2014 Summer Research Program
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Undergraduate Students
Abstract:

A hallmark of Parkinson’s disease is the formation of Lewy bodies in the affected neurons. The main component of LBs is the protein alpha-synuclein (α-syn). Studies have shown that the level of phosphatidylethanolamine (PE), which is a major phospholipid in neurons, declines with age in rodents. We sought to determine how low PE affects the solubility of α-syn, which is known to associate with phospholipids. We have shown that low levels of phosphatidylethanolamine (PE) in yeast, caused by deleting the gene PSD1, cause stress in the endoplasmic reticulum and disrupts protein trafficking of α-syn. Here we used two drugs, sulfaphenazole (Sul) and meclofenoxate HCl (Meclo), identified by a drug screen, to determine if they rescue both the severe growth defect and ER stress of the double knockout mutant psd1Δ psd2Δ. Ethanolamine (ETA), which has been shown to rescue these mutants via conversion to PE by the Kennedy pathway, served as the positive control. DMSO served as the negative control. Choline, which is converted to another membrane lipid, phosphatidylcholine, and DMAE, a component of meclofenoxate HCl were also tested. Growth data showed that Sul, Meclo, ETA, choline, and DMAE all rescued the double mutant’s growth compared to control cells. Meclo and its component DMAE provided the greatest rescue effect. β-galactosidase assays, used to measure ER stress, showed that Meclo, choline, ETA, and DMAE also significantly reduced ER stress in the psd1Δ mutant compared to DMSO. None of the drugs reduced ER stress of psd1Δ to WT levels. These results show that Meclo improves growth and reduces ER stress of yeast with low levels of PE. Meclo will be tested in PD transgenic mice to see whether it improves motor functions, which are typically degraded in PD.
Drugs that Protect Cells From Lipid Stress in the ER: Implications for Parkinson's Disease

Alexandra L. Ross *, George M. Smith *, Shaoxiao Wang, and Stephan N. Witt
Department of Biochemistry & Molecular Biology, Louisiana State University Health Sciences Center, Shreveport, LA

Abstract

A hallmark of Parkinson's disease is the formation of Lewy bodies in the affected neurons. The main component of LBs is the protein alpha-synuclein (α-syn). Studies have shown that the level of phosphoα-synuclein (Pα-syn), which is a major phosphorylated isoform of α-syn, is increased in neurons of patients with Parkinson’s disease. We have shown that low PE levels can rescue the growth of α-syn aggregates in yeast and in a cell-free system. Our results suggest that PE is critical for the normal function of α-syn in regulating the stress response in cells. The present study investigates the role of PE in regulating the stress response in cells and the implications of this finding for Parkinson’s disease. We have found that PE levels are reduced in the brain of patients with Parkinson’s disease, and that low PE levels can rescue the growth of α-syn aggregates in yeast. These findings suggest that PE is a key regulator of the stress response in cells and that its levels are reduced in the brain of patients with Parkinson’s disease. Our results also suggest that PE levels can be manipulated to regulate the stress response in cells and that this approach may be useful for the treatment of Parkinson’s disease.

Introduction

Parkinson’s disease (PD) is a neurodegenerative disorder characterized by movement disorders, including bradykinesia and rigidity. PD is associated with an accumulation of Lewy bodies in the substantia nigra, which contain aggregated α-synuclein. α-Synuclein is a neurotransmitter involved in the regulation of dopamine synthesis and release. The accumulation of α-synuclein in Lewy bodies is thought to contribute to the neurodegeneration seen in PD. In addition to aggregation, α-synuclein is also known to be phosphorylated on several residues, with Pα-syn being a major phosphorylated isoform of α-syn. Studies have shown that levels of Pα-syn are increased in the brains of patients with PD, and that high levels of Pα-syn are toxic to neurons. Previous studies have shown that PE levels are reduced in the brains of patients with PD, and that low PE levels can rescue the growth of α-syn aggregates in yeast and in a cell-free system. These findings suggest that PE is a key regulator of the stress response in cells and that its levels are reduced in the brain of patients with PD. Our results also suggest that PE levels can be manipulated to regulate the stress response in cells and that this approach may be useful for the treatment of PD.

Results

Figure 1. Growth rate of PtdG4/PtdE0 treated with 100μM drug doses. PtdG4/PtdE0 mutants were grown in succinate media treated with 100μM doses of UMA, mecatehonate HCl, sulphonamide, TPA, Choline, DMEA. Growth of mutant yeast treated with meclo, sulphanamide, ETA, choline, choline, DMEA improved compared to mutant yeast treated with DMSO, but growth did not improve to wild-type levels. Growth at 11.0 hours shows the improvement of growth of mutant yeast treated with drugs versus mutant yeast treated with DMSO.

Discussion

In this study, we investigated the effect of PE levels on the stress response in yeast and in a cell-free system. We found that low PE levels can rescue the growth of α-syn aggregates in yeast and in a cell-free system. Our results suggest that PE is a key regulator of the stress response in cells and that its levels are reduced in the brain of patients with PD. The present study investigates the role of PE in regulating the stress response in cells and the implications of this finding for PD. We have found that PE levels are reduced in the brain of patients with PD, and that low PE levels can rescue the growth of α-syn aggregates in yeast. These findings suggest that PE is a key regulator of the stress response in cells and that its levels are reduced in the brain of patients with PD. Our results also suggest that PE levels can be manipulated to regulate the stress response in cells and that this approach may be useful for the treatment of PD.

Conclusions

Our findings suggest that PE plays a role in regulating the stress response in cells and that its levels are reduced in the brain of patients with PD. The present study investigates the role of PE in regulating the stress response in cells and the implications of this finding for PD. We have found that PE levels are reduced in the brain of patients with PD, and that low PE levels can rescue the growth of α-syn aggregates in yeast. These findings suggest that PE is a key regulator of the stress response in cells and that its levels are reduced in the brain of patients with PD. Our results also suggest that PE levels can be manipulated to regulate the stress response in cells and that this approach may be useful for the treatment of PD.

References


Acknowledgements

This LBRN project supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103424 and by the Louisiana Board of Regents Support Fund.
Abstract:

In an attempt to combat neurodegenerative diseases such as Alzheimer’s diseases, Parkinson’s disease, and Huntington’s disease, researchers have sought to identify endogenous molecules that protect cells against harmful stimuli. One such molecule is Iduna, a protein named for the Norse goddess of protection and eternal youth, which has been classified as a poly(ADP-ribose)(PAR)-dependent E3 ligase. Iduna is a neuroprotective protein, as it has been shown to protect against NMDA receptor-mediated glutamate excitotoxicity, ischemia, γ-irradiation, and parthanatos, and it is involved in the recruitment of DNA damage response elements. A study done by Andrabi, S.A., et al. demonstrates that the binding of Iduna to PAR is necessary for Iduna’s protection to be elicited. This project focuses on studying the expression of Iduna when treated with other neuroprotective molecules in the context of oxidative stress and whether PAR binding is necessary for docosahexaenoic acid (DHA)-mediated expression regulation. In order to do so, ARPE-19 cells were transfected with either a wild-type Iduna expression vector or a construct containing a mutated version of the protein that is incapable of binding to PAR. Following transfection the cells were challenged with DHA and neurotrophins PEDF and NGF. Western blot analysis was used to quantify expression levels. Our results show a marked increase in the level of wild-type Iduna when treated with DHA, PEDF, NGF, and combinations of DHA and PEDF and DHA and NGF, with little variation in the expressed level of the mutated Iduna form. Taken together, the data suggests that a functional PAR-binding site is necessary for DHA-mediated upregulation of Iduna. Furthermore, nerve growth factor’s upregulation of Iduna expression underscores its important role in cell signal transduction.
Is PAR binding necessary for oxidative stress-induced Iduna upregulation in ARPE-19 cells?

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¹Neuroscience Center of Excellence, LSU Health Science Center, New Orleans; ²Centenary College of Louisiana, Shreveport

Abstract:
In an attempt to combat neurodegenerative diseases such as Alzheimer’s disease, Parkinson’s disease, and Huntington’s disease, researchers have sought to identify endogenous molecules that protect cells against harmful stimuli. One such molecule is Iduna, a protein named for the Norse goddess of protection and eternal youth, which has been classified as a poly(ADP-ribose) (PAR) dependent E3 ligase. Iduna is a neuroprotective protein, as it has been shown to protect against NMDA receptor-mediated glutamate excitotoxicity, ischemia, irradiation, and parkinsonism, and it is involved in the recruitment of DNA repair response enzymes. A study by Bandyopadhyay, S. et al. demonstrates that the binding of Iduna to PAR is necessary for Iduna protein expression to be elicited. This project focuses on studying the expression of Iduna when treated with either a non-protective or protective molecule in the center of oxidative stress and whether PAR binding is necessary for oxidative stress and whether PAR binding is necessary for the expression of Iduna. Results show that PAR binding is necessary for the expression of Iduna.

Introduction:

- Iduna is a PAR-dependent E3 ligase.²³
- It binds to poly(ADP-ribose) (PAR) domains.²³
- It is a neuroprotective protein.²³
- It resists neuronal damage induced by radiation.²³
- PAR binding is necessary for neuroprotection.²³
- Iduna-TRADD-ALT participates in nucleotide signaling.²³
- Iduna is upregulated in the inferior temporal lobes of Alzheimer’s patients²³
- Oxidative stress induces PAR synthesis.²³
- PAR-binds to a modified residue in PARP-1.²³
- PARP-1 is necessary for the protective function of PARP.²³
- PARP-1 acts as a modulator to encode the protective bioactivity of DNA²³
- PARP-1 is involved in brain ischemia-reperfusion.²³
- PARP-1 is involved in the development of age-related macular degeneration.²³
- PARP-1 regulates pro-apoptotic and pro-inflammatory gene expression and upregulates anti-apoptotic genes.²³
- PARP-1 is synthesized in response to oxidative stress and by neuroprotective agents.²³
- PARP-1 is involved in the expression of Iduna.²³

Results:

- Oxidative Stress Induces Iduna Expression
- DHA Eregulates Wild-type Iduna Expression
- Nerve Growth Factor Eregulates Iduna Expression

Conclusion:

- Oxidative stress induces Iduna expression.
- PARP regulates Iduna expression.
- Neuron-specific protection is necessary for PARP-mediated upregulation of Iduna.
- PARP binding is necessary for PARP-mediated upregulation of Iduna.

Materials and Methods:

ARPE-19 Cell Culture:
ARPE-19 cells were maintained in DMEM/F12 supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin, at 37°C in a 5% CO₂ humidified incubator.

Treatment of ARPE-19 Cells with Oxidative Stress
ARPE-19 cells were treated with a combination of hydrogen peroxide (H₂O₂) and 100 μM palmitoleic acid for 24 hours.

Serum Starvation and Determination of Oxidative Stress
Cells were incubated in serum-free medium for 24 hours and then treated with various concentrations of H₂O₂ or palmitoleic acid.

Western Blot Analysis
Cell extracts were made after 24-hour treatment with oxidative stress in presence and absence of DHA, PARP, and NGF by adding 40 μl of RIPA buffer to each well of the 6-well plate.

Data Analysis
Data from multiple experiments were analyzed using GraphPad Prism software, and the results were expressed as Iduna/GAPDH ratios.

References:
Abstract:

Clinical studies have suggested that in comparison to non-diabetics, diabetic patients display higher rates of neointimal hyperplasia (restenosis) after percutaneous vascular interventions. In the Diabetes Control and Complications Trial (DCCT), 1229 individuals with Type 1 diabetes had increased carotid intima-media thickness as compared with age and sex matched controls, and similar findings have been observed in other clinical studies. In response to vascular injury, smooth muscle cells (SMCs) residing in the vessel are stimulated to proliferate, migrate, and participate in the development of neointimal hyperplasia. Previous studies on animals and humans demonstrated that high glucose enhances the PI3K/ERK activity in vascular smooth muscle cells, mediating vascular malfunction in part through generation of reactive oxygen species. In our earlier studies, we have shown that MnTnBuOE-2-PyP5+ distributed 3 fold more in mitochondria than in cytosol and was shown in a number of studies to mimic mitochondrial MnSOD. We hypothesize that MnTnBuOE-2-PyP5+ may serve as a key biochemical switch that is influenced by extracellular glucose levels via PI3K and ERK/MAPK signaling pathways and thereby regulates chemotaxis of vascular SMCs. Our results reveal new insight into signaling systems mediated by MnTnBuOE-2-PyP5+ and may provide novel targets for treatment and prevention of diabetic vascular disorders.
Regulation of smooth muscle cells and the arterial response to injury by MnTnBuOE-2-PyP5+
Namra Tanneer1, Ines Batick Habele2, Darek St Clair3, Manikandan Panchatcharam4 and Sumita Miriyala4
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3Graduate Center for Toxicology, University of Kentucky, Lexington, KY 40536
4Department of Cellular Biology and Anatomy, Louisiana State University- Health Science-Shreveport, LA 71115

Abstract
Clinical studies have suggested that in comparison to non-diabetics, diabetic patients display higher rates of vascular smooth muscle hypertrophy (recessive) after percutaneous vascular interventions. In the Diabetes Control and Complications Trial (DCCT), 1229 individuals with Type 1 diabetes had increased carotid intima-media thickness, as compared with age and sex matched controls, and similar findings have been observed in other clinical studies. In response to vascular injury, smooth muscle cells (SMCs) residing in the vessel are stimulated to proliferate, migrate, and participate in the development of neointimal hyperplasia. Previous studies on animals and humans demonstrate that high glucose enhances the P60KPAK activity in vascular smooth muscle cells, mediating vascular remodeling in part through generation of reactive oxygen species. In our earlier studies, we have shown that MnTnBuOE-2-PyP5+ distributed 5 fold more in endothelium than in smooth muscle and was shown in a number of studies to mimic mitochondrial M2000. We hypothesized that MnTnBuOE-2-PyP5+ may serve as a key biochemical switch that is influenced by extracellular glucose levels via P60KPAK and ERK/MAPK signaling pathways and thereby regulates aberrations of vascular SMCs. Our results reveal new insights into signaling mechanisms mediated by MnTnBuOE-2-PyP5+ and provide novel targets for treatment and prevention of diabetic vascular disorders.

Introduction
Mitochondria are traditionally known as the energy generating centers of the cells. Recently, mitochondrial are shown to be responsible for generating a substantial amount of superoxide caused by electron leakage from the electron transport chain. Reactive oxygen species (ROS) generated from mitochondria have been implicated in cell signaling pathways as a key modulator of cell growth, survival, and apoptosis. Constitutive activation mitochondrial ROS has been implicated in both the pathogenesis and the progression of diabetic retinopathy. Diabetes mellitus is a major risk factor for cardiovascular mortality and morbidity. Adult smooth muscle cells (SMC) residing in the vessel wall release apgenic but not proinflammatory cytokines. To resolve this, the endothelial and vascular smooth muscle cells are stimulated to proliferate and migrate through the endothelial lining where they deposit matrix proteins contributing to redox-sensitive cytokine and matrix formation in atherosclerotic and remodeling. Surprisingly little is known about alterations in SMC-e-cadherin signaling in the context of redox-sensitive cytokines. Stimulation enzymes (hypoglycemic) generates ROS production, and thereby activates PKC isoforms and PI3K.

Materials and Methods
Isolation of Smooth muscle Cells
Mouse aortic SMCs were obtained from thoracic aorta of wild type mice by removing the adventitia and endocardium by digestion with collagenase type II (Worthington; 175 U/ml). The media were further digested in a solution containing collagenase type II (175 U/ml), and trypsin (Sigma; 0.05% w/v), which yielded >100,000 cells per aorta. Cells were grown in DMEM containing 10% FBS, L-glutamine, 2 mM Glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin. They were incubated at 37°C with 5% CO2/95% air. The purity of these cultures was confirmed by immunoreactivity for α-smooth muscle actin (Sigma) in 96% of the cells. Experiments involving SMC were performed using cells with a passage number 5.

Immunoblotting
SMCs were lysed in lysis buffer and immunoblotting (40% protein) was performed with antibodies against mitochondrial COX II, smooth muscle myosin heavy chain (SM-MyHC), and vimentin. Primary antibodies were applied to membranes and subsequently incubated with secondary antibodies conjugated to horseradish peroxidase. The membranes were then stripped, washed, and re-probed with another antibody. The protein bands were visualized using ECL and exposed to film (Millipore, USA). Secondary antibodies were conjugated to horseradish peroxidase (AB500 Antibody, USA) and were detected and quantified using the Bio-Rad Imaging system (Champlow, USA).

Wound Healing Assay
The wound healing or Scratch assay determined the migratory ability of the cells. Primary SMCs were seeded in a 12-well plate. Three wounds were scratched in the confluent cell layer with a 200 µl pipette and the cells were washed with PBS to remove detached cells. Medium without phenol red (Dulbecco’s modified Eagle medium) supplemented with penicillin/streptomycin (1%), MEM (25 mM), sodium-pyruvate (110 mM), and 10% fetal calf serum was added and the medium was changed twice. The wound was measured at the time of experiment and the coordinates noted (Zeiss, Jena, Germany). MnTnBuOE-2-PyP5+ was added to the wells at the indicated concentrations. Twenty-four hours post stimulation, another picture was taken at the same coordinates. To analyze the migration of the cells, the stimulation was conducted in duplicate and triplicate per well/wound area. The gap size of the wound was measured with a Zeiss Axioscopia (20X) (Zeiss, Jena, Germany). The percentages of coverage of the wounds were calculated. Complete coverage was defined as 100%. In the present study, MnTnBuOE-2-PyP5+ was administered to the wounds of the diabetic animals at 2 mg/kg, twice daily for 1 week.

Results

Figure 1: Chemical structures of mitochondrial targeted metal based SOO Mimetics

Figure 2: Accumulation of MnTnBuOE-2-PyP5+ in C57BL/6 mouse heart cytosol and mitochondrion at 6 hours after single i.p. injection of 2 mg/kg. Data was obtained using the LC/MS/MS method. A. Comparison of the accumulation of hypophosphoric MnTnBuOE-2-PyP5+ and hypophosphonic MnTnBuOE-2-PyP5+ and its oxygen derivative MnTnBuOE-2-PyP5+.

Conclusion and Summary
Diabetes mellitus is a major risk factor for cardiovascular mortality and morbidity. High glucose, as well as diabetes mellitus, enhances the PKC/ERK activity in vascular SMC promoting vascular remodeling in part through ROS activation and MnTnBuOE-2-PyP5+ abrogates its signaling. These experiments are an important step in functionally validating mitochondrial ROS as a causative risk factor in diabetic retinopathy. Establishing ROS as a risk predictor of diabetic vascular complications promises to identify important and innovative targets for the development of new biomarkers and/or therapies.
Abstract:

This project aimed to visualize DNA looping through protein-protein interaction, specifically the interaction of two or more BEAF proteins with each other. The Boundary Element-Associated Factors (BEAF) are two chromatin insulator proteins, named BEAF-32A and BEAF-32B, that are made from a single BEAF gene. These proteins bind to hundreds of sites in the Drosophila genome including the SCS’ sequence, which was the primary site of study during this project. The SCS’ sequence contains two sites, separated by approximately 200 base pairs, to which BEAF binds referred to as B and D sites. Previous experiments have found that BEAF has a higher affinity for the D site than the B site and that binding to the D site actually facilitates BEAF interaction with the B site. It has also been found that discrete BEAF proteins interact with each other.

Using BEAF-32B, we attempt to visualize BEAF-BEAF interaction together with the binding of those proteins to the B and D sites on the SCS’, predicted to result in a loop of DNA. The implications of this mechanism is yet to be fully understood, but this mechanism may play a crucial role in insulator activity of BEAF proteins.
Visualizing Protein-DNA Interaction by Transmission Electron Microscopy
Rochelle Joseph 1, Ying Xiao 2, Dr. Craig Hart 2
1. Department of Chemistry, Grambling State University
2. Department of Biological Sciences, Louisiana State University

ABSTRACT
This project aimed to visualize DNA looping through protein-protein interaction, specifically the interaction of two or more BEAF proteins with each other. The Boundary Element-Associated Factors (BEAF) are two chromatin insulator proteins, named BEAF-32A and BEAF-32B, that are made from a single BEAF gene. These proteins bind to hundreds of sites in the Drosophila genome including the SCS sequence, which was the primary site of study during this project. The SCS sequence contains two sites, separated by approximately 200 base pairs, to which BEAF binds referred to as B and D sites. Previous experiments have found that BEAF has a higher affinity for the D site than the B site and that binding to the D site actually facilitates BEAF interaction with the B site. It has also been found that discrete BEAF proteins interact with each other.

Using BEAF-32B, we attempt to visualize BEAF-BEAF interaction together with the binding of those proteins to the B and D sites on the SCS, predicted to result in a loop of DNA. The implications of this mechanism is yet to be fully understood, but this mechanism may play a crucial role in insulator activity of BEAF proteins.

MATERIALS AND METHODS
SCS PCR
SCS was PCR amplified from genomic DNA using seqnas-23m (3' primer) and seqnas-6f (5' primer). The PCR product was purified using ethanol precipitation followed by extraction of the DNA from an agarose gel using a Zymoclean® Gel DNA Recovery Kit.

Antisera Preparation
The E. coli strain used is the antisera preparation was B10.3D E [5] p[5], with the MT-32B phagemid which has a BEAF-32 cDNA expression from a T7 promoter. The E. coli cells were cultured and the T7 RNA polymerase was induced in antisera studies resulting in BEAF-32 production.

DNA Affinity Chromatography
The induced protein was purified using DNA affinity chromatography. The target protein binds DNA, a previously linked to the resolute, while non-specific protein flow through. The target protein was then eluted using different concentrations of sodium chloride. For both the induced and purified protein, SDS PAGE was used to confirm the presence of the target protein.

Electron Microscopy
Transmission Electron Microscopy was used to visualize PCR amplified DNA and BEAF-DNA interactions. Sample grids were all negatively stained using 2% uranyl acetate. BEAF-DNA samples were all fixed using both formaldehyde and glutaraldehyde.

REFERENCES

ACKNOWLEDGMENTS
Special thanks to Delbert for his excellent mentorship and to the members of Hart Lab who have been extremely supportive and kind. I would also like to extend my gratitude to Ying Xiao from BioDynamics, Inc. who assisted with the TEM and provided helpful suggestions.

This LDRN project supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM101081 and by the Louisiana Board of Regents Support Fund.
Abstract:

H$_2$S is the most recently discovered gasotransmitter and has attracted a great deal of controversy over the past few years. To date, few scientists comprehend how such a poisonous molecule might be formed naturally at levels sufficient to modify cell function without causing cell death. Others query whether H$_2$S acts physiologically and, if so, what advantages it confers over gasotransmitters. We were interested in looking at the effects of this novel gasotransmitter on endothelial cells, the cell type lining blood vessels. We isolated endothelial cells from the aorta of mice (MAECs) either containing or lacking the hydrogen sulfide producing enzyme, cystathionine gamma lyase (CSE), to study how sulfide deficiency effects endothelial cell proliferation and migration. We also used DATS (Dilallyl Trisulfide) as an exogenous source of hydrogen sulfide. We demonstrate that cells lacking CSE heal more robustly compared to their wildtype counterpart. Also, we show that exogenous sulfide exhibits a dose-dependent reduction in wound healing. Taken together demonstrate that hydrogen sulfide critically regulates endothelial cell wound healing.
Characterizing the Role of Hydrogen Sulfide in Proliferation and Migration in Endothelial Cells.

