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USE OF VARIOUS NONSTEROIDAL ANTI-INFLAMMATORY DRUGS IN CHRONIC MYELOID LEUKEMIA PROGRESSION

JOCELYN STOUDT
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Reviewed and approved* by the following:

K. Sandeep Prabhu
Professor of Immunology and Molecular Toxicology
Thesis Supervisor

Pamela A. Hankey Giblin
Professor of Immunology
Honors Adviser

* Signatures are on file in the Schreyer Honors College.

ABSTRACT

Chronic Myeloid Leukemia (CML) is responsible for 15% of all adult leukemias and current treatments do not eliminate the disease. CML is most common in older adults over age 65 and literature suggests that many older adults who take low doses of nonsteroidal anti-inflammatory drugs (NSAIDs) may be more prone to CML. Selenium is a micronutrient that the body needs for healthy function, and has been known to have chemoprotective effects on leukemia. This study examines how NSAIDs impact the role of chemoprotective properties of selenium, particularly in targeting a key population of leukemia stem cells that cause relapse of the disease. $Trsp^{fl/fl}Cre^{LysM}$ mice are unable to make selenoproteins in macrophages, and these mice are utilized to observe whether the protective effects of selenium are dependent on selenoproteins, or if free selenium can also have the same effect on CML when selenoproteins cannot be made. RAW 267.4 murine macrophages and bone marrow derived macrophages (BMDMs) isolated from C57BL/6 mice were used to answer this question. As previously described, selenium treatment increased GPX1 expression in murine RAW 264.7 macrophages. RNA and protein analysis of primary BMDMs showed varied expression of prostaglandin (PG) synthases that are downstream of cyclooxygenases. Further research is needed to determine the long-term consequences of this observation. *Trsp* WT and KO BMDMs produced Δ^{12}-PGJ_2 at different levels. Interestingly, *Trsp* KO BMDMs showed higher levels with NSAID treatment. This could affect CML patients with *Trsp* gene mutations. Lipid extracts generated from *Trsp* BMDMs will be used to treat experimental CML cells to determine the biological significance of these findings.

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Figure 5. Δ12-PGJ2 concentration was different in *Trsp* WT vs. KO BMDMs treated with selenium and NSAIDs. Femurs were plated across 12 plates and BMDMs from TRSP WT and KO mice were treated with Se +/- NSAIDs for 6 days. Media was changed and fresh treatments were added daily. On day 6, 100ng/ml LPS was added for 2 hours. Media was collected 24 hours post-LPS and LC-MS/MS MRM method analysis was conducted on isolated lipids from media. N=2-3. 15

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Chapter 1

Introduction

Chronic Myeloid Leukemia

Chronic Myeloid Leukemia (CML) is a form of leukemia that commonly occurs in older adults of an average age of 65. New cases of the disease are diagnosed in 1 in 100,000 patients every year [1]. CML is responsible for 15% of all adult leukemias. It is caused by a mutation in the 22nd chromosome at the breakpoint cluster region (BCR) and 9th chromosome at the Ableson murine leukemia gene (ABL). This causes the two chromosomes to translocate and recombine, creating the Philadelphia chromosome (Ph+), which contains the BCR-ABL fusion gene that is found in 95% of CML patients. BCR-ABL encodes a protein with dysregulated tyrosine kinase activity. The dysregulated activity of ABL protein, a tyrosine kinase, leads to uncontrolled growth of leukocytes [2]. Currently, a widely utilized treatment is imatinib, a tyrosine kinase inhibitor, which suppresses the disease, but does not kill the pre-existing leukemia stem cells. This treatment is effective at controlling the disease, but still largely results in disease relapse [3].

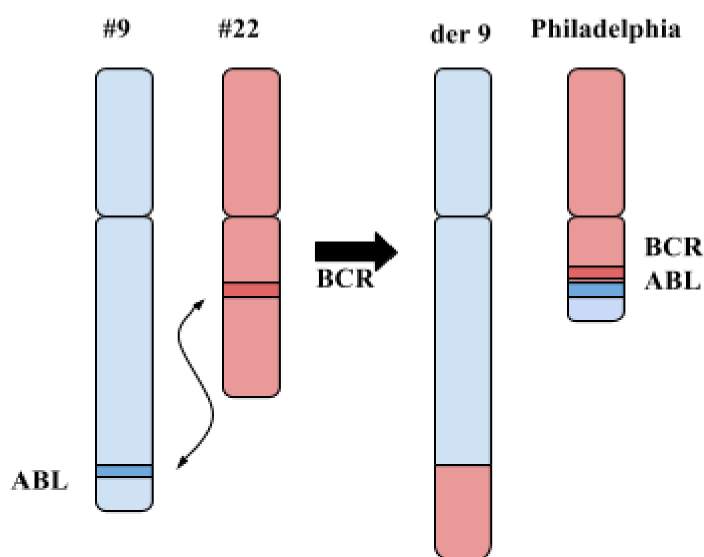

Figure 1. Translocation and recombination of Philadelphia chromosome.

