 2: Identify prodrugs that will adequately deliver the parent 5-LO inhibitor to the brain

Specific Aim 3: Identify synthetic routes to 5-LO prodrug imaging candidates that are amenable to radiolabeling with PET and/or SPECT radioisotopes

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Other Participating Researchers

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Expected Research Outcomes and Benefits

The expected outcome of the research project is to establish a successful, self-sustaining multidisciplinary center that will focus on the design, development and production of novel PET and SPECT radioligands. Temple University currently does not possess an internal resource for designing novel imaging radioligands. Establishment of such a resource would provide support to collaborators' clinical and basic research programs. The tools provided by this initiative will facilitate understanding the molecular basis of disease and provide invaluable information on the pharmacotherapeutic effect of new drug candidates. Such support would ultimately lead to improved diagnostic accuracy and optimized patient care. The center would also provide training opportunities for scientists who wish to study imaging ligand design and would attract high quality graduate students and postdoctoral candidates to Temple University. This initiative will serve as a foundation for seeking external funding and could lead to generation of intellectual property and the potential for commercialization of novel PET and SPECT ligands. Additionally, revenue can be generated by conducting studies for external researchers in the academic and industrial sectors on a contractual basis.

Summary of Research Completed

Selection of a 5-LO inhibitor candidate for evaluation as a potential CNS PET/SPECT imaging agent.

MC-200022 was previously identified as having suitable physicochemical and pharmacokinetic properties to be considered as the lead molecule for further evaluation as a potential PET/SPECT imaging ligand. In an attempt to arrive at compounds with even better ADME properties, the des-methyl analogs ZD-21138 (MC-200023) and MC-200022 (MC-200024) were prepared and tested (Table 1). Both compounds possess adequate 5-LO inhibitory potency and in silico physicochemical parameters (logP, TPSA). However, both compounds were surprisingly stable to metabolism in mouse and human liver microsomes. An in vivo pharmacokinetic study with MC-200023 confirmed that the microsomal stability was predictive of an unacceptable half-life in vivo, which was 3.6 hours for MC-200023. Therefore, MC-200022 was selected for further studies.

Acquisition of samples of radiolabeled ligand for autoradiography studies.

The next step was to obtain samples of tritiated MC-200022 to perform a more detailed in vitro assessment of the compound's ability to serve as an imaging agent. American Radiolabeled Chemicals Inc., St. Louis, MO, has provided 5 mCi of [³H]-labeled MC-200022 with a specific activity of 80 Ci/mmol for these studies.

Identification of collaborators who will perform the autoradiography studies and, if appropriate, PET labeling and small animal imaging studies.

We have established a collaboration with Dr. Jinbin Xu of Washington University, St. Louis to perform the autoradiography studies designed to ascertain the suitability of MC-200022 to function as a PET imaging ligand. In vitro data on MC-200022 are shown in Table 1. The [³H]-MC-200022 prepared by American Radiolabeled Chemicals has been sent to Dr. Xu's labs and a Material Transfer Agreement and Confidentiality Agreement are in place. Dr. Xu's group will perform the binding affinity, binding density, non-selective binding, immunohistochemistry and autoradiographic imaging studies on rat and human brain samples (normal and Alzheimer's disease patient-derived) needed to assess the potential of MC-200022 as a PET imaging ligand. If adequate resolution and non-specific binding are observed and if MC-200022 distributes to regions of the rat brain known to be rich in 5-LO, then the results will trigger the next phase of the project, which involves generation of [¹¹C]-MC-200022 and small animal imaging studies.

We have identified the Massachusetts General Hospital PET Imaging Core as the group who will generate [¹¹C]-MC-200022 and perform small animal imaging studies provided that MC-200022 displays suitable results in Goal 3 activities.

Preparation and evaluation of a series of zileuton-based prodrugs as a back-up strategy.

To support Aim 2 and as a back-up strategy to Aim 1, a prodrug approach was initiated. While not common, prodrug approaches have been successfully used to arrive at PET ligands for imaging in the CNS. Zileuton was chosen as the prototype parent drug for this aim since it possesses an in vivo half-life that is suitable for PET imaging (2 hours). The potency of zileuton is not adequate for PET imaging, but a study detailing the work that led to the more metabolically stable and more potent analog ABT-761 provides possible insight into how to increase 5-LO inhibitory potency and even further reduce in vivo half-life. Therefore, the strategy was to use zileuton as a prototype of the hydroxyurea scaffold and determine if a suitable prodrug could be designed. Encouraging results would then trigger an SAR study to identify more potent, rapidly clearing analogs that could be combined with the prodrug approach to deliver high, rapidly cleared CNS concentrations of the parent compound.