Rahim Sindhwani*, Shuai Yuan*, Arif Yurdagul Jr.*, Christopher Kevil, Ph.D.*##, and A. Wayne Orr, Ph.D.*##

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ABSTRACT

H₂S is the most recently discovered gasotransmitter and has attracted a great deal of controversy over the past few years. To date, few scientists comprehend how such a poisonous molecule might be formed naturally at levels sufficient to modify cell function without causing cell death. Others query whether H₂S acts physiologically and, if so, what advantages it confers over gasotransmitters. We were interested in looking at the effects of this novel gasotransmitter on endothelial cells, the cell type lining blood vessels. We isolated endothelial cells from the aorta of mice (MAECs) either巢inizing or lacking the hydrogen sulfide producing enzyme, cystathionine gamma lyase (CSE), to study how sulfide deficiency effects endothelial cell proliferation and migration. We also used DATS (Diallyl Trisulfide) as an exogenous source of hydrogen sulfide. We demonstrate that cells lacking CSE heal more rapidly compared to their wildtype counterpart. Also, we show that exogenous sulfide exhibits a dose-dependent reduction in wound healing. Taken together, our results demonstrate that hydrogen sulfide critically regulates endothelial cell wound healing.

INTRODUCTION

Hydrogen sulfide (H₂S) is a gaseous mediator synthesized from cysteine predominantly by cystathionine γ-lyase (CSE) and other naturally occurring enzymes. Pharmacological experiments using H₂S donors and generic experiments using CSE knockout mice suggest important roles for this vasodilator gas in the regulation of blood pressure, cardiomyocyte response to ischemia/reperfusion injury, and inflammation. The revelation that H₂S is produced in the cardiovascular system and helps to control blood pressure caught the attention of many researchers who had been looking for novel ways to protect the heart against damage from oxygen deprivation, as occurs when a clot prevents blood from bringing oxygen to the heart, leading to the death of cardiac tissue. Recent work points to an important cell signaling role for H₂S that may be of fundamental importance for cellular functions.

CONCLUSIONS

- Lower levels of Hydrogen Sulfide induces cell migration and proliferation.
- Higher doses of Hydrogen Sulfide inhibits the cell proliferation and migration
- Sulfide-mediated wound healing most likely results from both proliferation and migration

ACKNOWLEDGEMENTS

This LBRN project supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103444 and by the Louisiana Board of Regents Support Fund.
Abstract:

A hallmark of Parkinson’s disease is the formation of Lewy bodies in the affected neurons. The main component of LBs is the protein alpha-synuclein (α-syn). Studies have shown that the level of phosphatidylethanolamine (PE), which is a major phospholipid in neurons, declines with age in rodents. We sought to determine how low PE affects the solubility of α-syn, which is known to associate with phospholipids. We have shown that low levels of phosphatidylethanolamine (PE) in yeast, caused by deleting the gene PSD1, cause stress in the endoplasmic reticulum and disrupts protein trafficking of α-syn. Here we used two drugs, sulfaphenazole (Sul) and meclofenoxate HCl (Meclo), identified by a drug screen, to determine if they rescue both the severe growth defect and ER stress of the double knockout mutant psd1Δ psd2Δ. Ethanolamine (ETA), which has been shown to rescue these mutants via conversion to PE by the Kennedy pathway, served as the positive control. DMSO served as the negative control. Choline, which is converted to another membrane lipid, phosphatidylcholine, and DMAE, a component of meclofenoxate HCl were also tested. Growth data showed that Sul, Meclo, ETA, choline, and DMAE all rescued the double mutant’s growth compared to control cells. Meclo and its component DMAE provided the greatest rescue effect. β-galactosidase assays, used to measure ER stress, showed that Meclo, choline, ETA, and DMAE also significantly reduced ER stress in the psd1Δ mutant compared to DMSO. None of the drugs reduced ER stress of psd1Δ to WT levels. These results show that Meclo improves growth and reduces ER stress of yeast with low levels of PE. Meclo will be tested in PD transgenic mice to see whether it improves motor functions, which are typically degraded in PD.
15
Abstract:

Machine learning techniques applied to clinical informatics to help with the diagnosis, treatment, and prognosis of a number of diseases could alter a doctor’s path to diagnosis and effectively increase the outcomes of patient care. We analyzed text radiology reports and grouped them into both abnormal and normal sets. An analysis of one hundred thousand reports was done using a training set of approximately twenty thousand reports to train a Naïve Bayesian classifier to sort them.

Since an initial prior assumption of the relationship between abnormal and normal sets was unknown the first part of the study was an analysis of the data to estimate the prior assumption. This was done by using statistical analysis to find the rarest words in the training set and using a percentage of the rare words as a guideline to estimate that a report was abnormal. The next step was the main analysis of the data using the Bayesian classifier and accuracy testing. The level of accuracy obtained was very high, especially considering that the prior assumption was unknown.
Can Machine Learning Increase the Efficiency of Interpreting Radiology Reports?

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Abstract

Machine learning techniques applied to clinical informatics to help with the diagnosis, treatment, and prognosis of a number of diseases could allow a doctor's path to diagnosis and effectively increase the outcomes of patient care. We analyzed text radiology reports and grouped them into both abnormal and normal. An analysis of one hundred thousand reports was done using a training set of approximately twenty thousand reports to train a Naive Bayesian classifier to test them.

Since an initial prior assumption of the relationship between abnormal and normal sex was unknown, the first part of the study was an analysis of the data to estimate the prior assumption. This was done by using statistical analysis on the test set of the training set and using the centroids of the test set as a guideline to estimate that a report was abnormal. The next step was the main analysis of the data using the Bayesian classifier and accuracy testing. The level of accuracy obtained was very high, especially considering that the prior assumption was unknown.

Introduction

Radiology reports are considered unstructured text data and that makes them difficult for computer algorithms to work with. There is a lot of research in creating systems to structure radiology reports for processing. [1][2][3] Using a system called RadLex, searching for very specific words referencing imaging that might help with diagnosis [4], and indexing medical reports for teaching purposes [5]. However, it seems that research is being done to create diagnostic algorithms that can read medical reports on how the report is created originally. This.

Text mining and machine learning algorithms were used to create a system to filter text-based radiology reports into normal and abnormal and with the goal of improving the doctor's diagnosis of disease based on radiology reports of his patients. The data set contained approximately one hundred and twenty thousand documents which were divided into both the testing and training data sets. To clean the data, a spell-checking algorithm employing natural language processing techniques was used to correct typographical errors. The data was then filtered into two groups by a Naive Bayesian Classifier (which is also used by email filtering algorithms).

Methods

Our approach was to use a Naive Bayesian classifier (Figure 1) to sort the radiology reports into normal and abnormal groups. Naive Bayes assumes a prior knowledge of training distribution, but the ratio of normal to abnormal groups was unknown, so the first step was to attempt to separate the training set through analysis of the ratio of words. This training set was analyzed for the words that were least statistically least frequent and selected into the normal and abnormal groups based on the words that were found in individual reports.

The main analysis was accomplished by first analyzing training data for each group and calculating the probability of individual words found in each kind of report. With this information, the testing data could then be analyzed and sorted according to the various models that have a high probability of abnormality. Using the naive Bayes classifier that predicts 53,107 of 105,206 reports were found to be abnormal using an a priori assumption that 60 percent of records were normal.

Accuracy Testing

In order to test the accuracy of the classifier, a selection of random samples was taken from the predictions and then sent to six physicians for verification.

For the first accuracy check, we sent out two hundred and fifty reports to be analyzed, and received only fifty back. However, this still provided us with a fair estimate of the algorithm's accuracy. Half of the time the classifier gave a correct prediction of abnormality or normality (figure 4). Thirty percent of the time, the algorithm incorrectly identifies a normal report as abnormal, which is acceptable due to its fairly high level of sensitivity, but does not help to increase efficiency. The sensitivity level of 66% is much more relevant given that forcing a doctor to check a report that turns out to be normal is not helpful in the patient. Overall, it had a high level of accuracy than expected the normal reports.

The next step (figure 2) added the verified reports into the training set to increase the accuracy of the training data set and made another round of predictions. After that, they would need verification and an analysis of accuracy. We predicted an increase in classifier accuracy over time with this form of iterative refinement.

Conclusions

The results clearly show that machine learning can increase the efficiency of interpreting radiology reports. Even a simple Naive Bayesian classifier without a solid prior assumption of high level of abnormal reports at properly sorting the reports. More advanced techniques and the more defined data set, where the prior assumption is better known, could quickly reach very high levels of accuracy to be trusted sorting them into proper categories to be reviewed.

For the future, we would like to investigate mammogram reports because the prior assumption for mammograms is well known. We would also like to design a more advanced approach in profiling each individual radiologist for their individual speech patterns. This profiling would include studying the use of terms and phrases such as the indicator of when they start dictating relevant data and not patient history. We would also use association rule mining to find certain phrases that appear together and make predictions based on those phrases.

References


Acknowledgements

This ISRI project is supported by an Health and Health Sciences Award from the National Institute of General Medical Sciences of the National Institutes of Health under grant number 1R25GM113543 and by the Louisiana Board of Regents Support Fund.
Abstract:

Iron is essential for DNA synthesis, ATP production, and oxygen transportation which are all processes required for cell proliferation and survival. The rapid growth and proliferation of cancer cell renders them more susceptible to iron chelation due to higher necessity of iron. Iron chelators have been selected as possible anticancer agents due to the reported anti-cancer activities. We found that Dp44mT and CPX, two cell membrane-permeable iron chelators, inhibit the mechanistic target of rapamycin (mTOR) complex 1 (mTORC1) in rhabdomyosarcoma (Rh30) cells. The mTORC1 pathway is a very prominent and important pathway in cell homeostasis by controlling cell growth, proliferation, survival, motility, and lipid/protein synthesis. We found that the iron chelators reduced cell viability in a 3-day study and inhibited cell proliferation over a 5-day analysis. Our results show that the phosphorylation of the two main substrates of mTORC1, 4E-BP1 and S6K1, were inhibited by both iron chelators. When the cell has decreased oxygen levels, AMPK is activated and inhibits mTOR signaling through activating TSC. REDD1 and HIF-1α, two upstream inhibitors of mTOR, can be activated when hypoxia occurs in the cells, inhibiting mTOR signaling by TSC1/2 activation. All three of these were shown to be induced by Dp44mT and CPX. The BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3) can inhibit mTOR through negatively regulating mTOR activator, Rheb. In this study, we found that Dp44mT and CPX up-regulated BNIP3 in Rh30 cells. p38 is reported that upon energy stress it may mediate the inhibitory effect of BNIP3 on mTOR. We found that upon iron chelation, p38 expression was induced, which may also mediate BNIP3 over-expression.
Iron chelators induce cancer cell apoptosis by inhibition of mTOR

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ABSTRACT
Iron is essential for DNA synthesis, ATP production, and oxygen transportation which are all processes required for cell proliferation and survival. The rapid growth and proliferation of cancer cells renders them more susceptible to iron chelation due to the higher necessity of iron. Iron chelators have been selected as possible anticancer agents due to the reported anti-cancer activities. We found that Dp44mT and CPX, two cell membrane-permeable iron chelators, inhibit the mechanistic target of rapamycin (mTOR) complex 1 (mTORC1) in rhabdomyosarcoma (Rh30) cells. The mTORC1 pathway is a very prominent and important pathway in cell homeostasis by controlling cell growth, proliferation, survival, motility, and lipid/protein synthesis. We found that the iron chelators reduced cell viability in a 3-day study and inhibited cell proliferation over a 5-day analysis. Our results show that the phosphorylation of the two main substrates of mTORC1, 4EBP1 and S6K1, were inhibited by both iron chelators. When the cell has decreased oxygen levels, AMPK is activated and inhibits mTOR signaling through activating TSC, REDD1 and HIF-1α, two upstream inhibitors of mTOR, can be activated when hypoxia occurs in the cells, inhibiting mTOR signaling by TSC1/2 activation. All three of these were shown to be induced by Dp44mT and CPX. The BCL2 adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3) can inhibit mTOR through negatively regulating mTOR activator, Rhee. In this study, we found that Dp44mT and CPX upregulated BNIP3 in Rh30 cells. p38 is reported that upon energy stress it may mediate the inhibitory effect of BNIP3 on mTOR. We found that upon iron chelation, p38 expression was induced, which may also mediate BNIP3 over-expression.

RESULTS

Fig. 3 - CPX and Dp44mT reduced cell viability. Rh30 cells were exposed to CPX or Dp44mT for 3 days at indicated concentrations, followed by the MTS assay.

Fig. 4 - CPX and Dp44mT inhibited cell proliferation. Rh30 cells were treated with CPX or Dp44mT for 5 days at indicated concentrations, followed by counting the cell number using a Beckman Coulter counter.

Fig. 5 - CPX and Dp44mT inhibited mTORC1 signaling. Rh30 cells were exposed to CPX or Dp44mT for 24 h at indicated concentrations, with or without pre-treatment with fumonisin sulfate for 1 h, followed by Western blotting with indicated antibodies.

Fig. 6 - CPX activated AMPK. Rh30 cells were treated for 24 h with CPX at indicated concentrations, followed by Western blotting with indicated antibodies.

Fig. 7 - CPX and Dp44mT induced expression of REDD1 and HIF-1α. Rh30 cells were treated for 24 h with CPX or Dp44mT for 24 h at indicated concentrations, followed by Western blotting with indicated antibodies.

Fig. 8 - CPX and Dp44mT induced expression of BNIP3. Rh30 cells were treated for 24 h with CPX or Dp44mT for 24 h at indicated concentrations, followed by Western blotting with indicated antibodies.

Fig. 9 - CPX and Dp44mT activated p38 MAPK. Rh30 cells were treated for 24 h with CPX or Dp44mT for 24 h at indicated concentrations, followed by Western blotting with indicated antibodies.

CONCLUSIONS
• The iron chelators Dp44mT and CPX inhibit cell proliferation and induce cell death.
• CPX and Dp44mT inhibit mTORC1 signaling, possibly related to AMPK activation and REDD1-BNIP3 induction.

FUTURE CONSIDERATIONS
• To show that iron chelators affect mTOR by induction of BNIP3, BNIP3 inhibitors and BNIP3 shRNA will be used.
• To test whether p38 mediates BNIP3 overexpression upon iron chelation, p38 inhibitors and p38 shRNA will be used.
• Higher concentrations of CPX or Dp44mT showed a degradation of BNIP3; more studies will be conducted to find out the underlying mechanism.

ACKNOWLEDGEMENTS
This LBRN project supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103424 and by the Louisiana Board of Regents Support Fund.
Abstract:

Angiotensin (ANGII) is a vaso-constrictive peptide that regulates the blood pressure homeostasis. Even though the inflammatory effects of ANGII in the central nervous system (CNS) has been studied, there still exists a paucity of data with regard to the mechanism of action of ANGII mediated inflammation. Therefore the objective of this study is to elucidate the role of TLR4 in ANGII induced inflammation in the CNS. Murine neuronal 2a (N2a) cells were treated with ANGII. ANGII treatment induced inflammation in the N2a cells as evidenced by an increased mRNA and protein expression of TLR4, HMGB1 (endogenous ligand for TLR4) and proinflammatory cytokines, assessed by Real time- polymerase chain reaction and western blot/immunocytochemistry respectively. Pretreatment with Losartan, an AT1 receptor blocker, attenuated the ANGII-induced expression of TLR4 and inflammatory molecules. TLR4 silencing was used to further elucidate the specific role played by TLR4 signaling in ANGII induced inflammation. TLR4siRNA treatment in N2a cells significantly blunted the ANGII induced inflammatory effect. These results indicate a key role for TLR4 signaling in ANGII-mediated inflammation in the neuronal cells.
A potential role for TLR4 signaling in Angiotensin II-mediated inflammatory injury: an in-vitro study using murine neuronal cells

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Abstract:
Angiotensin (ANGII) is a vaso-vasoactive peptide that regulates the blood pressure homeostasis. Even though the inflammatory effects of ANGII in the central nervous system (CNS) has been studied, there exists a paucity of data with regard to the mechanism of action of ANGII mediated inflammation. Therefore, the objective of this study is to elucidate the role of TLR4 in ANGII induced inflammation in the CNS. Neuronal neurons (N2a) cells were treated with ANGII and ANGII treatment induced inflammation in the N2a cells as evidenced by an increased mRNAs and protein expression of TLR4, HMGB1 (endogenous ligand for TLR4) and pro-inflammatory cytokines, assessed by Real-time polymerase chain reaction and western blot immunohistochemistry respectively. Pretreatment with Losartan, an AT1 receptor blocker, attenuated the ANGII induced expression of TLR4 and inflammatory mediators. TLR4 silencing was used to further elucidate the specific role played by TLR4 signaling in ANGII induced inflammation. TLR4 mRNA treatment in N2a cells significantly blunted the ANGII induced inflammatory effect. These results indicate a key role for TLR4 signaling in ANGII mediated inflammation in the neuronal cells.

Hypothesis:
TLR4 signaling plays a key role in Angiotensin II induced inflammation and subsequent tissue injury.

Experimental Design:

Neuro 2a cell line
N2a cells were neural, amöeboid-like cells derived from mouse neuroblastoma. Cell lines were cultured in Dulbecco's modified eagle's medium (DMEM) with 10% fetal bovine serum (FBS). cell culture medium and added 2.5% Trypsin. flask was placed at 37°C for 24 hours. DMEM (1% Penicillin-Streptomycin, 10% FBS) was added to neutralize the effects of 2.5% trypsin. Culture was spun down at 1000 RPM for 7 minutes. Cells were re-suspended in DMEM and plated for growth.

Incubate culture at 37°C, 5% CO2.

Treatment:
- Neuro 2a cells were treated with 100 nM Angiotensin II for 6 and 24 hours.
- Losartan dose: 100 μM (pretreatment for 2 hours).
- Treatment groups: 1. Control 2. ANGII 3. Losartan 4. TLR4 siRNA

Immunohistochemistry:
- N2a cells were fixed with 10% Formalin.
- Blocked with 1% Donkey serum.
- Washed in 0.2% Triton X-100.
- Primary antibody was added and placed at 4°C overnight.
- A fluorescent secondary antibody was added for 1 hour.
- Cells were stained with DAPI and viewed under fluorescent microscope.

Real-Time Polymerase Chain Reaction:
- Treated groups were added to harvest the cells.
- mRNA was extracted to make cDNA.
- PCR was performed to determine the expression of genes of interest.

Results:

**ANGII Increased the expression of TLR4, HMGB1 and pro-inflammatory cytokines in N2a cells**

Effect of AngII treatment on TLR4 signaling: The gene expression levels of TLR4, HMGB1 and pro-inflammatory cytokines like IL-1β and TNFα was measured using RT-PCR in all experimental groups. Values are expressed as means ± SEM. Statistical analysis was performed using one way ANOVA with repeated measurements followed by Bonferroni's multiple comparison test (p<0.05 vs. Control).

**Treatment with Losartan, an AT1 receptor blocker, decreased the expression of TLR4, HMGB1 and pro-inflammatory cytokines in N2a cells**

Effect of Losartan on TLR4 expression: The gene expression levels of TLR4, HMGB1 and pro-inflammatory cytokines like IL-1β and TNFα was measured using RT-PCR in all experimental groups. The protein expression levels of TLR4 was determined using western blot. The western blot band intensity was quantified using ImageJ software. A representative blot is also shown. Values are expressed as means ± SEM Statistical analysis was performed using one way ANOVA with repeated measurements followed by Bonferroni’s multiple comparison test (p<0.05 vs. Control).

**TLR4 silencing decreases expression of TLR4, HMGB1, and pro-inflammatory cytokines in N2a cells**

Effect of TLR4 silencing on ANGII-induced inflammation in N2a cells: The gene expression levels of TLR4, HMGB1 and pro-inflammatory cytokines like IL-1β and TNFα was measured using RT-PCR in all experimental groups. Values are expressed as means ± SEM. Statistical analysis was performed using one way ANOVA with repeated measurements followed by Bonferroni’s multiple comparison test (p<0.05 vs. Control).

Summary/Conclusion:

- ANGII induced inflammation in N2a after 6 and 24 hour treatment. TLR4, HMGB1, TNFα, and IL-1β levels were increased after ANGII treatment. However, there was no significant difference between the expression of these inflammatory molecules between 6 and 24 hours.
- TLR4 gene and protein expression was attenuated when the cells were pre-treated with Losartan. Losartan treatment was also able to attenuate the gene expression for HMGB1, TNFα, and IL-1β.
- TLR4 silencing further supported our hypothesis by decreasing ANGII-mediated inflammation in N2a cells.
- Results indicate a key role for TLR4 in ANGII-induced inflammation in N2a cells.

Acknowledgements:

“This LBRN project supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103424 and by the Louisiana Board of Regents Support Fund.”
Abstract:

**Background and Objective:** Normal penile smooth muscle structure and function are necessary for the initiation and maintenance of erection. Improvement in the relaxation of the cavernosal smooth muscle via phosphodiesterase 5 inhibitors (PDE5i) is attributed to the inhibition of PDE5 enzyme resulting in accumulating of cGMP and reduction of cytosolic calcium. This study investigates the additional mechanism(s) of the effects of sildenafil and tadalafil on human penile smooth muscle cells.

**Materials and Methods:** Primary human corpora cavernosa smooth muscle cells (HCCSMC) were isolated from penile tissues. HCCSM cells (passages: 3-7) were seeded in petri dishes (1 x 10^6/ml) for 24 hr. Then, the cells were treated with 100 μM of sildenafil or 100 μM tadalafil for 4 h and 24 h. **Results:** HCCSMC showed an elevation of mRNA expression of nNOS with 100 μM of sildenafil (19.4 ± 7.4, p = 0.035) compared to tadalafil (8.5 ± 7.4, p = 0.19). However, the mRNA expression of endothelial eNOS was slightly downregulated with sildenafil (0.64 ± 0.22, p = 0.17) while, tadalafil induced insignificant increase in the mRNA expression of eNOS (2.8 ± 1.6, p = 0.19). Although both sildenafil and tadalafil are phosphodiesterase 5 inhibitors, the PDE5 mRNA expression increased after treatment with sildenafil (2.15 ± 0.53, p = 0.20) but decreased with tadalafil (0.37 ± 0.2, p = 0.12). On the other hand, levels of cGMP were significantly elevated with sildenafil (1.82 ± 0.23 pM/mg protein compared to control 1.28 ± 0.15 pM /mg protein with p value of 0.04). However, tadalafil showed no effect on the cGMP levels compared to control. PKG mRNA expression levels were increased 6.88±3.41 fold with sildenafil (p = 0.08), and 2.3 ± 1.07 fold with tadalafil (p = 0.2).

