

Report

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PROGRAM**

ABSTRACTS

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2015 PRSI ABSTRACTS

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- 11. Correlation of Cobalt Effects on Gene Expression with Intracellular Metal Ion Accumulation in an In Vitro Sertoli cell Model**
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Development of Methods for the Extraction and Analysis of Vinblastine, Losartan, and Citalopram from Tissue Culture Cells

Jessica Beamon¹ and David H. Eagerton²

Affiliations of all authors:

¹Hamilne Univeristy. 1536 Hewitt Ave, St. Paul, MN 55104

²Department of Pharmaceutical and Administrative Science, Presbyterian College School of Pharmacy. 307 N. Broad St, Clinton, SC 29325

Author Emails: Jessica Beamon, jbeamon01@hamline.edu. David H. Eagerton, deagerton@presby.edu

Contact Information:

Jessica Beamon, Hamline University. 1536 Hewitt Ave, St. Paul, MN 55104

David H. Eagerton, Ph.D., F-ABFT, Associate Professor, Department of Pharmaceutical and Administrative Science, Presbyterian College School of Pharmacy. 307 N. Broad St, Clinton, SC 29325

Abstract

A limitation to anticancer and antibiotic therapy is the development of resistance to the anticancer and antibiotic medications. One mechanism of resistance is the efflux pump. Efflux pumps are located in the cytoplasmic membrane in all types of cells. The efflux pumps are used for moving parent drugs and metabolites of medications in and out the cell. They also contribute to drug resistance diminishing the desired therapeutic or biological effect. An over expression of ATP binding cassette (ABC) transporters, which can increase efflux of drugs from cancer cells, thereby decreasing intracellular drug concentration. We hypothesize that over expression of these efflux pumps can be determined by measuring the intracellular levels of drugs after treatment. To do this we will develop the methods. We looked at the drugs Vinblastine, Losartan, and Citalopram but, primarily on Losartan and Citalopram. To develop this method we first used freeze thaw extraction using human cells of about 0.03mg of Citalopram and Losartan. From this small amount of cells we were able to see the drug on the mass spectrometer. This means that we have the correct parameter to detect our drugs. From this result we continued to test Citalopram and Losartan by diluting the sample and make a calibration curve. Our results showed that we did successfully developed a method with the correct parameters.

The Effects of Efavirenz on abcg2 (bcrp) Expression In Human Carcinoma Cells

Nikki Dominick, PharmD Candidate; Eddie Grace, PharmD

Affiliations of all authors:

Department of Pharmacy Practice, Presbyterian College School of Pharmacy. 307 N. Broad St, Clinton, SC 29325

Authors emails: Nikki Dominick, kndominic@presby.edu. Eddie Grace, EEGrace@presby.edu

Contact information

Nikki Dominick, Pharm.D. Candidate, Presbyterian College School of Pharmacy. 307 N. Broad Street, Clinton SC 29325

Eddie Grace, Pharm.D., Associate Professor of Pharmacy Practice, Department of Pharmacy Practice. Presbyterian College School of Pharmacy. 307 N. Broad Street, Office 305. Clinton SC 29325

Abstract

Human Immunodeficiency Virus (HIV) infects approximately 2 million people per year, worldwide. Highly Active Antiretroviral Therapy (HAART) has significantly prolonged the life span of HIV-infected individuals. HAART is a combination of two nucleoside reverse transcriptase inhibitors (NRTI), in addition to a non-nucleoside reverse transcriptase inhibitor (NNRTI), a boosted protease inhibitor, or an integrase strand transfer inhibitor (INSTI). Drug resistance occurs via the cytochrome P450 enzymes and drug efflux transporters. ATP-binding cassette (ABC) transporters are efflux transporters found in various cells throughout the body. Efavirenz, a NNRTI, is both a substrate and inducer to many ABC transporters. We hypothesize that efavirenz will induce the upregulation of ABCG2 efflux transporter expression in MCF-7 cells. The aim of the present study is to induce the ABCG2 gene resulting in an increased ABCG2 expression using therapeutic concentrations of efavirenz. We will demonstrate ABCG2 overexpression through PCR and mass spectrometry. It is expected that efavirenz will induce the ABCG2 gene at the conclusion of this study.

Keywords: Drug Resistance, HIV, antiretroviral, efflux transporter

Functional blockade of CD163 receptor overexpressed in macrophages impairs release of cytokines and the wound healing process

David Wilson Ferreira^{1,2}, Candler Paige¹, Cristina Ulecia Moron^{1,3}, E.A. Romero-Sandoval¹

Affiliations of all authors:

¹Department of Pharmaceutical and Administrative Sciences, Presbyterian College School of Pharmacy, Clinton, SC .