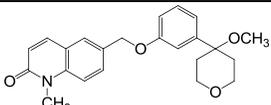
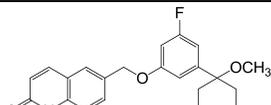
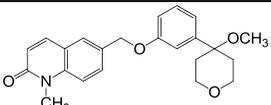
Since (R)-zileuton is considered to be the active enantiomer, the first step was to obtain suitable quantities of the stereoisomer. This was accomplished on a 10 g scale using supercritical fluid chromatography on a chiral HPLC column. (R)-zileuton was then converted to a variety of carbamate and carbonate analogs using the synthetic methodology outlined in Figure 1. Since lipophilicity plays a major role in CNS penetration, a range of lipophilicities was designed into the prodrug candidates.

Compounds were then screened for stability in C57bl/6 mouse plasma (the background strain for the murine Alzheimer's disease transgenic models like Tg2576 and 3xTg) on a 96-well platform at 37°C using procaine as a positive control. Data for a representative set of the prodrug candidates prepared to date are shown in Table 2.

As can be seen from Table 2, stabilities ranged from 0% remaining to 100% remaining after 1 hour @ 37C. Carbamates were more stable than corresponding carbonates and, with the exception of the morpholine derivative (Entry 8), steric bulk around the carbamate group enhanced stability. The prodrug derived from valine methyl ester was relatively stable (90% remaining after 1 hour), while a similar prodrug candidate derived from phenylalanine methyl ester was rapidly hydrolyzed by plasma. All compounds were concomitantly screened for aqueous solubility to insure that the plasma stability results were not influenced by solubility issues (data not shown).

Currently, efforts are underway to assess the stability of the prodrug candidates in mouse brain homogenates. Compounds that show rapid hydrolysis in brain tissue will be examined for their in vivo pharmacokinetic profiles in plasma and brain. Encouraging results in these studies would trigger the SAR campaign described at the beginning of this section.

Table 1. Data for MC-200022, MC-200023 and MC-200024.

			
	MC-200022	MC-200023	MC-200024
Potency vs 5-LO	IC ₅₀ = 18 nM	IC ₅₀ = 11 nM	IC ₅₀ = 20 nM
Microsomal Stability T _{1/2} , minutes	12	48	60
Protein Binding Free, Unbound Fraction	15%	11%	ND
Microsomal Partitioning Free, Unbound Fraction	71%	93%	ND
In Vivo PK – T_{1/2} (hrs)	2.7	3.6	ND
In Vivo PK – V_D (L/kg)	3	2.3	ND
Brain/Plasma Ratio	1.8	1.3	ND

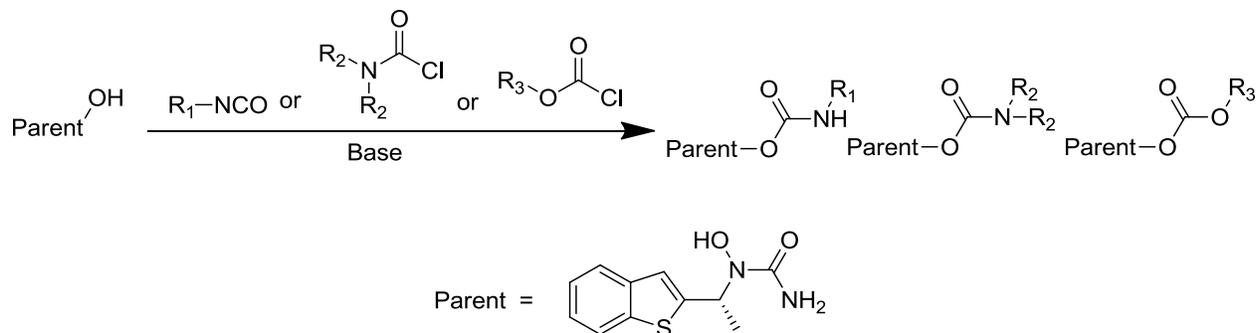
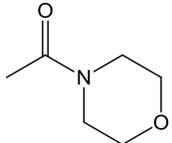
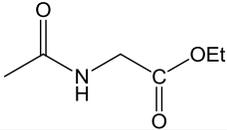
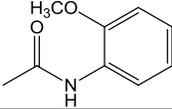
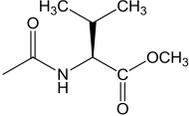
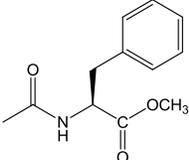