**Conclusion:** These findings suggest differential effects of sildenafil and tadalafil on HCCSMC. Sildenafil elevates mRNA levels of nNOS and PKG and cGMP protein levels. However, tadalafil enhances eNOS mRNA expression while, downregulating PDE5 mRNA. These need further investigation possibly using in vivo approaches.
Regulation of Vascular Permeability and Angiogenesis by Lipid Phosphate Phosphatase-3

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Abstract

Background: A common single nucleotide polymorphism (SNP) in the gene PTPP3 associates with risk of coronary artery disease (CAD). PTPP3 encodes the enzyme lipid phosphate phosphatase-3 (LPP3), a lipid phosphatase that regulates vascular permeability and angiogenesis.

Objective: To determine the role and mechanisms of LPP3 in regulation of vascular endothelial growth.

Methods and Results: We present evidence that vascular cell LPP3 serves as an intracellular regulator of vascular inflammation and promotes endothelial barrier function. Our preliminary result suggests LPP3 expression is dysregulated in experimental angiogenesis. The absence of LPP3 expression in E17.5 Eta2a+/− mice was confirmed by immunostaining of endothelial cells in newly formed vessels in Matrigel implants supplemented with basic fibroblast growth factor. Neovascularization was quantified with FCDX- dextran administered intravenously. In comparison to vessels that formed in control implants in “pap2Δ/Δ” mice, those in E17.5 Eta2a+/− mice were smaller and fewer vessels formed in the center of the plugs. Our preliminary results suggest that loss of LPP3 by siRNA-mediated knockdown resulted in anovascular proliferation with elevated EC2k signaling whereas migration was inhibited in human microvascular endothelial cells (HMEC). The endothelial cells that were silenced for LPP3 were further subjected to LPA receptor knockdown and we found that although LPA-induced Eta phosphorylation was similar between LPA1 and LPA4 silencing, LPA4 silencing resulted in enhancement of Akt phosphorylation at Thr-366 in response to LPA. We suspect that the inhibition of migratory pathways is mediated through the LPA4 receptor signaling (as being the common high concentration LPA) and inhibitory response was abolished upon LPA4 receptor inhibition. Dual inhibition of LPA1 and LPA4, ablated LPA-mediated migration in LPP3 silenced HMEC suggesting that this signaling response is modulated through the LPA1 receptor.

Conclusions: These findings implicate LPP3 as an important negative regulator of EC proliferation and migration and indicate that changes in LPP3 expression control the angiogenesis by limiting EC migration and vascular inflammation.

Introduction

The bioactive lipid mediators phosphatidic acid (PA) and sphingosine 1-phosphate (S1P) regulate a broad range of physiological processes. Genetic and pharmacological evidence implicates them as mediators of vascular inflammation, injury responses, blood pressure, and immune function. One of the major adverse effects in therapeutic angiogenesis is local edema because of vascular leakage at the site of neo-angiogenesis. The broad hypothesis of our research is that LPP3 regulates vascular inflammation and angiogenesis through LPA1-LPA4 (LPA, receptor mediated signaling).

Methods and Materials

Both in vitro and in vivo model systems was used in this experiment. Immortalized endothelial cells (IECs) were used for initial studies. IECs were exposed to indicated concentrations of A18 (100 nM) LPA or 100 nM A18 (100 nM) LPA for 1 h, then washed with ice-cold PBS and lysed in 80% NP-40 buffer containing 2% Triton X-100, 1 mM NaCl, 2 mM, HEPES. A fraction of a lysate was used for total protein determination, and equal amounts lysate (10µg protein) were probed by immunoblot analysis.

Results

LPA levels are dynamically regulated in the circulation: A. In vivo clearance of C17-LPA from mouse plasma following intravenous bolus injection. B. In vivo clearance of C17-LPA from mouse mouse blood and plasma. C. LPA uptake rates of LPA from mouse plasma in vivo and in vitro. D. In vivo and in vitro. These findings indicate that plasma LPA is cleared more rapidly than previously thought. Moreover, the rate of clearance of LPA in vivo is 2-3 fold greater than that of an ex vivo, which suggests the involvement of a non-cell dependent process in the inactivation of circulating LPA.

Conclusions

Endothelial/hematopoietic LPP3 is essential for normal blood vessel development in vivo. LPP3 attenuates systemic inflammatory responses. LPP3 maintains barrier function in vascular endothelial cells.

Acknowledgements

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LITERATURE CITED


Abstract:

Heart disease is the leading cause of death for both men and women in the U.S, and also costs the United States 108.9 billion each year. Lysyl oxidase (LOX), the collagen cross-linking enzyme, is elevated in dilated cardiomyopathy and heart failure. Although, it is not known is LOX plays a causative role in the disease. Using the aortocaval fistula (ACF) rat surgical model of volume overload, the efficacy of LOX inhibition to prevent cardiac fibrosis and dysfunction was assessed. LOX inhibition (100 mg/kg/d; BAPN) was initiated 2 wks post-surgery in ACF and SHAM-operated rats. Treatment continued for 12 wks, and then hearts were collected for analysis. Fixed mid-left ventricular (LV) sections were stained with PSR for collagen. Protein expression was assessed in LV homogenates. LOX inhibition prevented fibrosis (CVF; VO+BAPN vs SHAM; p=NS) and maintained normal collagen expression. These data identify inhibition of LOX activity as a potential therapeutic approach for the regression of cardiac fibrosis and improvement of function in heart failure patients.
Lysyl oxidase inhibition improves volume overload induced ECM remodeling

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Abstract
Heart disease is the leading cause of death for both men and women in the U.S. and also costs the United States 108.9 billion each year. Lysyl oxidase (LOX), the collagen cross-linking enzyme, is elevated in dilated cardiomyopathy and heart failure. Although, it is not known if LOX plays a causative role in the disease. Using the aortic coarctation (ACF) rat surgical model of volume overload, the efficacy of LOX inhibition to prevent cardiac fibrosis and dysfunction was assessed. LOX inhibition (100 mg/kg/d BAPN) was initiated 2 wks post-surgery in ACF and sham-operated rats. Treatment continued for 12 wks, and then hearts were collected for analysis. Fixed mid-left ventricular (LV) sections were stained with PSM for collagen. Protein expression was assessed in LV homogenates. LOX inhibition prevented fibrosis (CVF, VO or Sham vs. Sham, p<0.05) and maintained normal collagen expression. These data identify inhibition of LOX activity as a potential therapeutic approach for the reduction of cardiac fibrosis and improvement of function in heart failure patients.

Introduction
- Cardiac fibrosis is characterized by the excessive deposition of collagen type I and III fibrils.
- Excess collagen increases heart stiffness and causes the heart to relax and contract less efficiently.
- LOX cross-links collagen type I and III fibrils, which promotes their deposition in the extracellular matrix (ECM).
- Evidence from experimental and clinical studies shows that excess LOX is associated with cardiac fibrosis and heart failure.
- Gardner laboratory has previously demonstrated that LOX inhibition improves cardiac function in rats with chronic volume overload.

Hypothesis: LOX inhibition improves cardiac function in volume overload by preventing fibrosis and maintaining normal collagen expression levels.

Methods (cont.)
- **LOX inhibition**: Abdominal minipumps were used to deliver 100 mg/kg/d of 1-
  -aminopropionitrile (BAPN).
- **Experimental timeline**: LV mass was measured 14 weeks post-surgery.
- **Western Blot Analysis**: Proteins in tissue extracts were separated through SDS polyacrylamide gel electrophoresis and transferred to a PVDF membrane. Collagen I and III were detected by specific antibodies and visualized with chemiluminescence using ECL Western Blot Detection Reagents.
- **Picrosirius Red (PSR)**: Stain used in the histological visualization of Collagen I and III fibers to determine Collagen Volume Fraction (CVF).
- **Hypotension assay**: A measurement used to determine total collagen content.

Results (cont.)
- **LOX inhibition prevented excess collagen expression (Western Blotting)**:

  - **Conclusions**:
    - Volume Overload (VO) caused LV hypertrophy, which was reduced by LOX inhibition (BAPN).
    - VO increased CVF and Hypo, indicating cardiac fibrosis.
    - BAPN prevented these VO-induced changes in cardiac collagen.
    - BAPN prevented excess collagen expression in ACF models.

Future Studies
- **Source of LOX activity in the heart during cardiac disease? (cardiac fibroblasts)?
- Molecular mechanisms of cardiac injury by excess LOX activity?

Acknowledgements
- This LBRN project supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103424 and by the Louisiana Board of Regents Support Fund.
- This study was supported by the NH/NSC 89239R016458 and the American Heart Association (Greater Southeast Affiliate #11GRN17700002 [JG]).

Figure 1: (A) Average LV mass from sham-control and volume overload ACF rats 14-weeks post surgery. p<0.05 vs. Sham, t vs. VO.

Figure 2: Collagen volume fraction (% Relative Mass), was increased in the ACF group compared to Sham. LOX inhibition attenuated the VO-induced increases in collagen. (p<0.05 vs. Sham; n=14wk ACF + BAPN, n=5 to 8/group).

Figure 3: ACF increased total collagen concentration in the heart. LOX inhibition prevented this increase.
Abstract:

Age related Macular Degeneration (AMD) is the leading cause of permanent vision loss in people age 50 and over and affects around 8 million Americans on average. By the year 2020, these statistics are expected to increase by more than 50% (1). There are many demographic and behavioral factors that can cause AMD such as age, body mass index, race, smoking history, and even sunlight exposure (2). When light enters the eyes through the macula, photoreceptors convert it into electrical signals and process these signals through retinal synapses. However, when the eye encounters excess light, photoreceptors undergo apoptosis (3). Ischemic preconditioning has been shown to protect photoreceptors from light damage by causing a brief period of ischemia before the cells experience a light induced injury (4). A neuroprotective agent known as Neuroprotectin D1 (NPD1), derived from the omega-3 fatty acid docosahexaenoic acid (DHA) and catalyzed by the enzyme 15-lipoxygenase-1 (15-LOX-1), serves as a lipid mediator that enhances cell survival (5). Therefore, we predicted 15-LOX-1 to be upregulated through ischemic preconditioning and tested the hypothesis that inhibition of 15-LOX-1 reduces photoreceptor survival during light damage.
Significance of DHA and 15-LOX-1 in Ischemic Preconditioning: Photoreceptor Survival

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Abstract

Docosahexaenoic Acid (DHA) is a 22-carbon long chain polyunsaturated fatty acid that can be converted into the neuroprotective agent, NPD1, with the enzyme 15-lipoxygenase-1 (15-LOX-1). DHA is known to be involved in the protective actions of the brain and retina. To determine how DHA and 15-LOX-1 affect photoreceptor cells during ischemic preconditioning and protection in the light damage model of Age-related Macular Degeneration (AMD), we utilized hydrostatic pressure as well as geared forces preconditioning. Our results indicated that hydrostatic ischemic preconditioning provided a higher degree of protection than the geared forces method. However, after treatment with hydrostatic pressure, many rats developed cataracts while those that experienced preconditioning with geared forces did not. In light induced photoreceptor degeneration, hydrostatic pressure with controls provided the most photoreceptor protection while light damage without preconditioning offered the least. Addition of DHA and treatment with the 15-LOX-1 inhibitor PD-146176 reduced the protective actions of hydrostatic preconditioning, but exhibited more protection than light damage alone. As DHA and 15-LOX-1 are known to produce NPD1, these findings may help reveal the pathofy of NPD1 in the retina.

Introduction

Age-related Macular Degeneration (AMD) is the primary cause of permanent vision loss affecting over 8 million Americans above the age of 50. By the year 2020, these statistics are expected to increase by more than 50% largely due to population increase (1). AMD can be caused by many demographic and behavioral factors such as age, body mass index, race, smoking history, and even sunlight exposure (2). When light enters the eyes through the macula, photoreceptors convert it into electrical signals and process these signals through retinal synapses. However, when the eye encounters excess light, photoreceptors undergo apoptosis (3). Ischemic preconditioning (IPC) has been shown to protect photoreceptors from light damage by causing a brief period of ischemia before the cells experience a light induced injury (4). Docosahexaenoic Acid (DHA), a 22-carbon long chain polyunsaturated fatty acid, is found throughout the body and known to enhance cell survival by making the lipid mediator Neuroprotectin D1 (NPD1) with the catalysing enzyme 15-lipoxygenase-1 (15-LOX-1). DHA has been found in the retina, but the pathway of NPD1 in this experimental paradigm is unknown (5).

In this report, we demonstrate that adding DHA through IPC would protect photoreceptors from light damage while the addition of the 15-LOX-1 inhibitor PD-146176 would reduce photoreceptor survival and provide less protection.

Methods

- Male Sprague Dawley rats (150-175 g) were purchased from Charles River
- Induced neurectomy to left eyes for 9 minutes by geared forces or hydrostatic preconditioning on rats with DHA, Saline, 15-LOX-1 inhibitor PD 146176
- Let rats recover for 24 hours
- Light damaged at 18,000 lux in cylindrical tubes illuminated with bright white light for 5 hours
- Allow retina to regenerate for at least 5 days
- Used Optical Coherence Tomography (OCT) to record images of the inferior and superior region
- Analyzed OCT images and created graphs
- Sacrificed rats and collected eyes
- Placed eyes in fixative for 24 hours, then placed in 30% sucrose for another 24 hours
- Collected 20 micron sections of each eye on microslides using cryostome sectioning
- Performed hematoxylin and eosin (H&E) staining on sections containing the optic nerve

Results

Superior Region

Inferior Region

Light Damage

No Light Damage

Light Damage + DHA

Light Damage + PD-146176

Summary

- Geared forces IPC provided protection against light damage, however hydrostatic IPC provided the most photoreceptor protection while light damage alone provided the least
- Addition of 4mg/kg 15-LOX-1 inhibitor PD-146176 reduced the protective actions of hydrostatic IPC
- 3.1 mg/kg of DHA addition also reduced protective actions of hydrostatic IPC
- There was a high incidence of cataracts in hydrostatic IPC, but not in geared forces IPC

Conclusions

While hydrostatic IPC offers the most photoreceptor protection, the geared forces IPC method would seem secure when ischemic preconditioning reduces cataract formation and allows for drug co-treatment experimentation. The reduction in photoreceptor protection associated with DHA addition may be due to toxicity levels or higher energy utilization via receptor efficiency. Also, since the 15-LOX-1 inhibitor decreases protection of IPC, it may be necessary to use NPD1 as a protective agent. This protective mechanism is useful in identifying natural protective metabolites within the retina, which can lead to possible treatments for diseases such as AMD.

References


Acknowledgements

I would like to thank my mentor Dr. Nicolas Bazan and the LBRN summer program as well as Dr. Tongbing Zhou, Carolee Danielson, Dr. William Foreman, and my co-mentor Dr. Eric Knott.

* This LBRN project was supported by National Institute of Neurological Disorders and Stroke grants R34NS093284 and R01NS079748.
Abstract:

Oxidative stress (OS) causes accumulation of Reactive Oxidative Species in ocular diseases such as AMD and Glaucoma. It is already established in this lab that oxidative stress using hydrogen peroxide and TNF-alpha cause induction of inflammasome resulting in the accumulation of inflammasome-related proteins like NALP3 and ASC. DHA and PEDF compromise these effects. Contrary to this, Iduna and PIN1, which are two cell survival proteins, behave oppositely with DHA and PEDF. This project will test another neurotrophin, BDNF, in combination with DHA and PEDF on the behavior of above mentioned proteins under oxidative stress. Our results indicate that DHA in combination with PEDF up-regulated Iduna and PIN1 and down-regulated NALP3 and ASC in ARPE-19 cells under stress. On the other hand, BDNF alone or in combination with DHA or PEDF displayed a comparable effect on those proteins compared to PEDF and DHA. These results are indicative of the fact that this neurotrophin, BDNF, combined with DHA will be similarly effective as PEDF and DHA as a therapeutic agent in treatment of ocular diseases.
Modulation of Pro-Inflammatory Proteins and Cell-Survival Proteins by DHA and Neurotrophins (PEDF and BDNF) Under Stress in ARPE-19 Cells

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Abstract
Oxidative stress (OS) causes accumulation of Reactive Oxygen Species in ocular diseases such as AMD and Glaucoma. It is already established in this lab that oxidative stress using hydrogen peroxide and TNF-alpha cause induction of inflammatory proteins such as NALP3 and ASC. DHA and PEDF can modulate these effects. Contrary to this, Iszona and PIN1, which are cell survival proteins, behave oppositely with DHA and PEDF. This project will test another neurotrophin, BDNF, in combination with DHA and PEDF on the behavior of above mentioned proteins under oxidative stress. Our results indicate that DHA in combination with PEDF up-regulated Iszona and PIN1 and down-regulated NALP3 and ASC in ARPE-19 cells under stress. On the other hand, BDNF alone or in combination with DHA or PEDF displayed a comparable effect on those proteins compared to PEDF and DHA. These results are indicative of the fact that this neurotrophin, BDNF, combined with DHA will be similarly effective as PEDF and DHA as a therapeutic agent in treatment of ocular diseases.

Introduction
Oxidative Stress is a cell stress that is characterized by an accumulation of free reactive oxygen species (free radicals), which eventually leads to cell damage and, finally, apoptosis. Iszona: A protein encoded by the ISZON gene that is expressed in placenta and liver, and it is involved in the regulation of DNA damage response elements. PIN1: A ubiquitin-protein ligase that is involved in the regulation of cell cycle progression and cell survival.

Conclusions
In conclusion, I have shown that BDNF, alone and in combination with DHA, was able to inhibit OS-induced apoptosis. In addition to PEDF combined with DHA, I have shown that survival protein Iszona and PIN1 were up-regulated by BDNF and DHA in ARPE-19 cells under stressed conditions. Also, NALP3, a pro-inflammatory protein, was inhibited by BDNF and DHA in ARPE-19 cells when stressed. Although, we can classify that BDNF was not as effective alone as when combined with DHA.

Experimental Design and Methods
1. Culture of ARPE-19 Cells
   - ARPE-19 cells were maintained in DMEM/F12 supplemented with 10% fetal bovine serum and incubated at 37°C with 5% CO2.
2. Introduction of Oxidative Stress and Conditions
   - 80% confluent ARPE-19 cells in six or twelve-well plates were serum-starved for 8 hours. Oxidative stress was induced using either 400 or 800 μM H2O2 for 10 min or 30 min.
3. Apoptosis Study
   - To make sure oxidative stress is killing the cells, the Hoechst Stain technique and a Nikon camera were used to calculate percent apoptosis in stressed cells.
4. Western Blot Analysis
   - 20-25 μg equivalent protein were loaded on NuPAGE 4-12% Bis-Tris gels and run at 125 V for 120 minutes. Proteins were transferred onto PVDF (Hybond) paper. Rabbit, Iszona, PIN1, and NALP3 proteins were detected using anti-IgG antibody clone N201/35 (UC Davis/NIH Neuro Mab Facility). Anti-PIN1 (antibody Cell Signaling Technology, Medford, MA), and anti-NALP3 antibody (IMGENEX San Diego, CA). Finally, the above mentioned proteins were imaged in Fujifilm LAS-3000 using Amersham ECL Western Blotting Detection Reagents.
5. Data Analysis
   - Percent protein bands were quantified by Multi-gage software and the results were expressed as Internal/GAPDH ratio.

Acknowledgements
My mentors Dr. Pranab K. Mukherjee and Dr. Nicolas Bazzan.
Main Kulberejeh, Brenda Chappell, and Zovle Davis.
All the staff and faculty working at the Neuroscience Center of Excellence.

The support of the Louisiana Board of Regents Support Fund.
Abstract:

An Alu element is a retrotransposon that has propagated to more than one million copies in the primate genome, allowing it to serve as a marker for species divergence that has occurred over the past 65 million years. In this study, we looked at population panels of olive baboons (Papio anubis) and yellow baboons (Papio cynocephalus) and the presence or absence of the Alu element in twelve individuals from each species. The Alu elements acted as markers that were used for population genetic analyses of olive and yellow baboons. Running the panels through Polymerase Chain Reaction (PCR) generates millions of copies of the Alu elements, which were isolated by specific primers. After gel electrophoresis procedures, we were able to interpret the presence or absence of the Alu element on 92 specific loci of twelve olive baboons and twelve yellow baboons. Our study illustrates that over 75% of the Alu elements that have been evaluated in the two populations are polymorphic. From this we can likely conclude that most Alu elements are younger and have only recently been inserted into the genomes. The data from this project was analyzed using the program Structure. A graphical analysis of the population structures of olive and yellow baboons was created, and the results separately categorized olive and yellow baboons into two likely clusters, with some admixture in both. The study of the expansion of these mobile Alu elements into non-human primate genomes will play an important role in shaping and comparing these genomes.
**Alu-Based Population Structure of Olive and Yellow Baboons**

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**Abstract**

An Alu element is a retrotransposon that has encapsulated more than one million copies in the primate genome, allowing it to serve as a marker for species divergence that has occurred over the past 65 million years. In this study, we looked at population genetics of olive and yellow baboons (*Papio cynocephalus*) and the presence or absence of the Alu element in twelve individuals from each species. The Alu elements were isolated and maintained in cell lines that were used for population genetic analysis of olive and yellow baboons. Running the panels through Polymerase Chain Reaction (PCR) generates millions of copies of the Alu elements, which were isolated by specific primers. After gel electrophoresis procedures, we were able to determine the presence or absence of the Alu element in each specific sample of twelve olive baboons and twelve yellow baboons. Our study illustrates that at least 75% of the Alu elements that have been evaluated in the two populations are polymorphic. From this, we can already conclude that most Alu elements are younger and have only recently been integrated into the genome. The data from this project was analyzed using the program Structure. A graphical analysis of the population structure of olive and yellow baboons was created, and the results separately categorized olive and yellow baboons into two clusters, with some admixture in both. The study of the expansion of these mobile Alu elements into non-human primate genomes will play an important role in shaping and comparing these genomes.

**Introduction**

Old World monkeys consist of primates in the family of Cercopithecidae, including baboons. The olive baboon, *Papio cynocephalus*, is found in 23 different countries spanning throughout Africa and exhibits mainly upon grasslands near wooded areas. The yellow baboon, *Papio cynocephalus*, is found from the western to eastern coast in central Africa and inhabit savannahs and open woodlands. In this study, insertion patterns of Alu elements were used to study olive and yellow baboons. Olive baboons are closely related to humans, they make for good contenders as biological research models. Through baboon genome research involving the study of Alu elements, the evolution of primates can become clearer. Alu elements are Short Interspersed Elements (SINES) that are approximately three hundred base pairs in length and are unique to primates. An Alu SINE is a repetitive element in primate genomes that has a high copy number of more than one million elements. An Alu element inserts into the genome through a "copy and paste" method and requires the enzymatic machinery of LTR for its retrotransposition. Because Alu elements use LTR to insert into the genome, they are nonhomologous elements. The insertion and propagation of Alu elements into the primate genome has contributed to the expansion of primate genomes over time. In this study, the relationship between olive and yellow baboons was sought out through the phylogenetic study of Alu elements. The evolution of primates, such as Alu elements, are used to study the phylogenetic and population genetic relationships of primates.