Selenium

Selenium has been long known for its cytotoxic effect on leukemia cells [4]. It is a micronutrient that the body needs to maintain health. Selenium is found in the soil and incorporated into plants through it [5]. Humans consume selenium through sources such as meat, wheat, eggs, garlic, brazil nuts, and broccoli [6]. Studies have shown that a selenium supplemented diet has immunostimulatory effects, where it increases survival in HIV patients, has neuroprotective effects, positively affects fertility and reproduction and the thyroid, and can decrease cardiovascular disease [7].

Selenocysteine (Sec) is the 21st amino acid that is incorporated into selenoproteins. It is synthesized through the [Sec]tRNA, which is encoded by the [Sec]tRNA gene (*Trsp*). Incorporating Sec cotranslationally is the main way the body takes in selenium, but it can also be

nonspecifically incorporated during translation. There are also selenium binding proteins, which is another method of uptake in the body. There are 25 different selenoproteins that function in the human body [7]. Selenoproteins such as the glutathione peroxidases (GPXs), have the strongest antioxidant capabilities by protecting cells from reactive oxygen species (ROS). GPX converts the harmful free radicals into harmless compounds, for example, hydrogen peroxide to water. When there is too much ROS and not enough antioxidants, this can damage cellular components, including DNA, proteins, and lipids [11].

To be able to examine the role of selenoproteins in macrophages (bone marrow-derived), $Trsp^{fl/fl}Cre^{LysM}$ mice were used in this study. *Trsp* knockout (KO) mice lack the [Sec]tRNA gene which makes them unable to synthesize some selenoproteins in macrophages and monocytes as opposed to the wild-type (WT) that have normal selenoprotein function. GPX1 is one of the most affected selenoproteins with little to none made in the macrophages isolated from the KO mice [8,12].

Prostaglandins

Prostaglandins are lipids derived in the body that are involved in inflammation and resolution, apart from several physiological processes [17]. Series 2 PGs are produced from arachidonic acid by cyclooxygenase (COX), a necessary enzyme in this pathway. Previous studies have shown that selenium, via selenoproteins, influences whether macrophages activation could be biased towards pro-inflammatory or anti-inflammatory (reparative) phenotypes [13]. When macrophages were treated with selenium, levels of NF-kB were decreased, though high levels of selenium were shown to induce large amounts of endoplasmic reticulum (ER) and

oxidative stress on human leukemia cells in vitro [14]. This turns on a pro-apoptotic pathway and causes apoptosis of the cells affected. NF-kB is blocked at high concentrations of selenium while p53 and the caspase cascade are both activated [13, 14]. The caspase cascade and p53 both are involved in cell apoptosis through oxidative phosphorylation[3]. Thus, when selenium levels are elevated, these pro-apoptotic mechanisms terminate the leukemia cells [13, 14]. In healthy cells, however, selenium decreases oxidative stress in cells, which in turn decreases the amount of activated NF-kB. NF-kB causes the pro-inflammatory prostaglandins to be produced, such as PGE_2. As a result, by supplementing cells with selenium, a decreased level of pro-inflammatory prostaglandins will be made [9].

When supplemented with selenium, the pathway is shunted toward the anti-inflammatory pathway, creating the Δ^{12}-prostaglandin J_2 (Δ^{12}-PGJ_2) compound produced from PGD_2. Previous studies have shown that selenoproteins upregulate the expression and activity of hematopoietic PGD_2 synthase (HPGDS), resulting in more PGD_2 [3]. HPGDS catalyzes the conversion of PGH_2 to PGD_2. On the other hand, selenium supplementation decreases the macrophage expression of mPGES-1, microsomal PGE_2 synthase that catalyzes the conversion of PGH_2 to the pro-inflammatory PGE_2 [3].

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

NSAIDs are commonly used drugs used to relieve inflammation in the body. They block COX activity, which reduces inflammation through decreased prostaglandin production. NSAIDs used in this study included aspirin, ibuprofen, indomethacin, and naproxen that inhibit both COX-1 and COX-2. These types of NSAIDs reduce inflammation through COX-2, but

inhibition of COX-1 suppresses the prostaglandins produced necessary for stomach health, which can lead to gastric ulcers if taken in excess [15]. Previous research has shown that NSAIDs decrease the production of Δ^{12}-PGJ_2, the prostaglandin that selectively targets CML stem cells. As a result, NSAIDs may increase CML severity if there is less Δ^{12}-PGJ_2 produced [3].