²University of São Paulo – Ribeirão Preto Medical School, Ribeirão Preto, Brazil.

³University of Seville - Experimental Sciences Faculty, Seville, Spain.

Author's emails: David Wilson Ferreira, dwferrei@presby.edu, Candler Paige, capaige@presby.edu, Cristina Ulecia Moron, culeciamo@presby.edu, E. Alfonso Romero-Sandoval, asandoval@presby.edu

Contact information: David Wilson Ferreira, PhD Student. Department of Pharmacology. Ribeirão Preto Medical School - University of Sao Paulo. 3900 Bandeirantes Ave, Ribeirão Preto, SP – Brazil, 14049-900.

Abstract

Among the factors that influence postoperative pain, peripheral neuroimmune interactions play an important role in the development of pain and wound healing process after surgery. Macrophages exclusively express two molecules, namely CD163 and mannose receptors (MRs). Therefore, gene therapy using nanoparticles grafted with a mannose receptor ligand (mPEI) could be utilized to target monocytes/macrophages for the prevention or treatment of chronic pain since macrophages are involved in both the induction and the resolution of inflammation and tissue repair. Based on previous results in our laboratory showing that CD163-overexpressing macrophages stimulated with LPS adopted an M2-cellular phenotype, represented by a reduced release of the pro-inflammatory cytokine TNF- α , and an increase in the anti-inflammatory cytokine IL-10, as well as using an *in vitro* scratch assay model of keratinocytes + fibroblasts, CD163-overexpressing macrophages promoted a more efficient wound healing process, we aim to investigate whether these effects were induced exclusively by over-expression of CD163, and not the binding of the nanoparticle to the MRs, or even by other unspecific effects of the plasmid vector or the nanoparticle. We observed that neutralizing CD163 antibody affects the release of IL-10 (100.0 \pm 8.523% in pCD163 + isotype antibody vs. 62.34 \pm 5.163% in pCD163 + blocking antibody), but not TNF- α (100.0 \pm 5.652% in pCD163 + isotype antibody vs. 114.9 \pm 6.302% in pCD163 + blocking antibody), and impairs the efficient wound healing process induced by CD163-overexpressing macrophages. Therefore, using this pharmacological approach we could imply that M2 macrophages release anti-inflammatory molecules (through autocrine or paracrine mechanism), which in turn facilitate cell interaction among keratinocytes and fibroblasts, contributing to resolution of inflammation and a more efficient wound healing process. Finally, our data argue for the test of these hypotheses using *in vivo* animal models and future implementation of this approach in major surgeries to reduce the incidence of chronic postoperative pain.

Keywords: Macrophages, CD163 receptors, nanoparticles, wound healing, cytokines, chronic postoperative pain.

Characterization of Lymphocytes in the Cervical Mucosa

Becca Foster and Amy Messersmith

Affiliations of all authors:

Presbyterian College School of Pharmacy. 307 N. Broad St, Clinton, SC 29325

Authors emails: Becca Foster, rrfoster@presby.edu Dr. Amy Messersmith, amessersm@presby.edu

Contact information:

Amy Messersmith, Ph.D., Assistant Professor of Pharmaceutical Science & Safety Officer, Presbyterian College School of Pharmacy. 307 N. Broad St, Clinton, SC 29325
Becca Foster, PharmD. Candidate, Presbyterian College School of Pharmacy. 307 N. Broad St, Clinton, SC 29325

Abstract

Persistent HPV infection is the primary risk factor for cervical cancer progression. In order to fully understand the natural progression from transient HPV infection to clearance or persistence, it is necessary to understand the immune cells involved. T cells that secrete IL-17, named Th17 cells, have been recently identified in cervical cancer. Th17 cells produce pro-inflammatory cytokines and they have been found to play an important role in inflammation and autoimmune disease. There are also increased levels of regulatory T cells in cervical cancer, CIN 2, and CIN 3 patients. The aim of our study is to optimize immunohistochemistry procedures in order to determine the relative presence of Th17 cells and regulatory T cells in formalin-fixed, paraffin embedded cervical cancer tissue and to apply the staining technique to cervical tissue arrays. An immunohistochemistry technique was used on formalin-fixed, paraffin embedded cervical tissue samples to stain lymphocytes in order to visualize them using a light microscope. The procedure is still being optimized. Therefore, no lymphocytes were visualized at this time. We will continue to optimize our procedure until the lymphocytes can be visualized using a light microscope. Once the relative presence of the lymphocytes in CIN and cervical cancer are revealed, future experiments to understand the function of Th17 cells and regulatory T cells in cervical cancer are necessary.