Figure 1. General Synthetic Scheme for Zileuton Prodrug Synthesis

Table 2. Mouse Plasma Stability of Selected (R)-Zileuton Prodrugs.

Entry	R	logP	MW	Mouse Plasma Stability % Remaining @ 1 hr (37C)
1		2.84	294	0
2		3.22	308	1
3		3.67	322	4
4		2.25	293	76
5		2.66	307	86
6		2.81	307	71
7		3.64	335	100

8		2.29	349	27
9		2.38	365	100
10		3.82	385	100
11		3.19	393	90
12		3.89	441	6

Research Project 20: Project Title and Purpose

Egr-1 Tumor Suppressor of Chronic Myeloid Leukemia – Chronic myeloid leukemia (CML) is one of the most successfully treated human malignancies, using the tyrosine kinase inhibitor imatinib. In spite of remarkable results continued treatment is necessary to prevent relapse; drug resistance is also a problem. There is a need to identify molecular markers that can predict if an individual will be receptive to therapy, and if during therapy the individual starts to become resistant. There is also a need for therapies using alternative strategies that can be used in combination with imatinib to prevent resistance or even to promote a permanent cure. We have data that early growth response gene (Egr)-1 is a tumor suppressor of CML. Our studies should provide new information about the initiation and progression of CML; this knowledge can be used for prognosis and to design novel therapeutics.

Duration of Project

9/20/2011 – 9/19/2012

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

Research Project 21: Project Title and Purpose

Identification of IFN Inducible STAT2 Regulated Apoptotic Genes – Interferons- α/β (IFNs) are small proteins that are secreted by many types of cells. IFNs can cause tumor cell death or prevent tumor cell growth. This observation led to the approval of IFN for the treatment of certain types of cancer. Yet little is known about the mechanism of how tumor cells are eliminated by IFNs. STAT2 is one critical molecule IFNs activate to induce the expression of many genes whose protein products in turn elicit a biological response. Our recent work shows that deletion of STAT2 prevents the killing effects of IFN thus suggesting that STAT2 is important for promoting tumor cell death. The purpose of this project is to identify apoptotic (death inducing) genes induced by IFNs that are dependent on STAT2.

Duration of Project

9/20/2011 – 9/30/2012

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

Research Project 22: Project Title and Purpose

Integrin-Mediated Adhesion of Osteoblasts to CTGF Induces Intracellular Signaling and Differentiation – Proposed studies will establish that connective tissue growth factor (CTGF) can function as an ECM-associated (matricellular) protein and demonstrate that some of its bone anabolic activities reside in its interactions with a specific integrin ($\alpha_v\beta_1$) expressed on the cell surface of osteoblasts. These CTGF-integrin interactions can initiate intracellular signaling cascades that regulate cell growth and differentiation, thereby providing a mechanistic interpretation for some of the bone anabolic effects of CTGF. Proposed experiments are expected to generate novel information regarding CTGF-specific mechanisms of action in osteoblasts, and these data can ultimately be used to develop new therapeutic strategies to treat bone loss (osteoporosis).

Duration of Project

9/20/2011 – 6/30/2013

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

Research Project 23: Project Title and Purpose

Novel Targeted Drug Delivery System for Overcoming Chemoresistance – Chemotherapy, which is considered standard of care for breast cancer, often decreases tumor size allowing for subsequent breast surgery followed by radiation and further adjuvant chemotherapy. However, given that most current anticancer agents do not greatly differentiate between cancerous and normal cells, systemic toxicity and adverse effects associated with these chemotherapeutics limit their treatment efficacy. In addition, acquired drug resistance further decreases the treatment efficacy of the chemotherapy. Therefore, there is a critical need to develop more efficacious therapies or delivery methods that decrease systemic toxicity, side effects, and overcome the drug resistance in the patient.

Duration of Project

9/20/2011 – 6/30/2013

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.