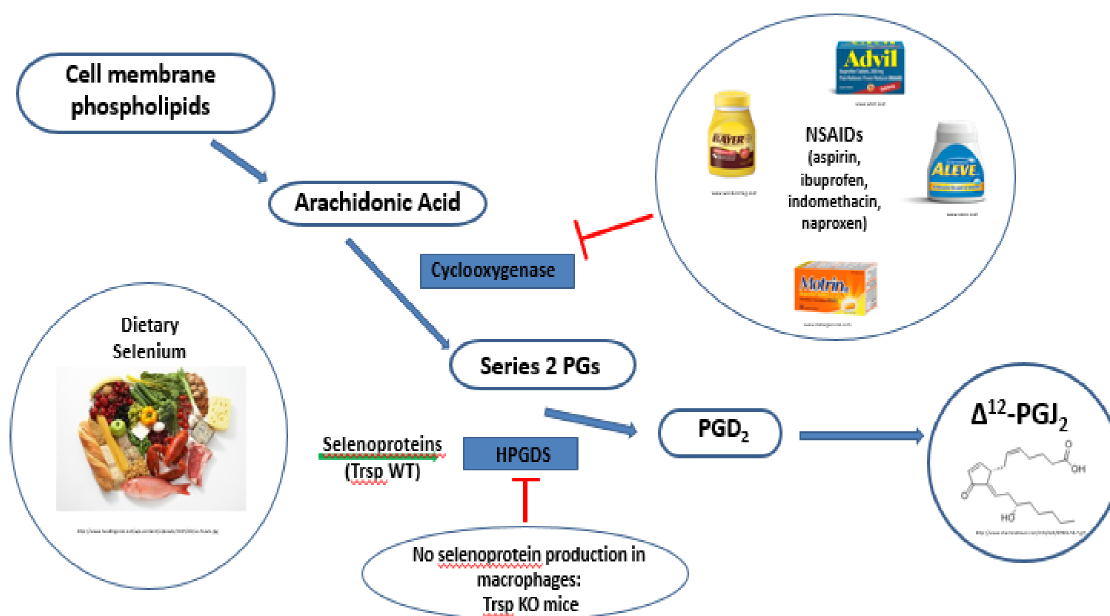

Figure 2. Arachidonic Acid Cascade

Statement of the Problem

Chronic myeloid leukemia (CML) is responsible for 15% of all adult leukemias and current treatments do not eliminate the disease. CML is most common in older adults over age 65 and literature suggests that many older adults who take low doses of non-steroidal anti-Inflammatory drugs (NSAIDs) may be more prone to CML [16]. Selenium is a micronutrient that the body needs for healthy function, and has been known to have chemoprotective effects on

leukemia [3]. This study examines how NSAIDs impact the role of chemoprotective properties of selenium in the form of selenoproteins, particularly in targeting a key population of leukemia stem cells that cause relapse of the disease. *Trsp*$^{fl/fl}$*Cre*^{LysM} mice are unable to make selenoproteins in macrophages [12], and these mice are utilized to observe whether the protective effects of selenium are dependent on selenoproteins, or if free selenium can also have the same effect on CML when selenoproteins cannot be made.

Chapter 2

Methods

Animals

C57bl/6 (n=3) and $Trsp^{fl/fl}Cre^{LysM}$ mice (n=4) were fed a selenium supplemented (Se-S; 0.4 ppm as selenite) or selenium deficient (Se-D; <0.01 ppm as selenite) diet. The semi-purified diets were purchased from Harlan. Mice were maintained on a 12-hour light cycle in a humidity and temperature controlled room. All procedures were preapproved by the Institutional Animal Care and Use Committee (IACUC) at The Pennsylvania State University (University Park, PA).

Bone Marrow Extraction and Cell Culture

Bone marrow derived macrophages (BMDMs) were extracted from mouse femurs. Bone marrow (BM) was flushed using a 25-gauge needle, strained through a 40 µm filter, and resuspended in DMEM media containing 10% (v/v) L929 fibroblast conditioned media, 5 % (v/v) fetal bovine serum, 100 units/mL penicillin, 100 µm streptomycin and 2 mmol/L L-glutamine. As a comparison to BMDMs, an immortalized murine macrophage-like cell line, RAW264.7, was purchased from American Type Culture Collection (ATCC) was used. BMDMs and RAW 267.4 cells were plated into 10 cm^2 plates with 10 mL of media and were treated with 0 nM or 250 nM selenium, and an NSAID (aspirin, ibuprofen, indomethacin, or naproxen) with an appropriate vehicle control. Cells were kept at 37°C with daily media changes

every day with specific treatments After 6 days, cells were stimulated with 100 ng/ml LPS (from *E. coli* serotype 0111:B4; Sigma Aldrich) for 2 hours, then washed twice with PBS, replaced with new media and harvested 24 hours later. Cells were resuspended in 10 mL of PBS in a 15 mL tube, centrifuged at 2000 rpm for 5 minutes and resuspended in 1mL of PBS. Clarified media was used for LC-MS/MS analysis (see below). The cell pellet was frozen at -80°C for later use in protein and RNA isolation.