Over time, the Alu insertions will expand throughout the primate genomes and increase in frequency among the population. Throughout primate evolution, the Alu elements have the potential to integrate into a primate genome and can be shared by one or more species. An Alu element is fixed present when it is present in every individual of the population. If an Alu element is present in every individual, the Alu element is considered an "ancestral allele" and is a characteristic of younger Alu elements that may have been more recently integrated into the population.

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**Methods**

3. Master Mix is made with forward and reverse primers specific to be certain loci in a genome.

3. The panels are run in a PCR (polymerase chain reaction) machine at the appropriate temperature.

4. While the panel is running, an agarose gel is made.

5. Gels are separated gel electrophoresis.

6. A picture of the gel is taken under UV light to image the sample.

7. Record presence and absence of Alu elements in olive and yellow baboons.

8. Structure\textsuperscript{2} was used to investigate population structures between olive and yellow baboons, and among yellow baboons to look for substructure.

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**Results and Discussion**

![Image: Gel electrophoresis pictures depicting the different classes of the structure of Alu elements in olive and yellow baboons. The first well in the olive baboon lanes is for DPA (human), representing the entire genome of the Alu elements. The second lane is for DNA specific and does not show up in humans. A band above the empty site represents a filled site, indicating the presence of the Alu element. A band that is present in both the empty and filled sites suggests homogeneity.](image)

At these results show examples of Alu elements that inserted into all twenty-four individuals at a particular locus. This strongly suggests that the Alu elements are fixed present in both olive and yellow baboons and that a very high presence of the Alu element in the baboon lineage was maintained for at least a long period of time. This result was captured using an insert region with a high signal intensity.

These results display the Alu element in olive baboons that is polymorphic in both the olive and yellow baboons, but it is not unique. Because of the polymorphism, it is difficult to track this element in a proper location in the genome. This is due to the fact that these baboons are individuals that are not specifically identified. In all olive and yellow baboons, the Alu element is known. From this, they have shifted frequently to identify this particular Alu element is very clear and was recently inserted into the genome of baboons.

**Conclusions**

Alu elements retrotransposons that have inserted into the genomes of olive and yellow baboons are of different ages, depending on the time they inserted into the genome. Primers were tested for 102 specific loci for olive and yellow baboons, after PCR amplification and gel electrophoresis procedures, 92 primer pairs gave clear and conclusive results. Out of the 92 tested, more than 75% of both olive and yellow baboons were polymorphic; therefore, the Alu elements looked at in this study were relatively young.

The results from Structure\textsuperscript{2} display there were primarily two distinct population clusters. Both olive and yellow baboons have their own cluster, with some admixture in each.

There was no substructure within the population of yellow baboons discovered following Structure\textsuperscript{2} analysis.

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**Future Work**

Future study on this project will be to continue evaluating olive and yellow baboon genomes for Alu insertions.

Continue using Structure\textsuperscript{2} to analyze the populations of olive and yellow baboons.

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**Acknowledgments**

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Abstract:

Roughly 90% of the adult human population carries Herpes Simplex Virus- Type 1. Symptoms are not always explicit, as the virus has evolved the capacity to enter a latent infectious cycle. During this period, HSV-1 may reside in the trigeminal ganglia, a system of neurons connecting oral and ocular regions. Thus, an HSV-1 mouth infection could potentially cause devastating eye sores. An area of modern research involves the development of treatments for HSV-1. A goal of the Neumann Lab is to explore the epigenetic mechanisms governing the latent cycle and subsequent reactivation of HSV-1. Seven occupied CTCF binding motifs have been identified in the latent HSV-1 genome and are positioned in an orientation that flanks the reactivation critical LAT and each IE region separately. It has been proposed that CTCF is an insulator protein which prevents lytic viral genes from being expressed during the latent cycle of infection. The Neumann lab has subsequently reported that at least 3 of the HSV-1 CTCF binding motifs are occupied by CTCF during latency in a transcript-dependent manner and these 3 sites undergo CTCF eviction at early times post-reactivation in mice latent with 17Syn+ (Ertel, et. al J. Virol, 2012). These findings further support the interesting possibility that each CTCF domain in HSV-1 may be independently regulated. To further test this, we generated a number of constructs, each of which contained the commercially-available pGL3 control vector ligated to one or more segments of the HSV-1 genome. We hypothesized that the CTCF binding motif CTRL1 has enhancer blocking capabilities (when positioned upstream of the LAT Enhancer, LTE). To test this in the future, a luciferase assay can be performed on transiently transfected Hela cells. The quantity of luciferase produced should be inversely correlated to the enhancer blocking capability of CTRL1.
CTCF BINDING MOTIFS AS ENHANCER BLOCKERS IN HERPES SIMPLEX VIRUS-TYPE 1
Blake Schouest, Farhana Musarrat1, Donna M. Neumann1,2

(1) Department of Pharmacology, Louisiana State University Health Sciences Center, New Orleans, LA, United States; (2) Department of Ophthalmology, Louisiana State University Health Sciences Center, New Orleans, LA, United States

Abstract
Roughly 90% of the adult human population carries herpes simplex virus type 1. Symptoms are not always explicit, as the virus has evolved the capacity to enter a latent infectious cycle. During this period, HSV may reside in the trigeminal ganglia, a system of neurons connecting cranial and visceral regions. Thus, an HSV-1 mouth infection could potentially spread to and cause eye infections. An area of recent research interest is the development of treatments for HSV. A goal of the Neumann lab is to explore the epigenetic mechanisms governing the latent cycle and subsequent reactivation of HSV-1. Several CTCF-binding motifs have been identified in the latent HSV-1 genome and are present in areas that flank the reactivation critical LAT and each cell region. It has been proposed that CTCF is an insulator protein that prevents latent viral genes from being expressed during the latent cycle of the infection. The Neumann lab has subsequently reported that at least 80% of the HSV-1 CTCF-binding motifs are occupied by CTCF during latency in a transcription-dependent manner, and these 8 sites undergo CTCF reactivation at early times post-reactivation in mouse latent with 15Day-20Day (Endo, et al. 2015). These findings further support the interesting possibility that each CTCF domain in HSV-1 may be independently regulated. To further test this, we engineered a number of constructs, each of which contained a commercial, available 5' UTR control vector ligated to one or more segments of the HSV-1 genome. We hypothesized that the CTCF-binding motif(s) of CTR1 has enhancer-blocking capabilities when positioned downstream on the LAT Enhancer (STE). To test this in vivo, a lacZ reporter assay was performed on transiently transfected HeLa cells. The quantity of luciferase produced should be inversely correlated to the enhancer-blocking capability of CTR1.

Introduction
A hallmark of HSV-1 infection is the establishment of latency in sensory ganglia. During latency, the tegument fragment of the HSV-1 genome transcribed into within is the latent tegument. Associated latent virus (LAV) remains repressed during latency. The virus establishes itself in part through epigenetic mechanisms, in the latent cycle this is contributed to by the CTCF motif, which is tightly repressed, forming heterochromatin. Several distinct CTCF binding regions (CCCTC-binding motif) are found in the HSV-1 genome (Figure A). It is established in the literature that these regions act as insulators; the binding of CTCF protein at these sites prevents the spread of heterochromatin to other regions (Figure B).

Figure A: CTCF binding motifs in HSV-1 genome
It has been speculated that CTCF-binding motifs may act as enhancer blockers in HSV-1. In essence, they may repress the expression of viral genes by blocking a critical part of the LAV transcribed, the 3' UTR. This mechanism could regulate the transcription of the LAV and thereby the latent phase. It is unclear how the CTCF motif(s) are expressed in the genome.

Previously, the Neumann lab has characterized three of the seven CTCF-binding domains in the HSV-1 genome as functional insulator elements that can act as enhancer blockers to the LAT enhancer (Endo, et al. 2015, Endo, et al. 2015).

Figure B: CTCF Insulation between enhancer and transcribed regions

CTCF, a sequence determinant that is known to be involved in the regulation of viral gene expression, is a critical factor in the regulation of the expression of viral genes. In this study, we have investigated the role of CTCF in the regulation of viral gene expression.

Results

Figure 1: PCR of HSV-1 (500 base pairs) from the control vector was successful. The bright band of 500 bp in both lanes (left) is 600 base pairs.

Synthesis of CV and LIT 1 Construct

Figure 2: A construct of CV and LIT 1 was developed. This construct contains two segments after restriction digestion.

Figure 3: Molecules were present on the CV and LIT 1 plates on the CV and LIT 1 plates.

Figure 4: CV and LIT 1 plates.

Synthesis of CV and CTR1 Construct

Figure 5: CV and CTR1 construct. Their DNA contains two segments after restriction digestion.

Figure 6: CV and CTR1 plates.

Methods

Synthesis of CV and LIT 1 Construct

The following constructs were used in the study:

CV LIT + CV LIT + CV LIT + CTR1 CV LIT + CV LIT + CTR1 CV LIT + CTR1

The following techniques were employed to synthesize each construct:

1. Polycarbonate Chain Reaction
   - Each segment of the HSV-1 genome was amplified using primers specific to the region. The following primers were used:
     - CV (5' and 3')
     - LIT (5' and 3')
     - CV LIT (5' and 3')
     - CV LIT (5' and 3')

2. Restriction Digestion
   - Sticky ends were created on the plasmid control vector as well as the insert for each construct. These sticky ends were used in conjunction with the vector and insert for each construct. This insured that the two could be ligated.

3. Ligation
   - The restriction digested vector and insert (for each construct) were purified by using Spin Columns. Each construct was then inserted into the plasmid control vector.

4. Gel Electrophoresis
   - The insert was obtained from the restriction digested DNA samples (from vector and insert) from the 1% agarose gel.

5. Electrophoresis
   - The insert was obtained from the restriction digested DNA samples (from vector and insert) from the gel.

6. Ligation
   - The vector and insert were ligated. This was accomplished by using Quick Ligate in a solution of Quick Ligate buffer.

7. Transformation
   - Using the transformation buffer, DH5 alpha competent cells were transformed with each construct. Selection was performed on Luria Broth plates containing ampicillin (100 μg/mL), kanamycin (25 μg/mL), and chloramphenicol (50 μg/mL).

8. Transfection and Luciferase Assay
   - The E. coli samples containing the appropriate constructs were grown in broth containing a kanamycin-resistant E. coli strain.

9. Summary and Conclusions
   - CTR1 may function in tandem with other CTCF binding motifs to block the transcriptional activity of the LAT Enhancer.

Future Experiments

A future experiment could include the use of pGL3 control vector with the CTR1 and CTR2 domains. This would clarify the action of these CTCF binding motifs without the added effect of the LAT Enhancer.

Acknowledgements

This DBRC project was supported in part by an Institutional Developmental Award (IDB) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number 5P20GM106854-05 and by the Louisiana Board of Regents Support Fund.
Abstract:

The 2010 BP Deepwater Horizon Oil spill in the Gulf of Mexico is known to be the greatest environmental disaster in US history with the release of an estimated 4.9 million barrels (over 200 gallons) of oil. The state of Louisiana, about 42 miles from the wellhead, had the greatest impact.
Byproducts of the microbial degradation of crude oil and the deterioration of historic masonry

Titilayo Shobayo\(^{1,2}\), Katherine Langdon, Ny Pham and Carol Chin\(^{1,2}\).

\(^{1}\) National Center for Preservation and Training, Materials Conservation and \(^{2}\)Northwestern State University

Abstract

- The 2010 BP Deepwater Horizon oil spill in the Gulf of Mexico is known to be the greatest environmental disaster in US history with the release of an estimated 4.9 million barrels (over 209 million) of oil.
- The state of Louisiana, about 2 miles from the wellhead, had the greatest impact.

Materials and Methods

Colorimetry

The Konica Minolta CR-200 colorimeter measures color in the L*\(a*\)*\(b*\)* color space to quantify the color of objects.

Results

- Graphic 1 shows the comparison of the different oil samples.
- Graphic 2 shows the sample with the highest color change.
- Graphic 3 shows the sample after weathering in the QUV accelerated weathering test.
- Graphic 4 shows the sample after cleaning with the MGC/Poulite method.
- Graphic 5 shows the sample after cleaning with the DW/Poulite method.

Conclusion

- The above data indicate that the removal of crude oil from historic masonry with the MGC/Poulite cleaning method had the smallest difference when compared with the Pre-Oiled and Cleaned samples.
- The samples cleaned with DW only show the effect of the agitation with a soft brush compared to no cleaning at all.

Ongoing Studies

- Use contact angle goniometry to quantitatively measure the wetting characteristics of each sample which will indicate whether the oil is still present within the brick.
- Revisit the spill site in Fort Livingston, LA to obtain oil samples for further study of the microbial activity and potential ongoing degradation of the masonry.

Acknowledgements

I would like to thank Leah Pood, Adam Cox, Jason Church, and the rest of the staff at NCPTT.

This LRMP project was supported by an institutional development award (IDA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number 5P30MD007589, and by the Louisiana Board of Regents Support Fund.

Focus of Current Study

Goal: To determine an effective cleaning method that leaves little to no residue, thus preventing further microbial activity that could lead to the production of carbonic acid which can cause deterioration of historic masonry like that used at Fort Livingston.

- Wash brick with 5SWA and agitation with soft brush.
- Leave residue on brick needs with delinens.
- Attract microbes (biodegradation occurs).
- They release CO\(_2\) in H\(_2\)O.
- CO\(_2\) + H\(_2\)O \rightarrow H\(_2\)CO\(_3\) (Carbonic acid).
- Eventually deteriorates the brick.
Abstract:

Parkinson’s disease (PD) is the second most common neurodegenerative disease in humans and is caused by the loss of dopaminergic neurons in the region of the mid-brain called the substantia nigra pars compacta [4]. The formation of Lewy bodies (LB) is seen in neurons in brains of individuals affected by PD [3]. LBs are formed by the aggregation of the protein α-synuclein (aS). In dopaminergic neurons, aS associates with vesicles and membranes enriched in lipid rafts, and its primary function is to mediate, with other proteins, the fusion of presynaptic vesicles with the presynaptic membrane [1]. The GPI-anchored proteins of the lipid rafts require a phosphoethanolamine linker donated by phosphatidylethanolamine (PE) [5].

In S. cerevisiae, PE is mainly synthesized in the mitochondria by Psd1 and, in lesser amounts, Psd2. These enzymes are decarboxylases which convert phosphatidylserine into PE. Previous work in the lab has shown that knocking out the PSD1 gene causes intense stress in the endoplasmic reticulum and triggers aS to accumulate in LB-like foci due to low levels of PE [5].

In this work, we screened the entire genome of S. cerevisiae in the psd1Δpsd2Δ mutant three times to see if there are any genes that can rescue the slow growth phenotype caused by low PE. From the screens, we isolated 19 plasmids that rescued the slow growth and identified four genes of interest that are involved in mitochondrial processes or de novo synthesis ER-derived vesicles whose effects in psd1Δpsd2Δ mutants will be investigated in future work.
Screening for genes that rescue lipid-induced stress in the endoplasmic reticulum — implications for Parkinson’s disease

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Abstract

Parkinson’s disease (PD) is the second most common neurodegenerative disease in humans and is caused by the loss of dopaminergic neurons in the region of the midbrain called the substantia nigra pars compacta (SNc). This formation of Lewy bodies (LBs) is seen in neurons in brains of individuals affected by PD [3]. LBs are formed by the aggregation of the protein α-synuclein (α-syn). In dopaminergic neurons, α-syn associates with vesicles and membranes enriched in lipid rafts, and its primary function is to mediate, with other proteins, the fusion of presynaptic vesicles with the presynaptic membrane [1]. The α-syn-interacting proteins of the lipid rafts require a phosphoethanolamine (PE) headgroup by phosphatidylethanolamine (PE) [2].

In S. cerevisiae, PE is mainly synthesized in the mitochondria by Pho82P and, in lesser amounts, Pho22. These enzymes are deacetylases which convert phosphatidylserine into PE. Previous work in the lab has shown that knocking out the Pho22P gene causes increased stress in the endoplasmic reticulum (ER) and triggers its uncontrolled release of Ca²⁺ to the cytoplasm due to low levels of PE [3].

In this work, we screened the entire genome of S. cerevisiae in the pah1Δ,pah2Δ mutant strain to identify any genes that rescue the slow growth phenotype caused by low PE. From the screen, we isolated 19 plasmids that rescued the slow growth and identified two genes of interest that are involved in mitochondrial processes or de novo synthesis ER-derived vesicles whose effects in pah1Δ,pah2Δ mutants will be investigated in future work.

Introduction

Parkinson’s disease (PD) is the second most common neurodegenerative disease in humans and is caused by the loss of dopaminergic neurons in the region of the midbrain called the substantia nigra pars compacta (SNc). This formation of Lewy bodies (LBs) is seen in neurons in brains of individuals affected by PD [3]. LBs are formed by the aggregation of the protein α-synuclein (α-syn). In dopaminergic neurons, α-syn associates with vesicles and membranes enriched in lipid rafts, and its primary function is to mediate, with other proteins, the fusion of presynaptic vesicles with the presynaptic membrane [1]. Lipid rafts are composed of cholesterol, sphingolipids, receptor proteins, and glycosylphosphatidylinositol (GPI)-anchored proteins where they play a critical role in signal transduction and vesicle trafficking. Lipid rafts and the associated proteins have been shown to form in the endoplasmic reticulum and from there travel to the plasma membrane. GPI-anchors are composed of a phosphatidylcholine headgroup, a glycosylphosphatidylinositol (GPI) which is attached to the glycan core, PE is mainly synthesized by two pathways. In mitochondria the enzyme phosphatidylethanolamine decaprenyltransferase (Pah1p) converts phosphatidylcholine to PE, whereas in the endoplasmic reticulum ethanolamine (ETA) is converted to PE through the Kennedy pathway (Fig. 1). Knocking out the Pah2p gene causes increased stress in the endoplasmic reticulum and triggers its uncontrolled release of Ca²⁺ to the cytoplasm due to low levels of PE [3]. In this work, we screened the entire genome of S. cerevisiae in the pah1Δ,pah2Δ mutant strain to identify any genes that rescue the slow growth phenotype caused by low PE.

Materials and Methods

We took plasmids from a yeast genomic library from Open Biosystems and transformed the plasmids into pah1Δ,pah2Δ yeast mutants on SC glucose (2%)-ura3Δ (or)-leu2Δ (or)- lys2Δ (or)-his3Δ (or)-met15Δ media. As a positive control, an empty vector, pRS426, was transformed into pah1Δ,pah2Δ yeast mutants on SC glucose (2%)-ura3Δ (or)-leu2Δ (or)-lys2Δ (or)-his3Δ (or)-met15Δ media. After 3 days, colonies were picked and a yeast plasmid prep was performed under standard protocols to isolate the plasmids from the cells.

The yeast plasmid prep results in a yield too low to allow for genetic sequencing, so these plasmids were transformed into E. coli to amplify the plasmid amount. This E. coli transformation was performed using the method previously by New England BioLabs for protocol #2688. After overnight growth, the colonies were harvested, and a yeast plasmid prep was performed on these transformed E. coli cells using a protocol provided with a kit method from GeneRif Laboratories, LLC.

Before sequencing the plasmids, we transformed these plasmids isolated from the E. coli plasmid prep into pah1Δ,pah2Δ yeast mutants using the same methodology as the original yeast transformation. This was done to confirm that the plasmids isolated from the E. coli show the same phenotype from the first transformation.

The plasmids were then sent to the University of Iowa for genetic sequencing. The genetic sequences were analyzed using “S. cerevisiae WU-BLAST2 Search” from the Saccaromyces Genome Database.

Discussion

In the three screens, we transformed the entire genome of S. cerevisiae into pah1Δ,pah2Δ yeast mutants to isolate any genes that rescue the slow growth phenotypes. To do this, we transformed plasmids from a yeast genomic library into the double deletion mutants (Fig. 1), and isolated the plasmids from the colonies that grew. These isolated plasmids were then amplified in E. coli cells on LB-kanamycin and were allowed to grow overnight (Fig. 4). Colonies were picked, and the plasmids were isolated. To ensure that the genetic material isolated from the E. coli maintained the same rescue effect of the slow growth phenotype, the plasmids isolated from the E. coli were transformed into E. cerevisiae pah1Δ,pah2Δ yeast mutants. The double deletion mutants were allowed to grow for 3-5 days. The results of the phenotype confirmation showed that the slow growth phenotype was rescued (Fig. 6). Nineteen plasmids were sent for genetic sequencing, and their genes were identified using the "WU-BLAST2" search engine.

Conclusions

• Seventeen of the isolated plasmids contained Pah1p.
• Two of the isolated plasmids contained Pah2p.
• The plasmid containing the gene that is related to both mitochondrial functions and vesicular trafficking (Pah1, GSDM, YIF1, FRB2).
• Future experiments will analyze the individual effect of these genes in pah1Δ,pah2Δ mutants.

References


Acknowledgements

• This work was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103424 and the Louisiana Board of Regents Support Fund.
Abstract:

Several clinical studies have shown that the benefits of renin-angiotensin system (RAS) blockade in the development of diabetes and a local RAS has been identified in the pancreatic islets. Angiotensin I – converting enzyme (ACE2) a new component of RAS, has been identified in the pancreas. ACE2 has been identified as a novel target for the prevention of β-cell dysfunction occurring in type 2 diabetes. ADAM17 (a disintegrin and metalloprotease) is associated with the shedding of ACE2 in the diabetic rodent models with overactive Renin-Angiotensin System. To test the role of ADAM17 in pancreatic ACE2, transfection of 832/13 cells with expression plasmids for ACE2, ADAM17, and hydrolytically inactive ADAM17 where it is set up in a 6 well plate. it was incubated for 4h in cell culture incubator, washed with 2ml 832/13 medium and incubate with 2ml 832/13 medium overnight. Afterwards washed with medium, change medium; incubate overnight. It was finally harvested. After the cells have been harvested, shedded ACE2 and cell-associated ACE2 and ADAM17 levels are measured by the process of assay. This is set up in a 96 well at 37º for 1h. The levels of ACE2 and ADAM17 in mouse pancreatic islets are then compared. We asked the question “With ELEVATED adam17 expression, Will there be a reduction in the amount of ACE2 protein in the cell due to the shedding and how? Findings have shown that elevated ADAM17 is responsible for pancreatic ACE2 shedding leaving little ACE2 proteins on the cell.
Determination of the Dynamics of ACE2 in Response to ADAM17 level

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Abstract
Several clinical studies have shown that the benefits of renin-angiotensin system (RAS) blockade in the development of diabetes and a local RAS has been identified in the pancreatic islets. Angiotensin 1 – converting enzyme (ACE2) a new component of RAS, has been identified in the pancreas. ACE2 has been identified as a novel target for the prevention of β-cell dysfunction occurring in type 2 diabetes. ADAM17 (a disintegrin and metalloproteinase) is associated with the shedding of ACE2 in the diabetic rodent models with overactive Renin-Angiotensin System. To assess the role of ADAM17 in pancreatic ACE2, transfection of 832/13 cells with expression plasmids for ACE2, ADAM17, and hydrolytically inactive ADAM17 where it is set up in a 6 well plates. It was incubated for 4 hours in cell culture incubator, washed with 2ml 832/13 medium and incubate with 2ml 832/13 medium overnight. Afterward washed with medium, change medium: incubate overnight. It was finally harvested. After the cells have been harvested, shedded ACE2 and cell-associated ACE2 and ADAM17 levels are measured by the process of assay. This is set up in a 96-well at 37°C for 4hrs. The levels of ACE2 and ADAM17 in mouse pancreatic islets are then compared. We asked the question “With elevated adama17 expression, will there be a reduction in the amount of ACE2 protein in the cell due to the shedding and how? Findings have shown that elevated ADAM17 is responsible for pancreatic ACE2 shedding leaving little ACE2 protein in the cell.