Keywords: cervical cancer, human papillomavirus, Th17, lymphocytes, immunohistochemistry.

P-glycoprotein Induction of Chemotherapy Naïve Cancer Cells via Non-chemotherapy P-glycoprotein Substrates

Lisa Gibbs, Christopher Farrell, Ph.D

Affiliation of all authors:

Department of Pharmaceutical and Administrative Sciences, Presbyterian College School of Pharmacy 307 N. Broad St, Clinton, SC 29325

Authors Emails: Lisa Gibbs, lgibbs@presby.edu. Christopher Farrell, clfarrell@presby.edu

Contact Information:

Lisa Gibbs. Pharmacy Student, Presbyterian College School of Pharmacy. 307 N. Broad St, Clinton, SC 29325.

Christopher Farrell, Ph.D. Department of Pharmaceutical and Administrative Sciences, Presbyterian College School of Pharmacy. 307 N. Broad St, Clinton, SC 29325.

Abstract

Multidrug resistance (MDR) is a huge barrier to cancer treatment with chemotherapy and targeted therapy. Overexpression of the protein transporter P-glycoprotein (P-gp) has been shown to be a major contributor to MDR. Certain P-gp substrates, such as anticancer agents and HIV-protease inhibitors, have been shown to induce MDR in cancer cells. We investigated if the P-glycoprotein substrates losartan (Cozaar) and citalopram (Celexa) would induce P-gp overexpression in Caco-2 cells. The cells were continuously given losartan or citalopram and passaged every 4-5 days for 2 months. The cells were monitored for P-gp overexpression by RT-PCR. P-gp overexpression has not been observed yet; the project is still on-going. Based on previous studies, the induction should be observed around month 8 or 9. If observed, this could be an important step toward further personalizing cancer treatment.

Keywords: P-glycoprotein, multidrug resistance, ABCB1, MDR1, Caco-2, losartan, citalopram

Birinapant, a SMAC Mimetic, Inhibits Cell Proliferation in Human Bladder Papilloma Cells via cIAP1 and cIAP2

Serge A. Afeli and Sarah Goines

Affiliations of all authors: Department of Pharmaceutical and Administrative Sciences, Presbyterian College School of Pharmacy, 307 N. Broad St, Clinton, SC 29325

Authors emails: sdgoines@presby.edu; afeli@presby.edu

Contact information:

Serge A. Afeli, Ph.D. , Associate Professor of Pharmacology, Department of Pharmaceutical and Administrative Sciences, Presbyterian College School of Pharmacy, 307 N. Broad St, Clinton, SC 29325

Abstract

Bladder cancer affects 74,000 people each year in the U.S.¹ The standard treatment for unresectable bladder carcinomas is a cisplatin-gemcitabine therapy, which is controversial due to chemoresistance.² Birinapant, a SMAC mimetic, induces apoptosis by targeting inhibitor of apoptosis proteins (IAPs) cIAP1 and cIAP2. It has proven successful against multiple types of cancers. We investigated the molecular fingerprints of cIAP1 and cIAP2 in human bladder papilloma cells (RT4 cell line), along with the expression of efflux transporter MRP5 (ABCC5). Furthermore, we evaluated birinapant's ability to inhibit RT4 cell proliferation in comparison to cisplatin and gemcitabine. The mRNA expression of cIAP1 and cIAP2 was detected using reverse transcriptase PCR. Cell growth optimization was determined using a dilution series and the MTT assay. Cells were treated with birinapant, cisplatin, and gemcitabine at various concentrations for 24 h and cell proliferation was assessed using the MTT assay. RT-PCR experiments detected mRNA messages for cIAP1, cIAP2, and MRP5 in RT4 cells. The dilution series experiments demonstrated that RT4 cell proliferation follows an exponential growth curve, and optimal growth of RT4 cells occurs between 1×10^5 cells and 1×10^6 cells/mL in a 96-well plate. Birinapant decreased RT4 cell proliferation in a concentration-dependent manner. Our data suggests that the inhibitory properties of birinapant in RT4 cells are mediated via inhibition of cIAP1 and cIAP2. Birinapant demonstrated similar inhibitory effects on cell proliferation as cisplatin suggesting similar efficacy. Therefore, birinapant could represent a novel chemotherapeutic option for bladder cancer.