RNA Isolation

One mL of Trizol was added to the cell pellet, homogenized, and incubated at room temperature for 5 minutes. After 5 minutes, chloroform was added to the tubes and capped and mixed vigorously for 15 seconds. Tubes were then incubated for 3 minutes at room temperature and centrifuged at 12000g for 15 minutes. The clear aqueous phase was extracted and transferred to a separate tube. 550 µL of 2-propanol was added and inverted 5 times. After the samples incubated at room temperature for 10 minutes, they were centrifuged at 4°C at 12000g for 15 minutes. Liquid was decanted from pellet and pellet was washed with 950 µL of 75% ethanol and centrifuged again at 4°C at 7500g for 5 minutes. Ethanol was removed from pellet and the pellet was air dried and resuspended in 25 µL of nuclease free water. RNA concentration was calculated using the absorption values determined using the Nanodrop.

cDNA Synthesis

Each cDNA sample was prepared using the appropriate RNA concentration, 2 µL 10X reverse transcriptase (RT) buffer, 0.8 µL 25X dNTP mix, 2uL 10X RT random primers, 4.2 µL

nuclease free water, and 1 μL reverse transcriptase. The samples were then run in the thermal cycler to synthesize cDNA.

Real Time PCR

Each sample was prepared using 0.5 μL cDNA, 0.5 μL of the designated probe, 5 μL Master Mix, and 4 μL water. Validated taqman probes for COX-2, HPGDS, and PTGES were used in an Applied Biosystems realtime PCR machine (model #7300). DDCt was calculated by taking Ct_{gene}-CT_{GAPDH} for each sample and graphed.

Protein Isolation and Estimation

A cocktail of the mammalian extraction reagent, MPER (Thermo Pierce), was prepared and added to the cell pellet using 1mL MPER, 5 μL aprotinin, 1 μL leupeptin and 1 μL pepstatinA. Samples were vortexed every 5 minutes for 20 minutes, then centrifuged at max speed for 10 minutes and the supernatant was collected. Protein concentrations were estimated using the BCA assay per the suggestions of the supplier (Thermo Pierce). The samples incubated at 37°C for 30 minutes and read at 560 nM in the SpectraCount plate reader against a BSA standard.

Western Blot

Samples were prepared at a concentration of 30 μg of protein, with water and SDS loading dye, totaling 24 μL. Samples were boiled at 95 °C for 5 minutes. 12.5 % separating and stacking gels were made and casters were placed on either side of the gel apparatus and the

middle section was filled with 1X running buffer (121.1g Tris base, 576 g glycine, 200 mL 20% SDS, 4 L ddH_2O). Samples and protein ladder were loaded into wells and the gel was run at 150V for 60 minutes.

When the gel was finished running, the gel was prepared for transfer between sponges, blotting paper, and the PVDF membrane. The gel apparatus, ice block, and stir bar were placed into the chamber and filled to the top with 1X TBS (193.6 g Tris base, 640g NaCl, 3.2 L ddH_2O, HCl, pH=7.6) Blotting was performed for 90 minutes at 100V.

Transfer of proteins from the gel on to the membrane was tested using Ponceau dye staining. 10 mL of a 5% milk solution in TBST was added and the membrane was incubated for 60 minutes at room temperature. Then the primary antibody was added and incubated overnight at 4°C. The blot was washed three times for 10 minutes each with TBST. After washing, the secondary antibody diluted in milk (anti-rabbit 1:2500, anti-mouse 1:5000) was added and incubated for 1 hour at room temperature on an end-to-end shaker. The blot was washed again 3 times for 10 minutes in TBST. Five mL of each chemiluminescent substrate was added and incubated for 5 minutes on the shaker. Then the membrane was placed in the cassette between two transparencies and a film was placed inside and exposed for the appropriate amount of time and developed.

Lipid Extraction

BMDM media was collected from cell culture as described earlier. For each sample, 5 mL methanol then 5 mL PBS was passed through the column dropwise. Then sample was acidified with 6N HCl and passed through the column 2 times. The column was then washed

with 5 mL PBS, then 5 mL hexane, and finally 5 mL of methanol was passed through the column and collected.