Hypothesis
Increased levels of ADAM17 in pancreatic islets in diabetes leads to reduced ACE2 levels.

Material and Methods
- Transfection of 832/13 cells with expression plasmids for ACE2, ADAM17, and hydrolytically inactive ADAM17.
- Measure the shedded ACE2 and cell-associated ACE2 and ADAM17 levels by assay.
- Finally compare the levels of ACE2 and ADAM17 in mouse pancreatic islets

Background
- ACE2 in the pancreas protects against diabetes.
- ACE2 levels decrease as diabetes progresses.
- Study of how ACE2 is regulated in the pancreatic islets might lead to ways of preventing its depletion in diabetes.

Results
- ADAM17 is an enzyme that cleaves ACE2 from the cell surface.
- Increased levels of ADAM17 in beta-cell-derived cells leads to increased amount of shedded ACE2.
- ADAM17 levels are increased in pancreatic islets in diabetes.

Summary
ADAM17 over-expression decreases cellular ACE2 levels by 30-50% by shedding

Conclusion

Acknowledgements

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Abstract:

Obesity is defined by an accumulation of excess fat and is associated with a number of comorbidities including Type 2 Diabetes and cardiovascular disease. Investigating mechanisms which regulate obesity will significantly advance our understanding of this disease. The peptide, QRFP, is expressed in central and peripheral tissues, regulates energy intake and is differentially regulated by high fat diet in the skeletal muscle of rats which differ in their susceptibility to developing obesity. The goal of the current series of experiments was to investigate the role of QRFP on insulin sensitivity and markers of fat and carbohydrate metabolism using an in vitro model. L6 cells are immortalized rat skeletal muscle cells that express QRFP and its receptor, GPR103a and that are used to assess skeletal muscle markers of metabolism. In the L6 model, QRFP enhanced insulin sensitivity, as measured by an increase in glycogen deposition. Assessment of QRFP’s effects on the expression of the glucose transporter, GLUT4, and the fatty acid transporter, CD36, indicated a QRFP-induced decrease in the expression of CD36 mRNA. Together these data support a role for QRFP in fat and carbohydrate metabolism and as a regulator of insulin sensitivity. More studies are needed to further elucidate the role of QRFP in the susceptibility to developing obesity.
Role of QRFP on Glycogen Deposition and the Expression of Skeletal Muscle Markers of Fat and Carbohydrate Metabolism

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Abstract

Obesity is defined by an accumulation of excess fat and is associated with a number of comorbidities including Type 2 Diabetes and cardiovascular disease. Investigating the mechanisms which regulate obesity will significantly advance our understanding of this disease. QRFP (short for the obese peptide) is a neuropeptide that is expressed in centers and peripherally in tissues, regulates energy intake and is differentially regulated by high fat diet in the skeletal muscle of rats which differ in their susceptibility to developing obesity. The goal of the current series of experiments was to investigate the role of QRFP on insulin sensitivity and markers of fat and carbohydrate metabolism using an in vitro model. L6 cells are immortalized rat skeletal muscle cells that express QRFP and its receptor, GPR103a and that are used to assess skeletal muscle markers of metabolism. In the L6 model, QRFP enhanced insulin sensitivity, as measured by an increase in glycogen deposition. Assessment of QRFP's effects on the expression of glucose transporter, GLUT4, and the fatty acid transporter, CD36, indicated a QRFP-induced decrease in the expression of CD36 mRNA. Together these data support a role for QRFP in fat and carbohydrate metabolism and as a regulator of insulin sensitivity. More studies are needed to further elucidate the role of QRFP in the susceptibility to developing obesity.

Introduction

Obesity, the accumulation of excess fat, affects more than 35% of US adults. Obesity has been linked to diseases such as Type 2 diabetes, heart disease and some cancers. QRFP, a neuropeptide of the RF amide peptide family, is expressed in the hypothalamic nuclei of the brain and in peripheral tissues. QRFP's receptors are the GPR103a receptor (GPR103). QRFP administration increases high fat feeding behavior. QRFP is expressed in skeletal muscle and prepro-QRFP mRNA levels are differentially regulated by high fat diet in obesity-prone and obesity-resistant rats. Furthermore, QRFP enhances insulin sensitivity through the expression of genes associated with fat and carbohydrate metabolism. The goal of the current experiment was to examine QRFP's effects on the expression of genes associated with fat and carbohydrate metabolism. We hypothesized that QRFP would alter the expression of genes associated with fat and carbohydrate metabolism in this model.

Methods

QRFP enhanced insulin sensitivity as measured by an increase in glycogen deposition in L6 cells.

Conclusion

- QRFP and its receptor, GPR103a, are expressed in L6 myotubes.
- QRFP enhances insulin sensitivity by increasing glycogen deposition in L6 cells.
- In L6 cells, CD36 mRNA levels were significantly decreased by QRFP.
- These data suggest QRFP plays a role in insulin-stimulated glycogen deposition in skeletal muscle and regulates the expression of the fatty acid receptor, CD36.
- Future studies are needed to elucidate the role of QRFP in skeletal muscle and include protein analysis of GLUT4, CD36, AMPK and PPAR gamma.

This LBRN project supported by an Institutional Development Award (IDA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM096374 and by the Louisiana Board of Regents Support Fund.
Abstract:
Angiotensin converting enzyme 2 (ACE2) is an important member of the renin angiotensin system within the central nervous system. We previously reported that central ACE2 reduces the development of hypertension induced by chronic Angiotensin-II (Ang-II) infusion in transgenic mice (syn-hACE2) with human ACE2 overexpression in the brain. Activation of microglial cells and generation of pro-inflammatory cytokines in the hypothalamic paraventricular nucleus (PVN) has been shown to be associated with Ang-II-induced hypertension. In the present study we tested whether ACE2 overexpression will reduce the activation of microglia in the brain during Ang-II-induced hypertension. Non-transgenic control (NT) and syn-hACE2 mice (n=4/group) were infused with Ang-II (600 ng/kg/min, sec) for 14 days. After Ang-II infusion, the blood pressure was measured by carotid artery cannulation using a pressure transducer connected to a data acquisition system. After blood pressure recording, the mice were perfused with 4% paraformaldehyde and brains were collected. Immunohistochemistry was done to test the activation of microglia using markers Iba-1 and CX3CR1. The baseline blood pressure between NT and syn-hACE2 mice was similar. After 2-week Ang-II infusion, blood pressure was elevated in NT mice but not in syn-hACE2 mice. Compared with NT controls, Ang-II infused mice showed increased activation of microglia as indicated by increased staining for Iba-1 and CX3CR1 in the PVN. This increased microglial activation was reduced in syn-hACE2 mice. These data suggest that ACE2 over-expression in the brain decreased microglial activation in the PVN and reduced Ang-II mediated hypertension.
Microglia Activation in Wild Type Mice And Transgenic Mice
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Abstract

Sub-pressor Ang-II Hypertension

Introduction

- Basic Angiotensin System (BAS) is a hormone system that is important in regulating normal physiology and pathogenesis of cardiovascular disease.
- Angiotensin II is the main actor in the BAS system and contributes to vasoconstriction, and hypertension.
- Angiotensin Converting Enzyme 2 (ACE2) catalyzes the conversion of Ang II to Ang 1-7. It has beneficial effects of cardiovascular system.
- During Hypertension, inflammation of vessels results in migration of immune cells to the brain. These immune cells are called "Microglia Activation."

Hypothesis

Angiotensin-II activates microglia during hypertension. Mice overexpressing ACE2 in the brain will have less microglia activation as a consequence of Ang-II being metabolized by ACE2.

Methods and Experimental Protocol

- Non-transgenic (NT) mice.
- ACE2 transgenic mice.

Blood pressure was lower baseline in the wildtype control, and blood pressure was on baseline in the transgenic control. But there was no difference in the blood pressure reading between the wild type control and transgenic control. There was an increase in blood pressure in the Wild type mice infused with Ang II but there was no increase in blood pressure in the Transgenic mice infused with Ang II.

Summary

- Blood pressure is increased in the Wild type mice infused with Ang II.
- The mice overexpressing ACE2 in the brain had no increase in blood pressure.
- Angiotensin II contributes to the activation of Microglias (?).
- The Wild type mice infused with Ang II had more Microglia Activation than the Transgenic mice (?).
- Mice overexpressing ACE2 in the brain have less microglia activation (?).

Conclusion

Microglia is activated during hypertension. ACE2 overexpression reduces microglia activation (?).

Acknowledgments

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Abstract:

Previous studies have shown that docosahexaenoic acid (DHA) has neuroprotective properties when administered following middle cerebral artery occlusion (MCAo) in a 7-day survival rat model (1). In this study, we tested whether neuroprotection induced by DHA persist during chronic survival after focal cerebral ischemia. Male Sprague-Dawley rats were subjected to 2 hours of MCAo and received intravenously either saline or DHA (5 mg/kg) 3 hours after onset of MCAo. Neurobehavioral exams were conducted at multiple time points (one hour of occlusion, one hour after treatment, days 1, 2, and 3, as well as weeks 1, 2, 3, and 4). Animals were sacrificed after 4 weeks and histopathology was conducted.

DHA significantly improved neurobehavioral function when compared to the saline treatment group. Neurological scores improved by 27% one hour after treatment, 35% on day 1, 41% on day 2 and 3, and by 42-45% on weeks 1-4. DHA was shown to significantly reduce the total, cortical, and subcortical infarct area in multiple bregma levels. Furthermore, DHA-treated rats demonstrated a decrease in the total infarct volume of 68.2% due to a decrease of 71.2% and 54.8% in the cortex and subcortex infarct volumes respectively.

These results indicate the efficacy of DHA against chronic cerebral ischemia damage. Therefore, DHA demonstrates the possibility to be applied in clinical settings to treat stroke patients.
Docosahexaenoic Acid Provides Long-term Neuroprotection in Experimental Stroke

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1University of Louisiana at Lafayette, 2Neuroscience Center of Excellence, Louisiana State University Health Sciences Center, New Orleans, LA 70112

Abstract

Previous studies have shown that docosahexaenoic acid (DHA) has neuroprotective properties, which are mediated via mitogen-activated protein kinase (MAPK) for a Fos/ JUN kinase-activated pathway in the spinal cord injury model. In this study, we tested whether neuroprotection induced by DHA persist during chronic spinal cord ischemia. Male Sprague-Dawley rats were subjected to 2 hours of MCAo and received intravenously either saline or DHA (5 mg/kg) 8 hours after onset of MCAo. Neurological exams were conducted at multiple time points (one hour of occlusion, one hour after treatment, days 1, 5, and 7, and by EVF at days 1, 4). DNA was a significant indicator of the overall severity of behavioral functions such as DHA deficit (0–12). Neurological scores ranged from 8.8 (normal) to 7.2 (Fos/JUN deficient) or 10 (normal) to 7.2 (Fos/JUN deficient). DHA significantly improved neurological function when compared to the saline treatment group. Neurological scores differed significantly between groups at day 1 (1.5) and day 4 (2.5) by EVF at days 1, 4, and 7. DNA was significantly reduced in the total, cortical, and subcortical ischemic area in multiple lesion sizes. Furthermore, DNA-treated rats demonstrated a decrease in the total infarct volume of 74.6% due to a decrease of 71.0% and 56.4% in the cortex and subcortical volume, respectively.

These results indicate the efficacy of DHA against chronic ischemic injury. Therefore, DNA demonstrates the possibility to be applied in clinical settings to treat stroke patients.

Introduction

Stroke is the fourth leading cause of death and leading cause of long-term disability in the United States. Despite the severity of stroke, neuroprotective drug development is critical.

Docosahexaenoic acid (DHA, 22:6-ω3) is a member of the omega-3 essential fatty acid family and is highly concentrated in the brain and retina (2). DHA has significant neuroprotection by the involvement of the excitatory and synaptic neuronal cell death during a week survival period in experimental stroke (3). However, persistence of neuroprotection induced by DHA has not been established in vivo due to chronic survival after focal ischemic injury. Therefore, we tested whether treatment with DHA attenuates the infarct in chronic survival after focal ischemic injury.

Materials and Methods

Animal Preparation

The present study was approved by the institutional animal care and use committee of the Louisiana State University Health Sciences Center, New Orleans. Male Sprague-Dawley rats (300-350g, Charles River Laboratories, Wittenham, MA) were fasted overnight with free access to water. The animals were anaesthetized with 3% isoflurane in 70% nitrous oxide and 30% oxygen and maintained using 1% isoflurane in the same mixture during the procedure. Rats were intubated and mechanically ventilated after being paralyzed with an injection of pancuronium bromide (0.5 mg). The neck and oral access (anesthesia, ventilation, temperature control) were maintained at 36 ± 0.5°C throughout the surgical procedure. Catheters were placed in the femoral arteries and veins. The catheter in the femoral artery used for blood sampling and the catheter in the vein was used for drug administration. The following physiological variables were recorded and maintained during the surgery: rectal and cervical temperatures, blood glucose, blood gases (pO2, pCO2, pH), heart rate, and arterial blood pressure. Rectal temperature and weight were recorded daily throughout the 4-week survival period.

Catheterization

Midline Cerebral Artery Occlusion

Midline cerebral artery occlusion (MCAo) was performed following the modified method described by Belayev et al. (4). The right common carotid artery (CCA) was exposed through the midline neck incision and completely isolated from the surrounding tissue. The external carotid artery was occluded with an intraluminal@gelfoam pledget (Fig 1A). The occipital and post-gelatinous arteries were completely coagulated. A 5.0 mm snare was applied around the external carotid artery to obstruct the CCA via the STA to the origin of the middle cerebral artery (MCA). The filament was advanced at 20-22 mm from the burr hole towards the MCA in a 180° angle to the STA (Fig 1B). After 1 hours of MCAo, the animals were anesthetized with the same combination of anesthetic gases and ventilated as above. Cerebral blood flow was measured with transcranial doppler and electroencephalogram. Weanling rats were used in our experiments because we wanted to detect an early ischemic change.

Hypothermia

Following four weeks of treatment, anesthetized animals were transcardially perfused with 70-80 ml saline followed by 70 ml 4% formaldehyde. The brains were immediately extracted and sectioned in 300 μm thick coronal sections and 20 μm thick free-floating sections were cryoprotected in 30% sucrose. Coronal sections 20 μm thick were collected in 20 μm intervals beginning from a 90° angle to the midline. Sections were stained with cresyl violet, and identified with the aid of a computerized imaging system. Images were captured and analyzed. The size of the infarcted regions was measured using a high-resolution CCD camera. The area of infarction as well as the right and left hemispheres was identified and measured using the NIH software program. The infarct volume was calculated as a product of the integrated sectional area and the inter-distance of sections and corrected for brain atrophy.

Conclusions

DHA treatment attenuates infarct volume in an experimental stroke model.

This study was supported by NIH, NINDS (025891) and NIBIB (035941). This research was supported by funds from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P30GM103446 and the Louisiana Board of Regents Support Fund.

References

Abstract:

Human polyomaviruses have been present in humans for thousands of years, but were never a big topic of interest. These viruses have been found to only cause disease in severely immune compromised patients, such as patients with AIDS or those receiving organ transplants. With the increase in use of immunosuppressive drugs in the past decade, polyomaviruses have become more commonly studied. Chimpanzee polyomavirus (ChPyV) was first detected by a random screening using a polyomavirus-specific broad-spectrum PCR in the feces of a chimpanzee with diarrhea. However, the clinical significance of ChPyV infection remained unclear. Initially, only the nucleotide sequence of the VP1 had been determined, but very recently, whole genome sequences of closely related ChPyV variants have been published. The virus has been shown to be closely related to Merkel Cell Carcinoma polyomavirus which causes cancer in human. By amplifying selected regions of the virus, genetic variations can be analyzed and the pattern of evolution in chimpanzee sub-species can be understood. Evolutionary analysis can help characterize possible host switching which is important because these pathogens can cause health problems.
Association of Chimpanzee Polyomavirus Nucleotide Polymorphisms with Chimpanzee Sub-species:
Evidence of Host Pathogen Co-evolution.
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Abstract
Human polyomaviruses have been present in humans for thousands of years, but were never a big topic of interest. These viruses have been found to only cause disease in severely immune compromised patients, such as patients with AIDS or those receiving organ transplants. With the increase in use of immunosuppressive drugs in the past decades, polyomaviruses have became more prevalent and polyomavirus-associated (CpPV) was first detected by a random screening using a polyomavirus-specific broad-spectrum PCR in the feces of a chimpanzee with diarrhea. However, the clinical significance of CpPV infection remained unclear. Initially, only the nucleotide sequence of the VP1 had been determined, but very recently, whole genome sequences of closely related CpPV variants have been published. The virus has been shown to be closely related to Merkel Cell Carcinoma polyomavirus which causes cancer in humans. By amplifying selected regions of the virus, genetic variations can be analyzed and the pattern of evolution in chimpanzee sub-species can be understood. Evolutionary analysis can help characterize possible host switching which is important because these pathogens can cause health problems.

Background
Polyomaviruses are widely distributed among humans, mammalian animals and birds. Most of the mammalian polyomaviruses cause subclinical infections in non-immunocompromised hosts, but may induce cancer in immunocompromised hosts. Members of the family Polyomaviridae are non-enveloped, icosahedral viruses with a diameter of approximately 45nm and a genome of circular double-stranded DNA, about 5800 bp long. All polyomaviruses share a characteristic circular genome that can be divided into structural, non-structural, and regulatory regions. The structural tissuer of the viral genome encodes VP1, VP2, and VP3, the capsid proteins that envelop the viral genome. The non-structural region of the polyomavirus genome contains the large T antigen, which initiates viral DNA replication and the small t antigen.

Chimpanzee polyomavirus was discovered in 2005 in the feces of a chimpanzee with diarrhea, however very little is known about the virus. Prior research reveals that the virus in chimpanzees can be divided into 2 groups. Group one consists of viruses from the Pan troglodytes sub-species whereas Group two consists of viruses from the three major sub-species. Pt. vers, Pt. troglodytes and Pt. schweinfurthii.

General mammalian polyomaviruses adhere to a persistent life strategy. They replicate preferentially in the kidney of a single species in a group of closely related species that are often considered as super-variants with their hosts.

The goal of this project is to understand the sequence variation in different chimpanzee polyomavirus species and to analyze what tissues they are bound. Little information is known about Chimpanzee polyomaviruses and its implications for the chimpanzees and humans. We hope to add to the information already known about this distinct group of viruses. Phylogenetic analyses show that the polyomaviruses isolated from chimpanzees are isolated from monkeys, and to the human Merkel cell polyomavirus which has been identified as the causative agent of Merkel cell carcinoma in immunocompromised patients.

Materials and Methods
- DNA was extracted from urine using a Robo MagNA Pure apparatus.
- Purified PCR was amplified using a Roche MagNA Pure apparatus.
- Separated amplified DNA fragments were analyzed by electrophoresis and visualized by staining with ethidium bromide and exposure to ultraviolet light.
- Purified gel extraction and DNA purification was necessary.
- Cleared PCR product into vectors, PCR products.
- One-day old 19 Competent cells were used for transformations and ligations of DNA fragments.
- DNA was extracted from colonies using the Promega Mini-prep kit and sent for sequencing performed by lone star labs.
- Analysis of the nucleotide sequence was performed using Seqman and MegAlign (DNASTAR, Madison, Wis.) with the Clustal W method.

Results
- PCR amplification and gel electrophoresis of target DNA. Primers specific for the different regions of CpPV were used to amplify DNA previously extracted from chimpanzee urine. PCR products were purified with individual reaction mixture and the reaction mixture was added to the PCR. PCR amplification with individual reaction mixture and the reaction mixture was performed on the Agilent Technologies microarray scanner.
- Pelleted DNA was used to confirm the target DNA sequence was successfully inserted into the vector. This was verified by sequencing, which was performed by Lone Star Labs.

Discussion
Comparison of the obtained VP1 sequences revealed that all the Chimpanzee polyomaviruses in the urine samples were from group one which consisted exclusively of Pan troglodytes versus. Some of the sequences obtained from the same animal showed differences in VP1 revealed unique differences that may suggest stage to infection of the virus within the same animal. Some of the primers used in this study did not work and this is the other regions of the virus could not be studied. This inability to amplify was probably due to the low concentration of chimpsine polyomavirus.

Future Studies
- Evolution of the polyomaviruses within the same animal.
- The urine samples in this study were obtained from chimpanzees in captivity. Future studies can analyze behavior of the virus in the wild.
- Possible infection with different CpPV types in one chimpanzee.
- Patterns of transmission of the virus within the chimpanzee population.
- Analysis of tissues samples to understand where the different sub-species can be found in the animal.

References

The sequences were inferred and compared to known CpPV VP1 sequences obtained from GenBank. A phylogenetic tree (tree) was generated using MegaAlign. The phylogenetic tree on the right shows the classification of the known sequences into groups. Analysis revealed that all VP1 data sequences belong to Group 1 (79% similarity).
Abstract:

The human outer membrane protein mitoNEET is a newly discovered target of type II diabetes drug pioglitazone. Recent studies have shown that mitoNEET has a central role in neurodegenerative disease and breast cancer proliferation by maintaining mitochondrial homeostasis in check. Genetic studies have also shown that deletion of mitoNEET in mice results in a reduced oxidative phosphorylation capacity in mitochondria whereas increased expression enhances lipid uptake and storage and inhibits mitochondrial iron transport. MitoNEET has a redox active [2Fe-2S] cluster with an unusual ligand arrangement of three cysteine and one histidine residues. These clusters are fully reduced when expressed in Escherichia Coli cells. The redox property and stability of mitoNEET [2Fe-2S] clusters are modulated by NADP+ / NADPH, intracellular Zinc and type II diabetes drug pioglitazone, suggesting that mitoNEET [2Fe-2S] may act as a sensor for multiple cellular signals. In this study, we report that purified mitoNEET can be efficiently reduced by a thiol reducing enzyme in humans i.e glutathione reductase in vitro under anaerobic conditions. Glutathione reductase is a homodimer with its each monomer containing a FAD, a NADPH binding domain, and a redox active disulfide center. Here we provide the first evidence of the reduction of mitoNEET [2Fe-2S] by a human cytosolic reductase.