Keywords: bladder cancer, birinapant, cisplatin, gemcitabine, inhibitor of apoptosis proteins, IAPs, SMAC mimetics, RT4 cells

The Impact of Social Support on Patients in a Pharmacist-Run Diabetes Education

Theodora B. Hampton, PharmD Candidate 2018; Eileen D. Ward, PharmD, BCACP;
Tiffany Threat, PharmD, CDE

Authors Affiliation: Department of Pharmacy Practice, Presbyterian College School of Pharmacy. 307 N. Broad St, Clinton, SC 29325

Authors emails: Eileen D. Ward, edward@presby.edu, Theodora B. Hampton, tbhampton@presby.edu, Tiffany Threat, tbthreat@presby.edu.

Contact information

Eileen D. Ward, PharmD, BCACP. Assistant Professor of Pharmacy Practice. Department of Pharmacy Practice. Presbyterian College School of Pharmacy. 307 N. Broad St, Clinton, SC 29325

Theodora Hampton. PharmD Candidate 2018. Presbyterian College School of Pharmacy. 307 N. Broad St, Clinton, SC 29325

Tiffany Threat, PharmD, CDE. Assistant Professor of Pharmacy Practice. Department of Pharmacy Practice. Presbyterian College School of Pharmacy. 307 N. Broad St, Clinton, SC 29325

Abstract

Examine the impact of social support on changes in A1C, LDL, and achievement of self-care goals for patients with diabetes attending a pharmacist-run diabetes self-management education (DSME) program. Methods: 42 eligible participants completed DSME program between January 2014 - January 2015. 30 participants completed follow-up and were divided into 2 groups, those who brought a support member to a DSME class, (n=6), and those who did not, (n=24). Supported participants were surveyed on number of classes attended with support, patient's relation to support member, and patient's ranking of the perceived support on a scale of 1-5. Baseline and follow-up lab data was collected from referring physician or a shared EMR system. Change in A1C and LDL were described as mean change and self-care goals achievement were analyzed using descriptive statistics. Data analysis was completed for those with available laboratory values. Results: Mean change in A1C for supported participants with available data, (n=4), was -3.92% compared to unsupported participants, (n= 21), which was -0.98%. Mean change in LDL for supported, (n=2), was -2mg/dL and -12mg/dL for unsupported, (n=16). Supported participants who set a goal in any of the categories achieved or continued those goals in all of the categories except one. Conclusion: Although study results are consistent with published data on the role of social support in diabetes management, the limitations of this initial study, such as small sample size impede the ability to confidently analyze the true impact of social support on diabetes management. Additional, larger studies are needed.

Keywords: Social support, type 2 diabetes, pharmacist-run diabetes education program, rural wellness center.

Development of Combinatorial Synthesis of PDF Inhibitors

Brad Leonard and Kristopher Virga

Affiliations of all authors:

Department of Pharmaceutical and Administrative Sciences, Presbyterian College School of Pharmacy, Clinton, SC

Authors emails: Brad Leonard: btleonard@presby.edu; Kristopher Virga: kgvirga@presby.edu

Contact information

Kristopher Virga, Ph.D., Associate Professor, Department of Pharmaceutical and Administrative Sciences, Presbyterian College School of Pharmacy, Clinton, SC

Abstract

Peptide deformylase is a metalloprotease that is responsible for removing the N-formyl methionine from the amino acid sequence allowing the protein to fold into its functional conformation. This is a potential target for antibiotics since inhibition of this enzyme will prevent bacteria from producing proteins leading to their eventual death. Actinonin, a naturally occurring PDF inhibitor, has poor bioavailability so the development of synthetic PDF inhibitors would provide a new series of antibiotics.² The development of a reliable combinatorial synthetic route of novel PDF inhibitors is the ultimate goal. This synthesis will alter involve alterations in alpha keto-acids, amino acids, and cyclic amines to generate a myriad of PDF inhibitors that will be compared to one another based on binding. A reductive amination involving ethyl 2-oxo-4-phenylbutyrate and histidine was attempted under varying conditions, but the major product recovered was a reduced version of the ethyl-2-oxo-4-phenylbutyrate. Two new reactions were derived that resulted in either inadequate yield or reduced starting material. Currently two new reactions are being performed to move forward with the synthesis of a PDF inhibitor.

Keywords: PDF Inhibitor, Actinonin, Combinatorial Synthesis, Peptide Formylase

Diabetic Neuropathy: Incidence, Complications, and Treatment in Diabetic Patients from the PCSP Wellness Center

Jessica A Prestia, PharmD Candidate; Jimmy L Pruitt, PharmD Candidate; Tiffany Threatt, PharmD; Sarah Wagner, PharmD; E Alfonso Romero-Sandoval, MD, PhD.