Mass Spectrometry

Each sample was blown down using liquid nitrogen and resuspended in 400 µL of ethyl acetate. 100 µL of the ethyl acetate was taken and placed in a new tube and blown down using liquid nitrogen. Finally, each sample was resuspended in 100 µL methanol and placed in the mass spectrometry vial, and ran in the LC-MS/MS system (Shimadzu LC20AD UFLC pumps and API2000 triple quadruple mass spectrometer). The machine was set to 200°C on negative mode and used a 70:30 methanol/H_2O, 0.1% acetic acid solvent system utilizing a 0.15 ml/min flow rate. Scan mode was set at *m/z* 100-350 and 333.5 for Δ^{12}-PGJ_2. Multiple reaction monitoring (MRM) from base ion to the daughter ion, 332.72 to 271.2 (M – H+) was used to create a standard curve. A standard curve was established as y=209.05x with an R^2 value of 0.9985.

Analysis

All results were analyzed using GraphPad Prism and expressed as biologic mean ± SEM.

Chapter 3

Results

Selenium supplementation increases Gpx1 expression in RAW 264.7 macrophages

To confirm selenoprotein amounts increase with selenium treatment, RAW 264.7 macrophages were treated with either 0 nM sodium selenite (Na_2SeO_3) or 250 nM Na_2SeO_3. They were treated for 8 days and harvested at day 1 (D1), day 4 (D4), and day 8 (D8). D1 +250 nM Na_2SeO_3 had a protein amount of 0.49 normalized against GAPDH. D4 +250 Na_2SeO_3 had an amount of 1.05, and D8 +250 Se had 1.30. GPX1 expression increased with the number of treatment days.

Densitometry 0 0.49 0 1.055 0 1.302

Figure 3. Selenium increases GPX1 in RAW 267.4 macrophages. Cells were treated with 0nM selenium (Se) or 250nM Se. Cells were harvested at day (D) 1, 4, and 8 for protein analysis by Western blot. N=1.

Differential expression of mRNA and protein in BMDMs after treatment with selenium and various NSAIDs

C57bl/6 murine BMDMs were co-treated with selenite and NSAIDs for 6 days. NSAIDs that were selected included aspirin (25 mmol/L), ibuprofen (20 μmol/L), indomethacin (10 μmol/L), and naproxen (50 μmol/ L). Each plate was treated with 10 μL of each NSAID. Cells were treated with LPS on day 6 and collected on day 7. Cell pellets were collected and frozen at -80°C. mRNA levels were analyzed as described earlier. Figure 4 shows that COX-2 expression was varied by the NSAIDs and selenium treatment. mPGES-1 (*Ptges*) mRNA expression was increased in aspirin with no selenium. In the case of Hpgds mRNA, the expression was increased in aspirin +Se and in indomethacin +Se treatment groups. The protein analysis via Western Blot showed various COX-2 and mPGES-1 expression with little difference in Hpgds protein expression.