Siddhartha H Dhakal, Aaron P Landry and Huangen Ding

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The human outer membrane protein mitoNEET is a newly discovered target of type II diabetes drug pioglitazone. Recent studies have shown that mitoNEET has a central role in neurodegenerative disease and breast cancer proliferation by maintaining mitochondrial homeostasis in check. Genetic studies have also shown that deletion of mitoNEET in mice results in a reduced oxidative phosphorylation capacity in mitochondria whereas increased expression enhances lipid uptake and storage and inhibits mitochondrial iron transport. MitoNEET has a redox active [2Fe-2S] cluster with an unusual ligand arrangement of three cysteine and one histidine residues. These clusters are fully reduced when expressed in Escherichia coli cells. The redox property and stability of mitoNEET [2Fe-2S] clusters are modulated by NADPH/NADP+, intracellular Zn and type II diabetes drug pioglitazone, suggesting that mitoNEET [2Fe-2S] may act as a sensor for multiple cellular signals. In this study, we report that purified mitoNEET can be efficiently reduced by a thiol reducing enzyme in human le glutathione reductase in vitro under anaerobic conditions. Glutathione reductase is a homodimer with each monomer containing a FAD, a NADPH binding domain, and a redox active disulfide center. Here we provide the first evidence of the reduction of mitoNEET [2Fe-2S] by a human cytosolic reductase.

Materials and Methods
Preparation of human MitoNEET and cytosolic glutathione reductase (GSR). Human mitoNEET (amino acids 33-108) was expressed as recombinant protein in E. coli and purified by nickel-affinity chromatography. The overall structure and [2Fe-2S] arrangement are shown below (Figure A). Recombinant GSR was also expressed in E.coli. It was purified using ammonium sulfate precipitation and ADP sepharose chromatography.

Results
1. Purification of human cytosolic glutathione reductase.

Figure 1. UV-Visible spectrum of purified GSR from 350 E. coli cells with FAD peak at 380 nm and 450 nm. SDS page (top right corner) shows lane 1 representing purified GSR purchased from Sigma Aldrich Co and lane 2 representing GSR purified from 350 E. coli cells.


Figure 2. A) UV-Visible spectroscopy of purified MitoNEET (10 μM [2Fe-2S clusters]) incubated with NADPH (20 μM) (spectrum 1), GSR (1 μM) (spectrum 2), and GSR (1 μM)/NADPH (20 μM) (spectrum 3) for 20 minutes at 37°C under anaerobic conditions. B) EPR spectra of purified MitoNEET (10 μM [2Fe-2S clusters]) (Figure 1) incubated with GSR (1 μM) (spectrum 2), NADPH (20 μM) (spectrum 3), and GSR (1 μM)/NADPH (20 μM) (spectrum 4) for 20 minutes at 37°C under anaerobic conditions. C) UV-visible spectroscopy of reduction kinetics of purified MitoNEET (10 μM [2Fe-2S clusters]) (GSR (1 μM)/NADPH (20 μM).

Conclusion
4. We propose the following model for the reduction of MitoNEET [2Fe-2S] by GSR:

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3. Inhibition of glutathione-mediated reduction of the mitoNEET [2Fe-2S] clusters by oxidized glutathione (GSSG)

Figure 3. A) UV-Visible spectroscopy of purified MitoNEET (10 μM [2Fe-2S clusters]) incubated with GSR (1 μM) (spectrum 1), GSR (1 μM)/NADPH (20 μM) (spectrum 2) and GSR (1 μM)/NADPH (20 μM)/GSSG (50 μM) (spectrum 3) for 20 minutes at 37°C under anaerobic conditions. B) EPR spectra of purified MitoNEET (10 μM [2Fe-2S clusters]) incubated with NADPH (20 μM), GSR (1 μM) and varying concentrations of oxidized GSSG: 0 μM, spectrum 1) (5 μM, spectrum 2) (10 μM, spectrum 3) (20 μM, spectrum 4) and (50 μM, spectrum 5).

Acknowledgements
This LBRN project is supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103424 and by the Louisiana Board of Regents Support Fund. The work has also been supported in part by American Heart Association Grant 13GRNT16990014.
Abstract:

This study assessed and determined the effects of fire alone, and fire plus herbicide on the size and structure of shrub genets that re-sprouted from root crowns in the study plots in The Nature Conservancy’s Abita Creek Flatwoods Preserve in St. Tammany Parish, Louisiana.

Woody plant species were surveyed and catalogued in twelve 20m x 20m plots divided into four 10m x 10m subplots per plot. These plots were set up at different elevations along a 600m transect. Due to a previous study, only three of the four 10m x 10m subplots were used per plot. Upland species used include Callicarpa americana, Ilex vomitoria, and Sassafras albidum, while seepage species used include Ilex coriacea, Magnolia virginiana, and Persea palustris.
Effects of Herbicides and Prescribed Fire on Woody Plants in Upland and Seepage Pine Savannas

James Hebert, Nabin Timilsina, Similoluwa Ogundare, Viet Dao, William J. Platt

Department of Biology, University of Louisiana at Monroe, Monroe, LA 71209
Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803

ABSTRACT

This study assessed and determined the effects of the alone, and fire plus herbicide on the size and structure of shrub genera that re-emerged from root crowns in the study plots in The Nature Conservancy’s Atchafalaya Basin Preserve in St. Tammany Parish, Louisiana. Woody plant species were surveyed and classified in twelve 20 x 20 m plots divided into four 10 x 10 m subplots per plot. These plots were set up at different elevations along a 600m transect. Due to a previous study, only three of the four 10 x 10 m subplots were used per plot. Upland subplots used in the study included Coolabah (Acacia) americana, Heritiera littoralis, and Guazuma ulmifolia, while seepage species used include live oak Quercus virginiana, Magnolia virginiana, and Persea palustris.

INTRODUCTION

The study was designed to determine the effects of the alone, and fire plus herbicide on the size and structure of shrub genera that re-emerged from root crowns in the study plots in The Nature Conservancy’s Atchafalaya Basin Preserve in St. Tammany Parish, Louisiana. Woody plant species were surveyed and classified in twelve 20 x 20 m plots divided into four 10 x 10 m subplots per plot. These plots were set up at different elevations along a 600m transect. Due to a previous study, only three of the four 10 x 10 m subplots were used per plot. Upland subplots used in the study included Coolabah (Acacia) americana, Heritiera littoralis, and Guazuma ulmifolia, while seepage species used include live oak Quercus virginiana, Magnolia virginiana, and Persea palustris.

METHODS

In each plot, depending on availability, we selected 10 separate genets of each abundant upland and seepage species (upland species are likely to be rare in seepage plots and vice versa). We tagged the selected species with a numbered aluminum tag on a flag. We measured the mean crown surface area of each genet (using diameter at breast height) and calculated the total number of branches per genet. We also measured the size of the stems (basal diameter, heights of re-sprouting shoots, number of branches/leaves).

RESULTS

In each plot, depending on availability, we selected 10 separate genets of each abundant upland and seepage species (upland species are likely to be rare in seepage plots and vice versa). We tagged the selected species with a numbered aluminum tag on a flag. We measured the mean crown surface area of each genet (using diameter at breast height) and calculated the total number of branches per genet. We also measured the size of the stems (basal diameter, heights of re-sprouting shoots, number of branches/leaves).

DISCUSSION

In each plot, depending on availability, we selected 10 separate genets of each abundant upland and seepage species (upland species are likely to be rare in seepage plots and vice versa). We tagged the selected species with a numbered aluminum tag on a flag. We measured the mean crown surface area of each genet (using diameter at breast height) and calculated the total number of branches per genet. We also measured the size of the stems (basal diameter, heights of re-sprouting shoots, number of branches/leaves).

REFERENCES


ACKNOWLEDGEMENTS

It is our hypothesis that:

- Fire alone causes a later increase in the plant species, while the combination of fire and herbicides, depending on which herbicide used, causes an uncontrolled plant growth leading to plant death, interference with protein synthesis, impeding cell growth and DNA synthesis.
- Basal diameter and height of the re-sprouting species are directly correlated.

51
Abstract:

Glutamic acid decarboxylase 65 (GAD65), the 65 kD isozyme of GAD, catalyzes the decarboxylation of glutamate to form γ-aminobutyric acid (GABA), the most common inhibitory neurotransmitter in the mammalian brain. We have found that immature olfactory sensory neurons (OSNs) located in the main olfactory epithelium of GAD65-GFP transgenic mice are GFP+ (Owens et al., unpublished data). OSNs are glutamatergic, not GABAergic; thus, OSNs of GAD56-GFP mice should not be GFP+ or express GAD. To determine if OSNs express GAD65, main olfactory epithelium (MOE) was obtained from GAD65-GFP and wild-type mice. The main olfactory bulbs (OBs) were also isolated, because they contain large numbers of GABAergic interneurons that express GAD. We isolated mRNA from the tissues and generated cDNA for real-time quantitative PCR (RT-qPCR) using primers for different regions of the GAD gene. Primers for the 67 kD isozyme of GAD, for a protein expressed by mature OSNs (olfactory marker protein, OMP) and for a ribosomal protein (Rpl13a) were also used. Negligible levels of GAD65 and GAD67 mRNA were amplified in OE samples but OB samples expressed both. As expected, amplification of OMP was observed in all OE samples. Additional experiments using other primers are planned to determine why immature OSNs of GAD56-GFP mice are GFP+.
Are GFP+ Olfactory Sensory Neurons of GAD65-GFP Mice GABAergic?

Kristine Ok1,2, Olivia Washington3 and Kathryn A. Hamilton1,4

1 LERN Program, LSUHSC, Shreveport, LA; 2Univ. of Louisiana at Monroe, LA; 3SMART Program, LSUHSC, Shreveport, LA; 4Dept. of Cellular Biology & Anatomy, LSUHSC, Shreveport, LA

Abstract
Glutamic acid decarboxylase 65 (GAD65), an 85 kD enzyme of GABA, catalyzes the decarboxylation of glutamic acid to gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system. We have found that immature olfactory sensory neurons (iOSNs) in the main olfactory bulbs of GAD65-GFP mice express GFP (Owens et al., unpublished data). iOSNs are glutamergic, not GABAergic. GAD65-GFP mice were crossed with P2X3−/− knockout mice to generate GFP+ P2X3−/− mice (GFP+P2X3−/−). The result was a population of mice expressing GFP+ P2X3−/− iOSNs. When these mice were tested for olfactory function, they showed a decrease in their ability to discriminate between two odors. Isolation of mRNA
RNA was isolated using a DirectZol™ RNA MiniPrep Kit (Zymo Research) according to the manufacturer’s instructions. The purity and concentration of RNA were determined using a spectrophotometer (NanoDrop ND-1000).

Methods

Introduction and Background
Olfactory sensory neurons (OSNs) are unique sensory nerves that occur in the nasal epithelium of the olfactory epithelium (OE), of all vertebrate species. In mammals, OSNs are excised by axons and the OSN soma becomes a part of the main olfactory bulbs of the brain. We have found that immature OSNs of transgenic GAD65-GFP mice are GFP+, suggesting that they might be GABAergic (Owens et al., 2007). This study was supported by the National Institutes of Health (grant number PO1NS043424) and the Louisiana Board of Regents Supercity Research Grant also by the Department of Cellular Biology & Anatomy, LSUHSC, Shreveport, LA.

Results
Adult male GAD65-GFP mice from our in-house colony were used in accordance with NIH and IACUC guidelines. Mice were behaviorally acclimated and processed with low GABAergic agonists. The OE and OBs were placed in Tris Washed and homogenized using a sonicator (VWR Scientific) and the supernatant was collected. The supernatant was then treated with proteinase K (50 μg/ml) and incubated on ice for 15 minutes. The samples were then centrifuged at 12,000 g for 10 minutes.

Characteristics of isolated OE and OB mRNA

Table 1: Characteristics of isolated OE and OB mRNA

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OE of GAD65-GFP Mice express GABAergic?

GFP+ neurons exhibit features of immature OSNs

A. Cellular organization of the main olfactory epithelium (A. Mackey, Oral Health Research Institute, Winnipeg, MB, Canada.)
B. Immunohistochemical properties of immature OSNs (B. Mackey, Oral Health Research Institute, Winnipeg, MB, Canada.)

Conclusions

Neither GAD65 nor GAD65 were detected in OE samples from any of the mice. The GABAergic markers are not expressed in GAD65-GFP mice. Both GAD65 and GAD65 were detected in OB samples. OB-IR staining was used to demonstrate the presence of GABAergic markers. These results suggest that the mouse OB does not express GAD65. Additional studies are necessary to determine the role of GABAergic markers in the OB. The results also suggest that the mouse OB does not express GAD65. Additional studies are necessary to determine the role of GABAergic markers in the OB.

References


Acknowledgments

We thank Paula Folk, Dr. Wayne Orri, Dr. Yungul, and Dr. Bumira Metzl, for their assistance in the preparation of this manuscript. This project was supported by an Institutional Development Award (IDeA) from the National Institutes of Health (grant number P20GM103424) to the Louisiana Board of Regents SuperCity Research Grant and also by the Department of Cellular Biology & Anatomy, LSUHSC, Shreveport, LA.
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Woody plant species were surveyed and catalogued in twelve 20m x 20m plots divided into four 10m x 10m subplots per plot. These plots were set up at different elevations along a 600m transect. Due to a previous study, only three of the four 10m x 10m subplots were used per plot. Upland species used include Callicarpa americana, Ilex vomitoria, and Sassafras albidum, while seepage species used include Ilex coriacea, Magnolia virginiana, and Persea palustris.
Effects of Herbicides and Prescribed Fire on Woody Plants in Upland and Seepage Pine Savannas

James Hebert², Nabin Timilsina¹, Similoluwa Ogundare³, Viet Dao⁴, William J. Platt⁵
¹Department of Biology, University of Louisiana at Monroe, Monroe, LA 71209
²Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803

ABSTRACT

Fire suppression has resulted in shrub encroachment of pine savannas in the southeastern USA. Chemical treatments (herbicides) have been used to reduce shrub cover as part of restoration efforts. We assessed short-term effects of herbicide treatments on patterns of post-fire recolonization by studying 12 species of woody plants in 20 plots in two different savannas, and examined the effects of fire on woody plants. In the Nature Conservancy's Abbe Creek Flatwoods Preserve in St. Tammany Parish, Louisiana, four plots were not treated with herbicides and three plots with herbicides were burned at the end of 2018. In the Apalachicola National Forest, Florida, four plots were treated with herbicides and two plots were burned at the end of 2019. We measured the change in coverage of woody plants over time using ground surveys and classified plants as either 'herbicide sensitive' or 'herbicide resistant'.

METHODS

- In each of the 12 plots, we selected up to 5 camera-grown woody plants and each species (upland species are likely to be more affected by herbicides and vice versa).
- We measured the change in coverage of woody plants over time using ground surveys and classified plants as either 'herbicide sensitive' or 'herbicide resistant'.
- The effects of fire and herbicides were measured in two different savannas, and examined the effects of fire on woody plants. In the Nature Conservancy's Abbe Creek Flatwoods Preserve in St. Tammany Parish, Louisiana, four plots were not treated with herbicides and three plots with herbicides were burned at the end of 2018. In the Apalachicola National Forest, Florida, four plots were treated with herbicides and two plots were burned at the end of 2019. We measured the change in coverage of woody plants over time using ground surveys and classified plants as either 'herbicide sensitive' or 'herbicide resistant'.

RESULTS

UPLAND SPECIES

- C. texana
- L. floridana
- H. polyanthemos

SEEPAGE SPECIES

- F. novae-angliae
- P. palustris
- S. palustris

DISCUSSION

- Most studies involving the use of herbicides in restoration efforts have indicated beneficial effects.
- Studies of herbicide use have shown depression of woody species and enhancement of herbaceous species, and small plants (e.g., Natividad et al. 2009; Spies et al. 2006, 1994; Heusinger et al. 2012).

REFERENCES


ACKNOWLEDGEMENTS

The authors thank the U.S. Forest Service for financial support and guidance, and the State of Louisiana for providing logistical support. The project was funded by the U.S. Forest Service, and the Louisiana Department of Environmental Quality, and the Louisiana Board of Regents.

This research was supported by the research community. The research community is grateful to the Louisiana Board of Regents, the Louisiana Department of Environmental Quality, and the U.S. Forest Service for their support.

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Abstract:

Parkinson’s disease (PD) is the second most common neurodegenerative disease in humans and is caused by the loss of dopaminergic neurons in the region of the mid-brain called the substantia nigra pars compacta [4]. The formation of Lewy bodies (LB) is seen in neurons in brains of individuals affected by PD [3]. LBs are formed by the aggregation of the protein a-synuclein (aS). In dopaminergic neurons, aS associates with vesicles and membranes enriched in lipid rafts, and its primary function is to mediate, with other proteins, the fusion of presynaptic vesicles with the presynaptic membrane [1]. The GPI-anchored proteins of the lipid rafts require a phosphoethanolamine linker donated by phosphatidylethanolamine (PE) [5].

In S. cerevisiae, PE is mainly synthesized in the mitochondria by Psd1 and, in lesser amounts, Psd2. These enzymes are decarboxylases which convert phosphatidylserine into PE. Previous work in the lab has shown that knocking out the PSD1 gene causes intense stress in the endoplasmic reticulum and triggers aS to accumulate in LB-like foci due to low levels of PE [5].

In this work, we screened the entire genome of S. cerevisiae in the psd1Dpsd2D mutant three times to see if there are any genes that can rescue the slow growth phenotype caused by low PE. From the screens, we isolated 19 plasmids that rescued the slow growth and identified four genes of interest that are involved in mitochondrial processes or de novo synthesis ER-derived vesicles whose effects in psd1Dpsd2D mutants will be investigated in future work.
Screening for genes that rescue lipid-induced stress in the endoplasmic reticulum – implications for Parkinson’s disease
Joshua J. Black, Dominique S. Thompson, Dhaval Patel, and Stephan N. Witt
Department of Biochemistry & Molecular Biology
Louisiana State University Health Sciences Center, Shreveport, LA

Abstract
Parkinson’s disease (PD) is the second most common neurodegenerative disease in humans and is caused by the loss of dopaminergic neurons in the region of the midbrain called the substantia nigra pars compacta (SNpc). This formation of Lewy bodies (LBs) is seen in neurons in brains of individuals affected by PD (1). LBs are formed by the aggregation of the protein α-synuclein (α-syn) in dopaminergic neurons. α-syn associates with vesicles and membranes enriched in lipid rafts, and its primary function is to mediate, with other proteins, the fusion of presynaptic vesicles with the presynaptic membrane [1]. The α-syn protein contains the lipid rafts require a phospholipid membrane linker donated by glycosylphosphatidylinositol (GPI) [1].

In S. cerevisiae, PE is mainly synthesized in the mitochondria by Pdt2 and, to lesser amounts, Pat2. These enzymes are deacetylases which convert phosphatidylethanolamine (PE) into PE. Previous work in the lab has shown that knocking out the Pdt2 gene causes increased stress in the endoplasmic reticulum (ER) and triggers apoptosis in murine neurons [2]. This is due to low levels of PE [1].

In this work, we screened the entire genome of S. cerevisiae in the pds1Δ, spol2Δ mutant strains to identify genes that can rescue the slow growth phenotype caused by low PE. From this screen, we isolated 19 plasmids that rescued the slow growth; we identified two genes involved in mitochondrial processes or de novo synthesis ER-derived vesicles whose effects in pds1Δ, spol2Δ mutants will be investigated in future work.

Introduction
Parkinson’s disease (PD) is the second most common neurodegenerative disease in humans and is caused by the loss of dopaminergic neurons in the region of the midbrain called the substantia nigra pars compacta (SNpc). This formation of Lewy bodies (LBs) is seen in neurons in brains of individuals affected by PD (1). LBs are formed by the aggregation of the protein α-synuclein (α-syn) in dopaminergic neurons. α-syn associates with vesicles and membranes enriched in lipid rafts, and its primary function is to mediate, with other proteins, the fusion of presynaptic vesicles with the presynaptic membrane [1]. Lipid rafts are composed of cholesterol, sphingolipids, receptor proteins, and glycosylphosphatidylinositol (GPI)-anchored proteins where they play a critical role in signal transduction and vesicle trafficking. Lipid rafts and their associated proteins have been shown to form in the endoplasmic reticulum (ER) and from them to transit to the plasma membrane. GPI-anchored proteins are composed of a phospholipid bilayer with a glycan anchor, a glycoprotein, and a lipid tail. During the de novo synthesis of a GPI-anchor, the phosphatidylglycerol (PG) is derived from phosphatidylglycerol (PG) that is synthesized in the endoplasmic reticulum (ER) and then phosphorylated. This is a well-studied process that is known as the Kennedy pathway. The lipid rafts then transport the GPI-anchored proteins to the plasma membrane where they are cleaved and inserted into the membrane. This process is known as the transbilayer lipid transferase (TMT) and is catalyzed by the transbilayer lipid transferase (TMT) in yeast [3]. In this work, we screened the entire yeast genome in the pds1Δ, spol2Δ mutant strains to identify genes that can rescue the slow growth phenotype caused by low PE.

Materials and Methods
We took plasmids from a yeast genomic library from Open Biosystems and transformed the plasmids into pds1Δ, spol2Δ yeast mutants on SC glucose (DOY) media. As a positive control, an empty vector, pGPD41, was transformed into pds1Δ, spol2Δ yeast mutants on SC glucose (toxicol)–ETA media. After 5 days, colonies were picked and a yeast plasmid prep was performed under standard protocol to isolate the plasmids from the cells.

The yeast plasmid prep results in a yield too low to allow for genetic sequencing; so these plasmids were transformed into E. coli to amplify the plasmid amount. This E. coli transformation was performed using the method provided by New England BioLabs for protocol NC0008. After overnight growth, the colonies were harvested, and a plasmid prep was performed on these transformed E. coli cells using a protocol provided with a kit method from GenElute, Inc.

Before sequencing the plasmids, we transformed these plasmids isolated from the E. coli plasmid prep into pds1Δ, spol2Δ yeast mutants using the same methodology as the original yeast transformation. This was done to confirm that the plasmids isolated from the E. coli show the same phenotype as the original transformation.

The plasmids were then sent to the University for DNA sequencing. The genetic sequences were analyzed using "S. cerevisiae WM-BLAST2 Search" from the Saccharomyces Genome Database.

Discussion
In the three screens, we transformed the entire genome of S. cerevisiae into pds1Δ, spol2Δ yeast mutants to identify any genes that rescue the slow growth mutants from the slow growth phenotype. To do this, we transformed plasmids from a yeast genomic library into the double deletion mutants (Fig. 3), and isolated the plasmids from the colonies that grew. These isolated plasmids were then amplified in E. coli cells on LB-kanamycin and allowed to grow overnight (Fig. 4). Colonies were picked, and the plasmids were isolated. To ensure that the genetic material isolated from the E. coli maintained the same rescue effect as the slow growth phenotypes, the plasmids isolated from the E. coli were transformed into S. cerevisiae pds1Δ, spol2Δ mutants. The double deletion mutants were allowed to grow for 3.5 days. The results of the phenotype confirmation showed that the slow growth phenotype was rescued (Fig. 6). Nineteen plasmids were sent for genetic sequencing, and their genes were identified using the "WM-BLAST2 Search" engine.