Affiliations of all authors: Department of Pharmaceutical and Administrative Sciences and Department of Pharmacy Practice, Presbyterian College School of Pharmacy. 307 N. Broad St, Clinton, SC 29325

Authors emails: Jessica A Prestia, japrestia@presby.edu. Jimmy L Pruitt, jlpruitt@presby.edu. Tiffany Threatt, tbthreatt@presby.edu. Sarah Wagner, sarah.abrams.wagner@gmail.com. E. Alfonso Romero-Sandoval asandoval@presby.edu

Contact information

Jessica A Prestia, PharmD Candidate; Jimmy L Pruitt, PharmD Candidate.

Tiffany Threatt, PharmD, Assistant Professor, Director of PCSP Wellness Center.

Sarah Wagner, PharmD, Assistant Professor. Department of Pharmacy Practice, Presbyterian College School of Pharmacy. 307 N. Broad St, Clinton, SC 29325

Alfonso Romero-Sandoval, MD, PhD, Director of Research, Associate Professor. Department of Pharmaceutical and Administrative Sciences, Presbyterian College School of Pharmacy. 307 N. Broad St, Clinton, SC 29325.

Abstract

The incidence of type 2 diabetes is higher in rural areas in the United States. We hypothesize that diabetic patients of a rural area of South Carolina (SC) will have a higher incidence of peripheral diabetic neuropathy (PDN) and painful PDN. We also hypothesize that the severity of PDN will be associated with higher blood glucose levels, higher prevalence of painful PDN and major comorbidities. Clinical records of diabetic patients of the PCSP Wellness Center were reviewed for data collection. The incidence of PDN and painful PDN was higher than the national average. Inconsistencies in glycemic values records prevented from correlating this variable with PDN. Painful PDN cases display a lower degree of PDN severity than non-painful PDN, even when segregated and associated with obesity and hypertension, but not with dyslipidemia. This suggests that dyslipidemia could be a risk factor for a more progressive PDN form. Only 1.92% of painful PDN cases were treated at AAN level A (pregabalin). The fact that the studied population represent a rural underserved area could explain the higher incidence of PDN and painful PDN. Our study shows that for this population painful PDN could represent a mild form of PDN, and that the association with dyslipidemia could represent a higher risk. Interestingly, these conditions could be readily identified by pharmacists. This suggests that pharmacists are well positioned to better monitor and manage these patients, which could result in a reduction of the incidence of PDN and painful PDN.

Keywords: Diabetes, peripheral diabetic neuropathy, painful neuropathy, underserved, rural, disparities.

Correlation of Cobalt Effects on Gene Expression with Intracellular Metal Ion Accumulation in an *In Vitro* Sertoli cell Model

Melèq Williams and Nancy Pedigo

Affiliations of all authors: Department of Pharmaceutical and Administrative Sciences, Presbyterian College School of Pharmacy. 307 N. Broad St, Clinton, SC 29325

Authors emails: Meleq Williams, meleqwilliams@gmail.com; Dr. Nancy G. Pedigo, ngpedigo@presby.edu

Contact information:

Meleq Williams: Claflin University, Orangeburg, SC

Dr. Nancy G. Pedigo: Department of Pharmaceutical and Administrative Sciences, Presbyterian College School of Pharmacy. 307 N. Broad St, Clinton, SC 29325

Abstract

Toxic heavy metal ions have harmful effects on the male reproductive system. Cobalt is a toxic metal ion that has adverse effects on spermatogenesis causing infertility. Sertoli cells are particularly involved with the regulation of the process of spermatogenesis. In prior studies, the toxic effects of cobalt were mitigated by the essential trace element zinc, exhibiting partial protection *in vivo*. The purpose of the present study is to determine the specific role of Sertoli cell regulation in the mechanism of cobalt reproductive toxicity. In our current experiments, we determined changes in intracellular cobalt and zinc ion concentrations in cobalt-treated Sertoli cells. Cobalt, but not zinc levels were altered. We also evaluated the alterations in gene expression of Sertoli cells treated *in vitro* with cobalt. Results indicate that there are a large number of genes which were significantly affected at each cobalt concentration as compared with controls (523 genes for [Co]= 10 μ M and 5512 genes for [Co]= 33 μ M). Significance level was set at a ≥ 2 -fold change, $p \leq 0.05$. This research will further define the role of the Sertoli cell in the detrimental reproductive effects of cobalt on spermatogenesis.

Keywords: Sertoli cell, Reproductive toxicity, Cobalt, Gene expression