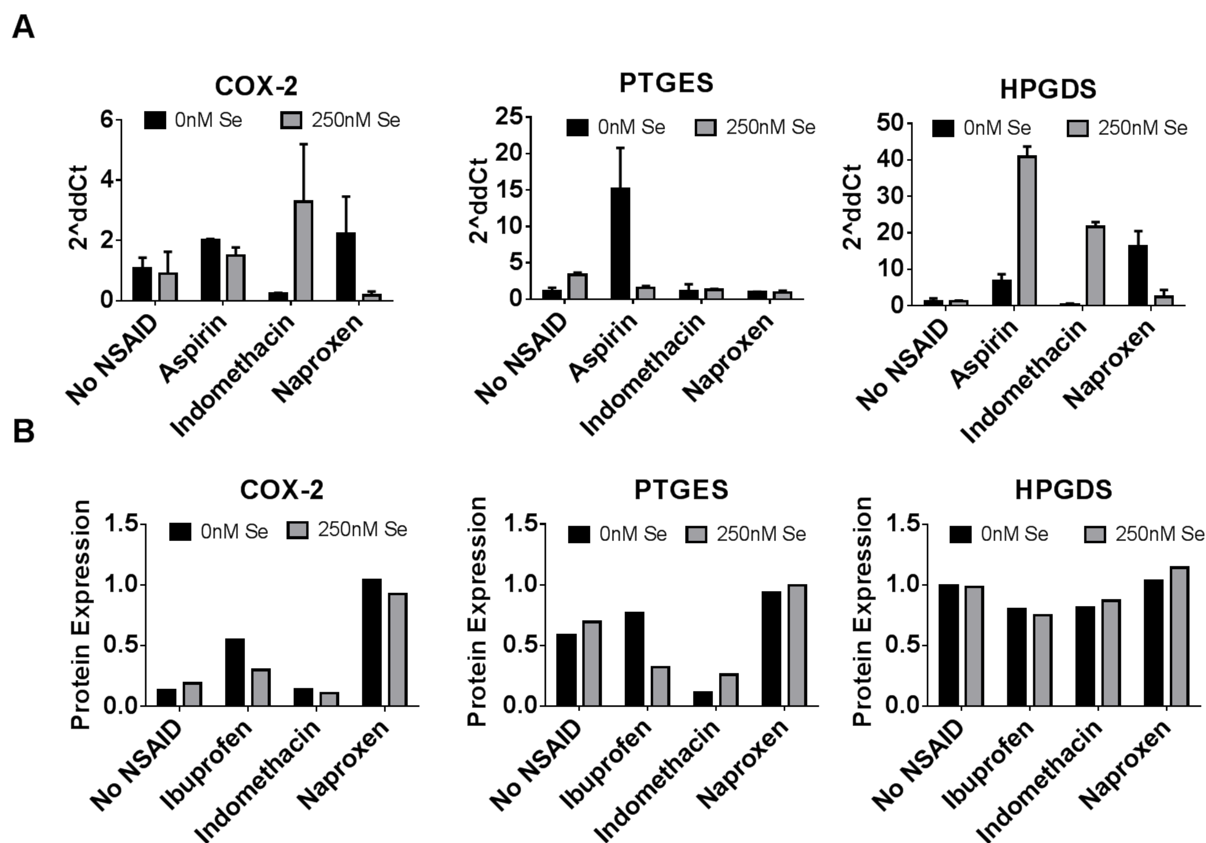

Figure 4. Differential expression of A) mRNA and B) protein in BMDMs after treatment with selenium and various NSAIDs. C57BL/6 murine BMDMs were treated with Se and NSAIDs for 6 days. Cells were collected on day 7. A) mRNA expression of COX-2, PTGES, and HPGDS by qRT-PCR. N=2. B) Protein analysis of COX-2, PTGES, and HPGDS by Western blot. N=1.

Δ^{12}-PGJ$_2$ concentration was different in *Trsp* WT vs. KO BMDMs treated with selenium and NSAIDs

BMDMs from *Trsp* WT and KO mice were treated with 250 nM selenite +/- NSAIDs for 6 days. Media was changed and fresh treatments were added daily. On day 6, 100ng/ml LPS was added for 2 hours. Media was collected 24 hours post-LPS and Liquid Chromatography- Mass Spectrometry with Multiple Reaction Monitoring (LC-MS/MS MRM) method analysis was

conducted on isolated lipids from media. *Trsp* KO mice showed higher amounts of Δ^{12}-PGJ_2 than *Trsp* WT.