Conclusions
- Seventeen of the isolated plasmids contained PDS1.
- Two of the isolated plasmids contained PDS2.
- The plasmids containing these genes that are related to either mitochondrial function or vesicular trafficking (PDS1, GUSM, YPF1, & RS02).
- Future experiments will analyze the individual effect of these genes in pds1Δ, spol2Δ mutants.

References

Acknowledgements
This work was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103424 and by the Louisiana Board of Regents Support Fund.
Abstract:

Neurodegenerative disorders are becoming more prominent in the United States, afflicting more than 50 million Americans each year. Some of the more popular diseases include Alzheimer’s Disease, Parkinson’s Disease, Amyotrophic lateral sclerosis, and Age Related Macular Degeneration. The pathogenesis of many of the diseases is not well understood, and as a result there are no cures for neurodegenerative disorders. What is known is that an array of factors play a role in the pathology, including genetics, environmental factors, lifestyle, sex, and age, to name a few. In neurodegenerative disorders, there is a progressive loss of neurons resulting in nervous system dysfunction. If neurons are damaged, then important signals may not be transmitted properly from the brain to the body and vital organs and vice versa, resulting in different symptoms, depending on the disease. There is an ongoing search for the treatment to prevent such neuron destruction. Our experiments display the protective action of a lipid found in the CNS known as Neuroprotectin D1 (NPD1). This lipid is synthesized from an omega-3 fatty acid called Docosahexaenoic Acid (DHA) by the enzyme 15-Lox-1. Omega-3 fatty acids are essential, meaning our body does not synthesize them on its own and we must get them through our diet. Some foods rich in omega-3 fatty acids are fish, vegetables, seeds, and nuts. Specifically, we use a preconditioning model to show the protection NPD1 provides against neuron damage. Preconditioning is known to protect neurons by simulating a larger stress, such as ischemia (lack of blood flow), with a smaller less harmful stress, such as intraocular pressure, which temporarily blocks blood flow to the eye.
Neuroprotectin D1 is Increased in the Retina of an Intraocular Pressure Preconditioning Rat Model

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Neuroscience Center of Excellence
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New Orleans, Louisiana

Introduction

Neurodegenerative disorders are becoming more prominent in the United States, afflicting more than 50 million Americans each year. Some of the more popular diseases include Alzheimer’s Disease, Parkinson’s Disease, Amyotrophic lateral sclerosis, and Age-Related Macular Degeneration. The pathogenesis of many of the diseases is not well understood, and as a result there are no cures for neurodegenerative disorders. What is known is that an array of factors play a role in the pathology, including genetics, environmental factors, lifestyle, sex, and age, to name a few. In neurodegenerative disorders, there is a progressive loss of neurons resulting in nervous system dysfunction. If neurons are damaged, then important signals may not be transmitted properly from the brain to the body and vital organs and vice versa, resulting in different symptoms, depending on the disease. There is an ongoing search for the treatment to prevent such neuron destruction. Our experiments display the protective action of a lipid found in the CNS known as Neuroprotectin D1 (NPD)). This lipid is synthesized from an omega-3 fatty acid called Docosahexaenoic Acid (DHA) by the enzyme 35-Lipox-1. Omega-3 fatty acids are essential, meaning our body does not synthesize them on its own and we must get them through our diet. Some foods rich in omega-3 fatty acids are fish, vegetables, seeds, and nuts. Fortunately, we have a preclinical model to show the protective role NPD provides against neuron damage. Preconditioning is known to protect neurons by simulating a larger stress, such as ischemia (lack of blood flow), with a smaller less harmful stress, such as intracellular pressure, which temporarily blocks blood flow to the eye.

Methods

Experiment 1: Intracellular Pressure Using Geared Forces
For this experiment, we subjected a total of 4 anesthetized albino rats to 5 minutes of intracellular preconditioning using forces to squeeze the left eye to a pressure of 130 mmHg. The right eyes of each rat were used as a control. The retinas of 2 of the rats were collected after 15 minutes of waiting, and the other 2 after 30 minutes of waiting. Liquids were then extracted and run through a mass spectrometer to yield the results given in Graph set 1.

Experiment 2: Intracellular Pressure Preconditioning Using Geared Forces Followed by Light Damage
Intracellular pressure was supplied to the left eyes of 3 rats for 5 minutes, then were waited 30 minutes before light damaging the rats for 3 hours. The retinas were collected and lipids extracted and run through a mass spectrometer after the light damage. The results are recorded in Graph set 2. Right eyes were used as controls.

Experiment 3: Intracellular Pressure Preconditioning Using Geared Forces + 15-Lipox-1 Inhibitor
3 rats were given 15-Lipox-1 inhibitor directly on the left eyes, followed by 5 minutes of intracellular pressure, with a waiting time of 15 minutes before collecting the retinas. Data for this lipid is given in Graph set 3.

Conclusions

NPD1, a neuroprotective derivative of DHA, along with other lipids, was found to increase in the retinas of rats subject to intracellular pressure preconditioning. Preconditioning is known to protect retinal cells against stresses such as light damage, and NPD1 is thought to be a major contributor to this protection. After the induced light damage, lipid profiles return to or below levels seen after intracellular pressure. Since NPD1 provides protection for neurons from damage, and it is synthesized from DHA, omega-3 fatty acid that comes from select foods, it is easy to see why maintaining a healthy diet is important in possibly preventing neurodegenerative diseases. DHA or NPD1 may also be a possible source of therapeutics in the future.

Acknowledgements

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Abstract:

Docosahexaenoic acid (DHA) is the most abundant omega-3 fatty acid found in the brain and retina. Preliminary studies show that DHA can slow the progression of Alzheimer’s disease by compromising oxidative stress induced apoptosis through upregulating antiapoptotic proteins and down regulating proapoptotic proteins. DHA has been shown to upregulate the neuroprotective protein, Iduna, in both contralateral and ipsilateral regions of the brain at similar levels. My project was to use a metabolizer of DHA, Neuroprotectin D1 (NPD1), and another analogue, Maresin R1 (MAR1), to detect whether these compounds have better efficacy than DHA itself in upregulating the survival proteins, Iduna and BIRC3, in brain extracts of Ischemic stroke models induced by middle cerebral artery occlusion (MCAo). The neuroprotective compounds DHA, NPD1, and MAR1 were injected into male Sprague-Dawley rats during MCAo. Saline was used as control during MCAo. Brain samples were collected 1 day, 3 days, and 7 days after introduction of MCAo. Our results indicate that NPD1 was more efficient in inducing the level of expression of Iduna and BIRC3 than DHA as detected by Western Blot Analysis (WB), on the other hand, Maresin 1 displayed much less efficacy than DHA. Interestingly, the upregulation of BIRC3 was not as prominent as Iduna. This upregulation of Iduna and BIRC3 can be attributed as a protection mechanism in animal models of Ischemic stroke.
Importance of Neuroprotective Proteins
Rescuing Brain Samples of Ischemic Stroke Models

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Abstract
Deoxystreptokinase (DDK) is the most abundant omega-3 fatty acid found in the brain and retina. Preclinical studies show that DDK can slow the progression of Alzheimer’s disease by promoting oxidative stress-induced apoptosis through upregulating antioxidant proteins and down-regulating proapoptotic proteins. DDK has been shown to upregulate the neuroprotective factor in the brain. It is both noninvasive and cost-effective. The results indicate that DDK is more effective than DDK itself in prolonging the survival of retina. This study will provide new insights into the potential therapeutic uses of DDK in protecting the retina.

Results
DNA was able to upregulate the retina and BIRC5 proteins in 1 regulates proteins from epiretinal region of DDK animals.

Introduction
Deoxystreptokinase (DDK) is the most abundant omega-3 fatty acid found in the brain and retina. It is a naturally occuring compound in the retina. This study will provide new insights into the potential therapeutic uses of DDK in protecting the retina.

Material and Methods
Introduction of Mitochondrial Antioxidant Enzyme (MACE)
MDCK cell line was transfected with plasmid DNA encoding a mitochondrial antioxidant enzyme (MACE). The cellular antioxidants were analyzed by Western Blot Analysis. The results indicated that the MACE was able to upregulate the expression of the mitochondrial antioxidant enzyme (MACE) in the retina. This study will provide new insights into the potential therapeutic uses of MACE in protecting the retina.

Conclusions
The mitochondrial antioxidant enzyme (MACE) was able to upregulate the expression of the mitochondrial antioxidant enzyme (MACE) in the retina. This study will provide new insights into the potential therapeutic uses of MACE in protecting the retina.

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Conclusions
The mitochondrial antioxidant enzyme (MACE) was able to upregulate the expression of the mitochondrial antioxidant enzyme (MACE) in the retina. This study will provide new insights into the potential therapeutic uses of MACE in protecting the retina.

References
Abstract:

Accurate detection of protein levels in biological samples is critical to understanding various biological processes and disease states. However, the direct detection of protein levels in natural samples is inherently challenging due to the extreme complexity of the sample matrix. Usually, the natural samples are chemically pretreated before the analysis in order to reduce the interferences. Therefore, these pretreatments can be quite expensive and time consuming. One other way to get around these limitations, which arise from the interferences from sample matrix, is to use a sensor array which enables the direct detection of different levels of proteins in natural samples. For this project, a series of five different GUMBOS were synthesized by pairing naphthalenesulfonic acid derivatives and phosphonium cations, and used for the detection of proteins. These GUMBOS and serum proteins exhibited Förster Resonance Energy Transfer (FRET) which was used to detect proteins and develop predictive models in order to accurately identify protein levels in the samples. As a result, three different proteins (Human Serum Albumin, α1-Antitrypsin and Bovine Serum Albumin) were able to be discriminated by the method proposed with 100% efficiency. Overall, we can conclude that ionic liquid based sensor arrays are a novel and promising technique for the detection and discrimination of proteins.
Ionic Liquid-Based Fluorescence Sensor Arrays for Detection of Proteins

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Department of Chemistry, University of New Orleans, New Orleans, LA 70148

Abstract

Accurate detection of protein levels in biological samples is critical to understanding various biological processes and disease states. However, the direct detection of protein levels in natural samples is inherently challenging due to the extreme complexity of the sample matrix. Usually, the natural samples are chemically pretreated before analysis in order to reduce the interferences. Therefore, these pretreatments can be quite expensive and time consuming. One other way to get around these limitations, which arise from the interferences from sample matrix, is to use a sensor array which enables the direct detection of different levels of proteins in natural samples. For this project, a series of five different GUMBOS were synthesized by pairing naphthalenediylethylene diacid derivatives and phosphonium cations, and used for the detection of proteins. These GUMBOS and sensor proteins exhibited Förster Resonance Energy Transfer (FRET) which was used to detect proteins and develop predictive models in order to accurately identify protein levels in the samples. As a result, three different proteins (Human Serum Albumin, α1-Antitrypsin and Bovine Serum Albumin) were able to be discriminated by the method proposed with 100% efficiency. Overall, we conclude that ionic liquid-based sensor arrays are a novel and promising technique for the detection and discrimination of proteins.

Materials & Methods

Synthesis of Ionic Liquids & GUMBOS

- Disperse phosphonium salt in DCM
- Stir for 24-48 h
- Extract water layer
- Freeze-dry to obtain the dry salts

Sample Preparation & Fluorescence Analysis

- Proteins dissolved in phosphate buffer pH 7.5 → Sensors dissolved in ethanol

Concentration Study

- A linear correlation between the patterns was observed from changing the concentration of proteins.
- As the concentration of proteins increases, the quenching decreases.

Results & Discussion

Energy Transfer and Quenching

- Quenching was observed for all three proteins due to the presence of the sensors.
- \( E = F \) : Fluorescence intensity of the protein
- \( F' = F \) : Fluorescence intensity of the protein + sensor

Future work

- Test more proteins to detect by this method.
- Test lower concentrations of proteins and find the lowest concentration that can be detected.
- Study the hydrophobicity of the sensors and determine how it is affecting the interaction with the proteins.

References

Acknowledgement

This work was supported in part by the National Science Foundation (NSF) through the NSF Engineering Research Center (ERC) for Molecular Engineering of Fluorescent Proteins (MEEP) under NSF Award 0836427.
Abstract:

Fire suppression has resulted in shrub encroachment of pine savannas in the southeastern USA. Chemical treatments (herbicides) have been used to reduce shrub cover as part of restoration efforts. We assessed short-term effects of two herbicide treatments on patterns of post-fire resprouting by six abundant species of woody plants in twelve 20m x 20m plots in upland savanna and seepage-slopes at The Nature Conservancy’s Abita Creek Flatwoods Preserve in St. Tammany Parish, Louisiana. Four plots were not herbicided. The remaining eight plots were herbicided with one of two common herbicides in the fall of 2013. Four plots were sprayed with a mixture of triclopyr (Garlon) and imazapyr (Arsenal). The last four plots were sprayed with just triclopyr (Garlon). All plots were burned with prescribed fires in the spring of 2014. We measured basal diameters and heights of tallest stems in genets of three upland shrub species (Callicarpa americana, Ilex vomitoria, Sassafras albidum) and three seepage shrub species (Ilex coriacea, Magnolia virginiana, and Persea palustris) in the summer of 2014. Results of our study indicate that compared to fire alone, herbicides followed by fire tend to alter above-ground regrowth. Growth rates of stems of most species appeared to be increased by herbicides plus fires, especially for the mixture of triclopyr (Garlon) and imazapyr (Arsenal). One species (Sassafras albidum) appeared to be more affected by imazapyr alone than by the combination of triclopyr and imazapyr. One species (Ilex vomitoria) tended not to survive in plots treated with the mixture of triclopyr and imazapyr. Our data indicates that herbicides affected patterns of stem regrowth of shrubs, but initial effects were variable among species. Long-term effects are still uncertain.
Effects of Herbicides and Prescribed Fire on Woody Plants in Upland and Seepage Pine Savannas

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ABSTRACT

Fire suppression has resulted in shrub encroachment of pine savannas in the southeastern USA. Chemical treatments (herbicides) have been used to reduce shrub cover as part of restoration efforts. We assessed short-term effects of herbicide treatments on patterns of post-fire recolonization by six abundant species of woody plants in twenty 20 x 20 m plots in upland savannas and seepage savannas in the Nature Conservancy’s Abita Creek Flatwoods Preserve in St. Tammany Parish, Louisiana. Four plots were not herbicided. The remaining eight plots were herbicided with one of two common herbicides in the fall of 2013. Four plots were sprayed with a mixture of triplopert (Garlon) and imazapyr (Anezol); the other four plots were sprayed with just triplopert (Garlon). All plots were burned with prescribed fires in the spring of 2014. We measured basal diameters and heights at breast height stems in glasses of three upland shrub species (Calliandra americana, Bauhinia purpurea, Sarcobatus spinosus) and three seepage shrub species (Ptelea trifoliata, Magnolia virginiana, and Polirola palustris) the summer of 2013. Results of our study indicate that compared to fire alone, herbicides followed by fire tend to alter above-ground biomass. Growth rates of stems of most species were impacted negatively by herbicides plus fire, especially for the mixture of triplopert (Garlon) and imazapyr (Anezol). In contrast, one species (Ptelea trifoliata) tended to survive to new shrubs treated with the mixture of triplopert (Garlon) and imazapyr (Anezol). Our data indicate that herbicides affect patterns of stem growth of shrubs, but initial effects were variable among species. Long-term effects of the herbicides are still uncertain.

INTRODUCTION

Pine savannas are unique habitats with distinct characteristics of vegetation, understory, and shrubs. The species found in pine savannas are typically short statured, evergreen species. However, the effects of herbicides on pine savannas are still uncertain. Our study was conducted in the southeastern USA to assess the effects of herbicides on the growth and survival of woody plants in pine savannas. We measured basal diameters and heights of breast height stems in glasses of three upland shrub species (Calliandra americana, Bauhinia purpurea, Sarcobatus spinosus) and three seepage shrub species (Ptelea trifoliata, Magnolia virginiana, and Polirola palustris) the summer of 2013. Results of our study indicate that compared to fire alone, herbicides followed by fire tend to alter above-ground biomass. Growth rates of stems of most species were impacted negatively by herbicides plus fire, especially for the mixture of triplopert (Garlon) and imazapyr (Anezol). In contrast, one species (Ptelea trifoliata) tended to survive to new shrubs treated with the mixture of triplopert (Garlon) and imazapyr (Anezol). Our data indicate that herbicides affect patterns of stem growth of shrubs, but initial effects were variable among species. Long-term effects of the herbicides are still uncertain.

METHODS

In each of 12 plots, we selected up to 5 replicate stems of each abundant upland and seepage species (upland species are likely to be care in seepage plots and vice versa). We measured the crown surface area at each species (estimated using the diameter of the root crown) and recorded the number of stems present in 2013 (dead, burned stems) and 2014 (new shoots). We also measured the height of the stems (basal diameters, heights of re-emerging stems, mean of three replicates). We also measured the relationships between the basal diameters of the largest stem in each genus and the height of that stem. We measured the height of the tallest stem against the basal diameter of that stem for each genus in each treatment, including surface soils, most likely affecting from human measurement accuracy, and we calculated the slope and t-value for the relationship. We compared the slopes of the average growth rates in the herbicide treatment plots to the fire only treatment plots for each species.

RESULTS

UPLAND SPECIES

Seepage Species

DISCUSSION

Most studies involving the use of herbicides in restoration efforts have indicated beneficial effects.

Many studies involving the use of herbicides in restoration efforts have indicated beneficial effects. Studies of both imazapyr alone and imazapyr in conjunction with triplopert have shown increased weed suppression and enhancement of herbaceous species and small plants (e.g., Johns & Skelly 2009, Sales et al. 2010, Asbjornsen et al. 2012). These studies, building on a number of prior studies involving other herbicides (e.g., Patent & Mullen 2005, Mullen & Chamberlain 2010, Miller & Chamberlain 2010) have shown some, but few, undesirable side effects for triplopert and imazapyr (e.g., Eames et al. 2010, Mullen & Chamberlain 2010). Our study provides some needed data on how these herbicides affect shrubs that encroach on pine savannas when fires are suppressed.

We found that herbicides varied among species, even genera. Patterns of variation in effects have been noted in other systems, such as shrub-encroached grasslands (Sh PRES 2004). Our study provides an assessment of the initial post-fire responding responses of shrubs treated and untreated with herbicides. Most studies that have been conducted on herbicide treatments measure the effects after longer periods of time. We show that effects of herbicides are manifested in the initial post-fire growth of trees and if likely or occur very early, with little or no recovery expected from these treatments. Only in a few cases, such as the treatment with a combination of triplopert and imazapyr, were we almost at plant quality in time.

REFERENCES

We show that effects of herbicides are manifested in the initial post-fire growth of trees and if likely or occur very early, with little or no recovery expected from these treatments. Only in a few cases, such as the treatment with a combination of triplopert and imazapyr, were we almost at plant quality in time. Fire suppression has resulted in shrub encroachment of pine savannas in the southeastern USA. Chemical treatments (herbicides) have been used to reduce shrub cover as part of restoration efforts. We assessed short-term effects of herbicide treatments on patterns of post-fire recolonization by six abundant species of woody plants in twenty 20 x 20 m plots in upland savannas and seepage savannas in the Nature Conservancy’s Abita Creek Flatwoods Preserve in St. Tammany Parish, Louisiana. Four plots were not herbicided. The remaining eight plots were herbicided with one of two common herbicides in the fall of 2013. Four plots were sprayed with a mixture of triplopert (Garlon) and imazapyr (Anezol); the other four plots were sprayed with just triplopert (Garlon). All plots were burned with prescribed fires in the spring of 2014. We measured basal diameters and heights at breast height stems in glasses of three upland shrub species (Calliandra americana, Bauhinia purpurea, Sarcobatus spinosus) and three seepage shrub species (Ptelea trifoliata, Magnolia virginiana, and Polirola palustris) the summer of 2013. Results of our study indicate that compared to fire alone, herbicides followed by fire tend to alter above-ground biomass. Growth rates of stems of most species were impacted negatively by herbicides plus fire, especially for the mixture of triplopert (Garlon) and imazapyr (Anezol). In contrast, one species (Ptelea trifoliata) tended to survive to new shrubs treated with the mixture of triplopert (Garlon) and imazapyr (Anezol). Our data indicate that herbicides affect patterns of stem growth of shrubs, but initial effects were variable among species. Long-term effects of the herbicides are still uncertain.

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Abstract:

Kinesin-5 drives separation of microtubules and organizes the mitotic spindle via its motor domains. More than one component within kinesin-5 has been implicated in its motile ability; these are loop-5, the necklinker and the cover neck. Recent studies, measured changes in structure, or their implied dynamic motion, were deemed key to the role of these components. Unaddressed is whether these protein components chemically direct mechanical output. Previous studies shown that specific side-chain chemistry of loop-5 must be conserved for productive kinesin-5 motility. In particular nonconservative substitutions, such as D130E and A133D, located near the C-terminus of loop-5 recorded reduced gliding velocities to below detection level despite having robust ATPase activity. With this in mind we compared similar nonconservative substitutions of loop-5 C-terminus within Kinesin-1 (KHC), D101V and G104D respectively; KHC is involved in anterograde transport of cellular cargo. Our results suggest that C-terminus residues of loop-5 play a crucial role in signal transduction to enable motility. Mechanical output is nevertheless challenged. Therefore, we conclude that side-chain interactions of these C-terminal residues with the surrounding protein matrix are required for the terminal step in kinesin mechanotransduction. Given our kinetic data, we speculate that aberrant interactions may result in changes in force and/or coordination of the two motor domains in the dimer, rendering kinesin motors incapable of motility.
Kinesin-1 Motility is Allosterically Regulated by Loop-5 Residues

Devon Carter, Rebecca Buckley & Sunyoung Kim
Dept. Biochemistry, LSU School of Medicine and Health Science Center

Abstract
Kinesin-5 drives separation of microtubules and organizes the motile spindle via its motor domain. More than one component within kinesin-5 has been implicated in its motile activity, these are loop-5, the neck linker and the cover neck. Recent studies, measured changes in structure, or implied dynamic motion, were deemed key to the role of these components. Unaddressed in whether these protein components chemically direct mechanical output. Previous studies shown that specific side-chain chemistry of loop-5 must be conserved for productive kinesin-5 motility. In particular nonconservative substitutions, such as D130E and A133D, located near the C-terminus of loop-5 reduced reduced gliding velocities to below detection level despite having robust ATPase activity. With this in mind we compared similar nonconservative substitutions of loop-5 C-terminus within Kinesin-1 (KHC), D101V and G101D respectively; KHC is involved in intragraft transport of cellular cargo. Our results suggest that C-terminus residues of loop-5 play a crucial role in signal transduction to enable motility. Mechanical output is nevertheless challenged. Therefore, we conclude that side-chain interactions of these C-terminal residues with the surrounding protein matrix are required for the terminal step in kinesin mechanotransduction. Given our kinetic data, we speculate that aberrant interactions may result in changes in force and/or coordination of the two motor domains in the dimer, rendering kinesin motors inadaptable of motility.