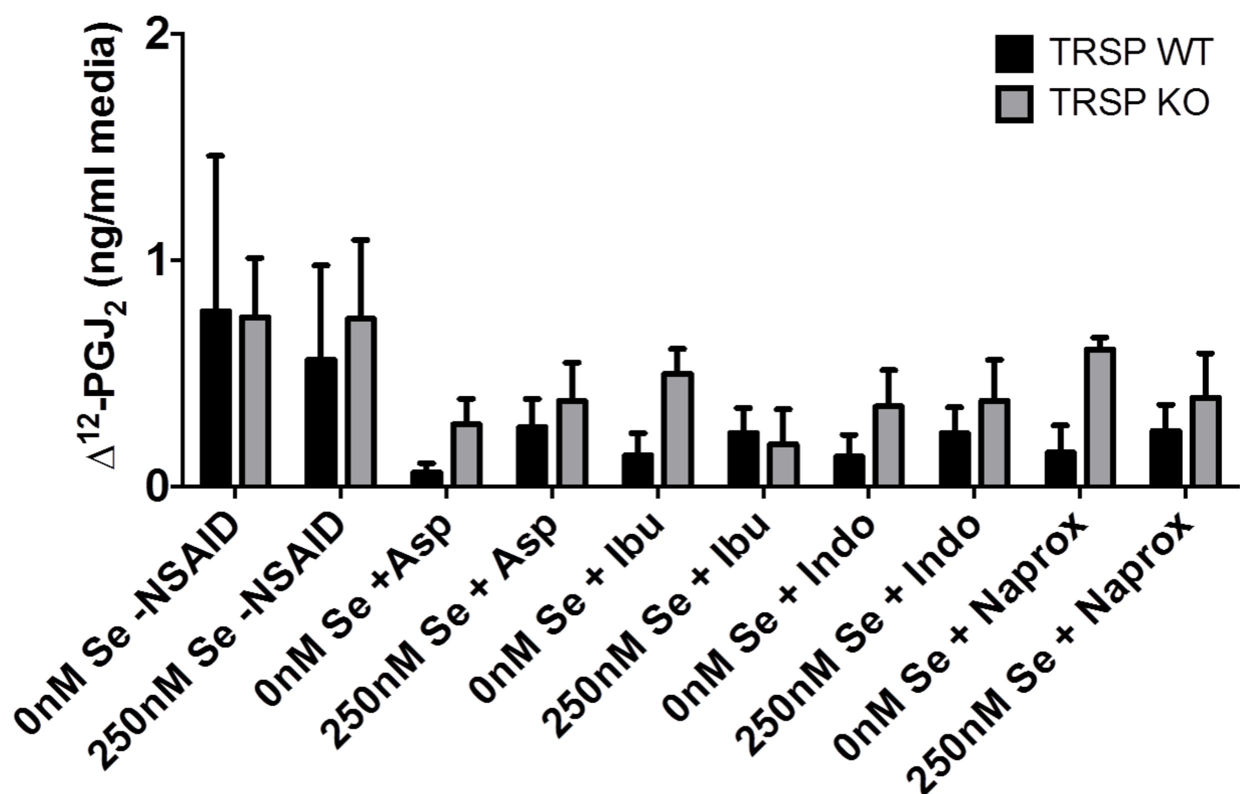

Figure 5. Δ12-PGJ2 concentration was different in *Trsp* WT vs. KO BMDMs treated with selenium and NSAIDs. Femurs were plated across 12 plates and BMDMs from TRSP WT and KO mice were treated with Se +/- NSAIDs for 6 days. Media was changed and fresh treatments were added daily. On day 6, 100ng/ml LPS was added for 2 hours. Media was collected 24 hours post-LPS and LC-MS/MS MRM method analysis was conducted on isolated lipids from media. N=2-3.

Chapter 4

Discussion

This project investigated the effect of selenite and NSAIDs on Δ^{12}-PGJ_2 in $Trsp^{fl/fl}Cre^{LysM}$ and C57BL/6 mice. RAW 267.4 murine macrophages were also used and treated with selenite. It was found that there is a positive correlation between number of treatment days and levels of Gpx1 expression in the cells. The macrophages incorporated the selenite into selenoproteins, which was confirmed using western blot in Fig. 3. Even though it is known that Gpx1 expression is highly responsive to exogenous addition of selenite in the media, the study was performed to make sure that the cells used for this project were responsive to exogenous selenium treatment.

Bone marrow-derived macrophages were cultured with and without selenite, and with various NSAIDs. mRNA and protein expression of several genes were measured. As shown in Fig. 4, COX-2 mRNA varied with the different NSAIDs. In indomethacin, levels of COX-2 were higher in the selenite treated cells. In naproxen, the 0 Se cells were higher in COX-2 expression. mPGES-1 levels were low among all treatments except aspirin. In aspirin, mPGES-1 levels were high in the selenium deficient cells, which suggests those macrophages may have produced higher PGE_2 amounts, a pro-inflammatory prostaglandin. Hpgds levels were found to be higher in 250 nM Se treated cells with aspirin and indomethacin. Naproxen was the opposite with 0 Se expression being higher than the Se treated expression.

In the Western blots, COX-2 expression was overall higher in the naproxen treated samples and slightly higher in the ibuprofen Se-deficient cells. mPGES-1 levels were fairly consistent except for selenite treated ibuprofen and both indomethacin treatments that both had slightly lower mPGES-1 expression levels. Ibuprofen with selenite could be slightly lower since with selenite the arachidonic acid pathway was shunted toward the PGD_2 and not the PGE_2 [3].

Finally, Hpgds levels remained constant across all treatments. Overall, mRNA expression varied with the different treatments. Needless to say, further research must be done to determine the long-term consequences of these results.

Results of the LC-MS/MRM analysis showed unexpected results. It was thought that in the *Trsp* KO mice, the levels of Δ^{12}-PGJ_2 would be lower because there would be no selenoproteins produced in the KO mice, leading to less Hpgds activity and Δ^{12}-PGJ_2 production. Interestingly, it appeared Δ^{12}-PGJ_2 levels were actually higher in the KO BMDMs. While this needs to be repeated to confirm these observations, a possible explanation is that since there was complete knock-out of selenoproteins, which are known for their antioxidant properties, increased stress on cells upon LPS treatment have inflicted much higher levels of intracellular oxidative stress due to lack of selenoproteins. Such an increased stress on the cells could have increased the endogenous production of Δ^{12}-PGJ_2 to compensate and mitigate increased inflammation to effect resolution. While this is interesting and conflicts with our hypothesis, further work is essential before we conclusively prove this. In addition, the lipid extracts from *Trsp* KO BMDMs will be used to treat experimental CML cells to determine the biological significance of these findings to examine if the increased Δ^{12}-PGJ_2 causes any apoptotic effects in CML LSCs, as reported earlier [3]. Further and more comprehensive research must be performed to understand the true meaning of these findings. Alternatively, use of other mouse genetic models where only a few selenoproteins have been knocked out could serve as a potential model.