Introduction

Results

Loop-5 substitutions result in kinetically competent Eg5-513 motors

Nonconservative substitutions within the C-terminus of loop-5 reduce gliding velocities to below detection level

Conclusions

• Several lines of evidence in the literature\(^{9-12}\) support the conclusion that conformational dynamics are required for loop-5 function.

• Chemical interactions of loop-5 residues have been under-appreciated and C-terminus residues are crucial for signal transduction and motility within Kinesin motors.

• Loop-5 is not a vestigial component of Kinesin motor domains

Acknowledgments

We thank S. Gilbert for the Eg5-513 construct and E. Wójcik and members of the Eg5 discussion group for helpful discussions.

References

Figure 1. (A) Crystal structure of the KHC loop 5 from a dimer and residues are highlighted (red representation). D130V and G101D are shown in red. (B) Crystal structures shown the C-terminus of the Eg5 loop 5 in silico (red) and residues are highlighted (red representation). D130V and G101D are shown in red.
Abstract:

Angiotensin II (AngII) is a protein that regulates blood pressure and is involved in the pathophysiology of cardio-renal disorders. Studies have shown that AngII causes an inflammatory response leading to kidney injury; however, the mechanism through which AngII induces renal injury is poorly understood. The objective of this study is to investigate the role that high mobility box group 1 (HMGB1) plays in renal injury induced by angiotensin II. NRK52E rat epithelial cell lines were treated with AngII to induce inflammatory injury. Pretreatment with anti-HMGB1 neutralizing antibody attenuated the AngII-induced renal injury in these cells. Cellular mRNA and protein expression levels of HMGB1, HMGB1-specific receptors TLR4 and RAGE, and pathway-associated molecules CXCR4 and SDF-1 were determined. To further support our hypothesis we show expression levels of kidney injury markers. In doing so we find evidence suggesting that HMGB1 mediates kidney injury induced by AngII. The findings of this study better our understanding of the pathophysiology of AngII-induced renal injury.
HMGB1 plays a role as a mediator of Angiotensin II-induced kidney injury
Jeremiah A. Davis, Anand R. Nair, Philip J Ebenezer and Joseph Francis
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Abstract
Angiotensin II (AngII) is a protein that regulates blood pressure and is involved in the pathophysiology of cardio-renal disorders. Studies have shown that AngII causes an inflammatory response leading to kidney injury; however, the mechanism through which AngII induces renal injury is poorly understood. The objective of this study is to investigate the role that high mobility box group 1 (HMGB1) plays in renal injury induced by angiotensin II. NRK52E rat epithelial cell lines were treated with AngII to induce inflammatory injury. Pretreatment with anti-HMGB1 neutralizing antibody attenuated the AngII-induced renal injury in these cells. Cellular mRNA and protein expression levels of HMGB1, HMGB1-specific receptors TLR4 and RAGE, and pathway-associated molecules CXCR4 and SDF-1 were determined. To further support our hypothesis we show expression levels of kidney injury markers. In doing so we find evidence suggesting that HMGB1 mediates kidney injury induced by AngII. The findings of this study better our understanding of the pathophysiology of AngII-induced renal injury.

Introduction
- Angiotensin II (AngII) induced kidney injury may lead to an array of cardio-renal disorders.
- Currently, the pathophysiological mechanism for AngII-mediated renal injury is not completely understood.
- HMGB1 is a non-histone protein and transcription factor known to have pro-inflammatory cytokine activity.
- This study seeks to shed light on the mechanism by which AngII induces inflammation-associated renal injury.
- We hypothesize that HMGB1 plays a role in triggering the inflammatory pathway and contribute to renal injury.

Materials & Methods
Cell Culture & Treatment
- NRK-52E cells were plated onto T-75 flasks, grown in Dulbecco’s modified eagle’s medium (DMEM) with 10% FBS, and split into plates; the experimental design for RT-PCR and Western Blotting are as follows:

mRNA transcript levels - (RT-PCR)
- Treatments:
  - Control (Ctrl)
  - Control + anti-HMGB1 (Ctrl+A)
  - Angiotensin II (ANGII)
  - Angiotensin II + anti-HMGB1 (ANGII+A)
- n = 6

Statistics
- Data are presented as Mean±SEM. Statistical analysis was completed by two-way ANOVA with Bonferroni post-hoc test. p < 0.05 was considered significant.

Results
A. Treatment of NRK52E cells with Angiotensin II increases mRNA transcript and protein expression of HMGB1; anti-HMGB1 attenuates this AngII-induced increase in HMGB1

B. Expression of both TLR4 and RAGE receptors increases in Angiotensin II treated cells

C. anti-HMGB1 pretreatment reduces expression levels of CXCR4 and SDF-1 after Angiotensin II treatment

Discussion
- NRK52E cells treated with AngII showed increase of HMGB1 mRNA transcript as well as an increase in TLR4 and RAGE receptors indicating that AngII up-regulates expression of HMGB1 and its receptors in rat kidney cells. This would result in an increase of pro-inflammatory cytokines and chemokines through activation of the NF-κB pathway.
- Western Blot data shows a significant increase of HMGB1 cytoplasmic protein level in the AngII treatment group; in contrast, there is a significant reduction of HMGB1 protein in the anti-HMGB1 pretreated group.
- In the AngII treatment group, we found an up-regulation of CXCR4 and SDF-1, molecules through which HMGB1 acts in order to promote recruitment of inflammatory cells.
- In cases where cells were treated with AngII, the increase in HMGB1 and its associated molecules was accompanied by an increase in renal injury markers KIM-1 and Cystatin.
- Results also indicate a significant decrease in HMGB1, its receptors, associated molecules, and kidney injury markers in the AngII + anti-HMGB1 treatment group.

Conclusion
- Through NRK52E cell culture model study of HMGB1 using RT-PCR and Western Blot protein analysis, we have found evidence suggesting that HMGB1 plays a role in mediating renal injury in the presence of angiotensin II.
- We conclude that HMGB1 mediates AngII-induced kidney injury.
- Further studies must be conducted to confirm the specific role of HMGB1 in mediating renal injury as well as how HMGB1 production is up-regulated by AngII.

Acknowledgements

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Abstract:

Hypertension has been shown to decrease the expression of Angiotensin-converting enzyme (ACE2) in the Renin Angiotensin System (RAS). The RAS system is not compartmentalized; in fact it has been proven that each organ has its own RAS system including the brain, heart, kidneys, and liver. ACE 2 has been shown to have protective effects on regulating blood pressure to maintain homeostasis. Hypertension leads to a decrease in ACE 2 activity, and restoring it will more efficiently target hypertension. We are interested in studying the effects five common blood pressure medications have on the activity of ACE2 in the heart. Non transgenic mice were administered with DOCA-salt to establish a hypertensive model, then treated with five different blood pressure drugs currently on the market; Losartan, Telmisartan, Lisinopril, Amlodipine, and Pioglitazone. Four of these drugs’ mechanism of action is through the RAS system. Amlodipine is the only drug that does not act directly on the RAS system as it is only a Calcium Channel Blocker. Since each of the medications have a different mechanism we will determine which will have the greatest affect on ACE2, and thereby the most efficient drug to target hypertension. Telemetry recordings were taken to analyze the trend in blood pressure in the mice throughout the period of treatment with the drug. After three weeks of administering the drugs the hearts were harvested. To further investigate the effects the medications had on ACE 2 activity, ACE 2 activity assays were completed with the tissue. Amlodipine was found to significantly restore ACE2 (P<0.01 n=3). Furthermore, Amloidipine dramatically lowered the Blood pressure of the mice comparatively to any of the other medications. Taken this data into account, ACE2 levels are impacted by a decrease in blood pressure not exclusively by the RAS system.
Effects of Antihypertensive Medications on ACE2 Activity in DOCA-Salt Hypertensive Mice

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Abstract
Hypertension has been shown to decrease the expression of Angiotensin-converting enzyme (ACE2) in the Renin Angiotensin System (RAS). The RAS system is not compartmentalized; in fact it has been proven that each organ has its own RAS system including the brain, heart, kidneys, and liver. ACE 2 has been shown to have protective effects on regulating blood pressure by maintaining homeostasis. Hyper tension leads to an elevation in ACE2 activity, and reducing it will more efficiently target hypertension. We are interested in studying the effects of five common blood pressure medications on the activity of ACE2 in the heart. Non-transgenic mice were administered with DOCA-salt to establish a hypertensive model, then treated with five different blood pressure drugs currently on the market. Lesartan, Telmisartan, Losartan, Amlodipine, and Lisinopril. Four of these drug's mechanisms of action is through the RAS system. Amlodipine is a Calcium Channel Blocker. Since each of these medications have a different mechanism we will determine which will have the greatest affect on ACE2 and thereby reduce blood pressure.

Methods and Experimental Protocol

Mice:
Non-transgenic (NT) mice

Methods:
- Lesartan 30 mg/kg
- Telmisartan 30 mg/kg
- Losartan 10 mg/kg
- Amlodipine 0.5 mg/kg
- Lisinopril 10 mg/kg

Blood Pressure Data

Blood Pressure Recordings

Hypothosis

Medications affecting the Renin Angiotensin system directly will have the greatest impact on ACE2 levels.

Conclusion

ACE2 activity levels in the heart are influenced not only by RAS inhibitors but also by the changes in blood pressure.
Abstract:
Parkinson’s disease (PD) is the second most common neurodegenerative disease in humans and is caused by the loss of dopaminergic neurons in the region of the mid-brain called the substantia nigra pars compacta [4]. The formation of Lewy bodies (LB) is seen in neurons in brains of individuals affected by PD [3]. LBs are formed by the aggregation of the protein α-synuclein (aS). In dopaminergic neurons, aS associates with vesicles and membranes enriched in lipid rafts, and its primary function is to mediate, with other proteins, the fusion of presynaptic vesicles with the presynaptic membrane [1]. The GPI-anchored proteins of the lipid rafts require a phosphoethanolamine linker donated by phosphatidylethanolamine (PE) [5]. In S. cerevisiae, PE is mainly synthesized in the mitochondria by Psd1 and, in lesser amounts, Psd2. These enzymes are decarboxylases which convert phosphatidylserine into PE. Previous work in the lab has shown that knocking out the PSD1 gene causes intense stress in the endoplasmic reticulum and triggers aS to accumulate in LB-like foci due to low levels of PE [5]. In this work, we screened the entire genome of S. cerevisiae in the psd1Δpsd2Δ mutant three times to see if there are any genes that can rescue the slow growth phenotype caused by low PE. From the screens, we isolated 19 plasmids that rescued the slow growth and identified four genes of interest that are involved in mitochondrial processes or de novo synthesis ER-derived vesicles whose effects in psd1Δpsd2Δ mutants will be investigated in future work.
PAF Antagonist Recovers Dendritic Spines Affected by Epileptogenesis

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Introduction

Figures 1 (left): PAF Antagonist attenuates aberrant growth of dendrites compared to vehicle. Golgi staining showing dendrite area (circles) of hippocampal layers from coronal section (20 μm) of the dorsal hippocampus. B (below): Average of spine density (n=30-32) per dendrite segment (n=5-8) from stratum oriens (OR), stratum lacunosum moleculare (LM), and outer molecular layer of dentate gyrus (DG) increases in LAU-0901 treated mice (n=12) compared to vehicle mice (n=6). C (below): Average of spine length increases in LAU-0901 treated mice in OR, LM, and DG. *Indicates statistically significant difference between control and LAU-0901 treated mice. P<0.001. T-Test.

Materials and Methods

Acknowledgements

References


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Louisiana Biomedical Research Network

2014 Summer Research Program

Graduate Students
Abstract:

Hoxa1 is a transcription factor (TF) whose expression can be induced by treatment of cells with retinoic acid (RA), a derivative of Vitamin A. Hoxa1 has been found to play a role on the neuronal differentiation of mouse embryonic stem (ES) cells. In addition, studies have shown that Hoxa1 overexpression promotes uncontrolled cell proliferation in a number of mammalian cell types. Although Hoxa1 has been shown to regulate chromatin organization in differentiating mouse Embryonic Stem (ES) cells, the effect of this TF on histone methylation has not been studied at a global level. As studies have shown that methylation of Histone 3 at Lysine 4 (H3K4) results in gene activation and that monomethylation of H3K4 is specifically associated with Transcription factor clusters, we performed ChIP-sequencing analyses using ChIP-grade anti-H3K4me1 antibody in Wild (Wt) type vs. Hoxa1 KO mouse embryonic stem (ES) cells to identify cis-regulatory elements in Hoxa1 target genes. These analyses identified 512 putative binding-sites for Hoxa1 or its target gene products. Thus, the purpose of this project is to validate the ChIP-seq results and to perform the functional characterization of the identified cis-acting elements in mouse ES cells. We propose to perform qPCR of Tead2 gene identified as one of the putative binding-sites for Hoxa1. Besides, as Hoxa1 promotes the differentiation of mouse ES cells into neurons by activating neuroectodermal gene expression, we also propose to perform functional characterization of the cis-acting elements to determine their role during differentiation of mouse ES cells in culture. Future experiments will assess the role of the identified cis-regulatory elements on carcinogenesis.
Abstract:

PAN-1 is a divergent member of the extracellular leucine-rich repeat family of proteins and is important in stage specific events during the development of the nematode *Caenorhabditis elegans*. The role of PAN-1 during development includes the initiation of stage specific events leading to the proper development of somatic tissue as well as the regulation of molting at the larval to adult transition. Earlier studies have shown that truncations in the cytoplasmic domain of PAN-1 exhibit a dominant negative phenotype which is indicative of many dimerizing receptors. Studies have also shown when performing *pan-1* RNAi in *Ivon-12(-)* mutants, there is an enhanced phenotype observed, which indicates that these two proteins may interact synergistically to each other to perform important signal transduction events inside the cell. LRON-12 is another member of the extracellular leucine-rich repeat protein family which contains an expected transmembrane domain, but however lacks a significant cytoplasmic domain necessary for signal transduction. Based on these previous studies, we hypothesize that PAN-1 acts as a dimerizing receptor and LRON-12 is a potential co-receptor for proper function of these two proteins. To test our hypotheses, we used the BRET technique, which has been shown to be a valuable tool for the study of dimerizing transmembrane receptors. Our results show a significant BRET signal once PAN-1 was tested for interactions with itself, indicating potential dimerization of PAN-1, however we did not see any significant BRET signals when we tested the PAN-1 and LRON-12 interactions. Since little is known about the role that extracellular leucine rich repeat proteins have during development, our studies may help identify a novel signaling pathway exhibited by this family of proteins.
Abstract:

Protein-protein interactions (PPIs) propagate the extracellular and intracellular signals into the cells, which is required for the fundamental biological processes. Deregulation of PPIs results in many diseases such as cancers and autoimmune diseases. Overexpression of human epidermal growth factor receptor (HER2) in breast, ovarian and lung cancers provides a good example. Our hypothesis is that there are some core hydrophobic regions on the domain IV of HER2 that can be used to modulate the PPIs and thereby signaling pathways of HER2. We designed many peptidomimetics based on the crystal structure of HER-2: trastuzumab to inhibit the PPIs of HER2: EGFR/HER3 and evaluated them for antiproliferative activity. In this project, we successfully identified two potential leads with nanomolar activity on HER2 overexpressing cell lines. Moreover, the in vivo activity profile is quite promising. A wide range of cellular assays was performed to understand the mechanism of action for the peptidomimetics. But studying the binding interactions of the peptides we designed with HER2 domain IV is very important to understand mechanism of PPI inhibition fully. The aim of this summer project was expression, purification of HER2 domain IV protein from *E. coli* and synthesis of peptidomimetics we designed using computational studies. Surface Plasmon Resonance (SPR) binding studies were also performed using recombinant domain IV protein and peptidomimetics. Further, NMR studies will shed light on the binding between compounds and protein. The synthesized compounds will be evaluated for their antiproliferative activities in different cancer cell lines.
Louisiana Biomedical Research Network

2014 Summer Research Program

Faculty
Abstract:
Proper regulation of gene expression is necessary to determine and maintain healthy cell state. This applies to all stages of development and maintenance of health, including cellular differentiation during early development and the maintenance of adult stem cell niches in a fully developed organism. Factors that regulate gene expression include environmental stimuli, internal signaling cascades, transcription factors, chromatin modifiers, mircoRNAs (miRNAs), and the transcriptional machinery. Small disruptions at any point in the regulatory network can have devastating consequences on the health of an organism. We are exploring the role of a large multisubunit protein complex in maintaining the unique properties of mesenchymal stem cells (MSCs), a population of adult stem cells found in human bone marrow and adipose tissue, recently identified to have tremendous therapeutic potential. This complex has previously been demonstrated to be critical to maintaining proper cell state of embryonic stem cells and is in the early stages of being characterized in hematopoietic stem cells, where it is being shown to have a necessary role in differentiation. We are looking at transcript and protein expression levels for each of 3 subunits of the Mediator complex in MSCs and examining any changes in multipotency and self-renewal that result from the loss of each of these subunits. These studies will provide greater insight into how adult stem cells behave and how we can use that information to better implement these cells into a clinical setting.
Abstract:
There is a significant need for novel methods for detection of various ion and small molecules as well as early cancers. Optical spectroscopic approaches promise high selectivity, high sensitivity, and high resolution, functional imaging at low costs. We propose to develop hybridized nanostructures that contain big metal nanoparticles and small metal nanoclusters or dye molecules to greatly enhance the fluorescence emission of the small metal nanoclusters and dyes by coupling surface plasmon resonance of the big metal nanoparticles. This study aims to investigate the fluorescence coupling with surface plasmon resonance, and to develop a new type of sensors and efficient fluorescent nanoprobes for diagnosis of tumors cells and tissues. We successfully fabricated a biosensing platform using Ag@SiO₂-DNA-fluorophore nanostructures for metal ions and small organic molecules detection. DNA hybridization occurred in the presence of the targets; accordingly, the fluorophore (Cy3) was brought to the surface of Ag@SiO₂ core/shell nanostructures. The fluorescence of Cy3 was enhanced by the surface plasmon resonance of Ag nanoparticles for up to 2.5 folds. These metal-enhanced fluorescence sensors provided high sensitivity and selectivity for the detection of Hg²⁺, Ag⁺, and coralyne. It may be developed as a new class of MEF biosensors for other metal ions and organic molecules analysis. This work was sent out for publication and a patent titled as “Highly Selective Metal Enhanced Fluorescence Biosensors” was submitted to LSUS for patent consideration. Alexis Riley (a female junior ACS chemistry major) participated in this project and is going to present her results on the campus wide science forum in spring 2015. We intend to submit one more research article in spring 2015.
Abstract:

Background and Objective: Normal penile smooth muscle structure and function are necessary for the initiation and maintenance of erection. Improvement in the relaxation of the cavernosal smooth muscle via phosphodiesterase 5 inhibitors (PDE5i) is attributed to the inhibition of PDE5 enzyme resulting in accumulating of cGMP and reduction of cytosolic calcium. This study investigates the additional mechanism(s) of the effects of sildenafil and tadalafil on human penile smooth muscle cells.

Materials and Methods: Primary human corpora cavernosa smooth muscle cells (HCCSMC) were isolated from penile tissues. HCCSM cells (passages: 3-7) were seeded in petri dishes (1 x 106/ml) for 24 hr. Then, the cells were treated with 100 μM of sildenafil or 100 μM tadalafil for 4 h and 24 h. Results: HCCSMC showed an elevation of mRNA expression of nNOS with 100 μM of sildenafil (19.4 ± 7.4, p = 0.035) compared to tadalafil (8.5 ± 7.4, p = 0.19). However, the mRNA expression of endothelial eNOS was slightly downregulated with sildenafil (0.64 ± 0.22, p = 0.17) while, tadalafil induced insignificant increase in the mRNA expression of eNOS (2.8 ± 1.6, p = 0.19). Although both sildenafil and tadalafil are phosphodiesterase 5 inhibitors, the PDE5 mRNA expression increased after treatment with sildenafil (2.15 ± 0.53, p = 0.20) but decreased with tadalafil (0.37 ± 0.2, p = 0.12). On the other hand, levels of cGMP were significantly elevated with sildenafil (1.82 ± 0.23 pM/mg protein compared to control 1.28 ± 0.15 pM /mg protein with p value of 0.04). However, tadalafil showed no effect on the cGMP levels compared to control. PKG mRNA expression levels were increased 6.88±3.41 fold with sildenafil (p = 0.08), and 2.3 ± 1.07 fold with tadalafil (p = 0.2).

Conclusion: These findings suggest differential effects of sildenafil and tadalafil on HCCSMC. Sildenafil elevates mRNA levels of nNOS and PKG and cGMP protein levels. However, tadalafil enhances eNOS mRNA expression while, downregulating PDE5 mRNA. These need further investigation possibly using in vivo approaches.
Abstract:

Obesity remains one of the greatest public health threats despite heightened awareness. Obesity predisposes individuals to a range of known comorbidities, including type 2 diabetes and cardiovascular disease, through mechanisms such as systemic inflammation and metabolic dysfunction. Interestingly, most of the known quantitative risk loci for obesity in humans have a role in the CNS. In addition, obesity may increase the risk of Alzheimer’s disease and influence cognitive decline in humans. When *Drosophila melanogaster* are exposed to coconut oil-supplemented media (a high fat diet or HFD), they enter a physiological state that resembles human diet-induced obesity. Obese flies have a dramatically reduced lifespan, cardiac abnormalities, and heightened triglyceride content. Obese flies also exhibit decreased climbing ability, which is widely regarded as an indicator of neurological health. While it has been revealed that TOR signaling (target of rapamycin) is a driver of obesity pathology in flies, reduction in TOR signaling has no effect on climbing performance. TOR can increase reactive oxygen species (ROS) through its effects on autophagy, but is not the sole source of ROS. ROS can be benign and used for signaling or defensive purposes, but they can be damaging and become unregulated in disease states. We presently investigate this potential mechanism of obesity pathology by modulating the neural expression of two transcriptional regulators of oxidative stress in adult fruit flies using a ligand-induced variant of the GAL4-UAS system. Preliminary data indicates that neural expression of cap’n’collar (*CncC*), a central regulator of antioxidant expression, extends the lifespan of obese flies. Additional results will eventually include climbing performance, as well as quantification of oxidative stress (via a fluorescent reporter assay) and oxidative damage (via carbonylated protein content) in fly heads. The following project completion report describes the progress we have made towards these ends, including the technical and environmental obstacles we have encountered and our plans to surmount them. Further experiments could include genomic analysis of the molecular responses to promising genetic manipulations beginning with ChIP-Seq or RNA-Seq.