BIBLIOGRAPHY

1. Hehlmann R., H. Andreas Hochhaus, M. Baccarani. 2007. Chronic Myeloid Leukaemia. *Lancet.* 370: 342–50.

2. O'Brien S., C.N. Abboud, M. Akhtari, J. Altman, E. Berman, D.J. DeAngelo,; S. Devine, A.T. Fathi, J. Gotlib, M. Jagasia, J.O. Moore, J. Pinilla-Ibarz, J.P. Radich, V. V.B. Reddy, N.P. Shah, P.J. Shami, D.S. Snyder, M. Wetzler, and F. Yunus. 2012. Chronic Myelogenous Leukemia. *JNCCN.* 10.1: 64-110.

3. Gandhi U.H., N. Kaushal, S. Hegde, E.R. Finch, A.K. Kudva, M.J. Kennett, C.T. Jordan, R.F. Paulson, and K.S. Prabhu. 2014. Selenium suppresses leukemia through the action of endogenous eicosanoids. *Cancer Res.* 74.14: 3890-3901

4. Batist G., A.G. Katki, R.W. Klecker, Jr., and C.E. Myers. 1986. Selenium-induced Cytotoxicity of Human Leukemia Cells: Interaction with Reduced Glutathione. *Cancer Res.* 46: 5482-5485.

5. Papp L.V., J. Lu, A. Holmgren, and K.K. Khanna. 2007. From Selenium to Selenoproteins: Synthesis, Identity, and Their Role in Human Health *Antioxid. Redox Signal.* 9: 775–806.

6. Finley, J. 2006. Bioavailability of Selenium from Foods. *Nutrition Reviews.* 64.3: 146-151.

7. Rayman, M. 2012. Selenium and Human Health. *Lancet.* 379: 1256–1268.

8. Kumaraswamy E., B.A. Carlson, F. Morgan, K. Miyoshi, G.W. Robinson, D. Su, S. Wang, E. Southon, L. Tessarollo, B.J. Lee, V.N. Gladyshev, L. Hennighausen, and D.L. Hatfield. 2003. Selective Removal of the Selenocysteine tRNA[Ser]Sec Gene (*Trsp*) in Mouse Mammary Epithelium. *Mol Cell Biol.* 23.5: 1477–1488.

9. Gandhi, U.H., N. Kasushal, K.C. Ravindra, S. Hedge, S.M. Nelson, V. Narayan, H. Vunta, R.F. Paulson, K.S. Prabhu. 2011. Selenoprotein-dependent up-regulation of hematopoetic prostaglandin D_2 synthase in macrophages is mediated through the activation of peroxisome proliferation-activated receptor (PPAR) γ. *J Biol Chem.* 286: 27471-27482.

10. Ricciotti, E., and G.A. FitzGerald. 2011. Prostaglandins and Inflammation. *Arterioscler Thromb Vasc Biol.* 31(5): 986–1000.

11. Tinggi, U., 2008. Selenium: its role as antioxidant in human health. *Environ Health Prev Med.* 13:102–108.

12. Nelson S.M., A.E. Shay, J.L. James, B.A. Carlson, J.F. Urban Jr., and K.S. Prabhu. 2016. Selenoprotein Expression in Macrophages Is Critical for Optimal Clearance of Parasitic Helminth Nippostrongylus brasiliensis.. *J Biol Chem*. 291(6):2787-98.

13. Guan, L., B. Han, J. Li, Z. Li, F Huang, Y. Yang, C. Xu. 2009. Exposure of human leukemia NB4 cells to increasing concentrations of selenite switches the signaling from pro-survival to pro-apoptosis. *Ann Hematol*.88.8:733-742.

14. Uguz, A.C., M. Naziroglu, J. Espino, I. Benjarano, D. Gonzalez, A.B. Rodriguez, J.A. Pariente. 2009. Selenium modulates oxidative stress-induced cell apoptosis in human myeloid HL-60 cells through regulation of calcium release and caspase-3 and -9 activities. *J Membrane Biol.* 232: 15-23.

15. Steele V.E., E.T. Hawk, J.L. Viner, R.A. Lubet. 2003. Mechanisms and applications of non-steroidal anti-inflammatory drugs in the chemoprevention of cancer. *Mutat Res.* 523-524: 137-144.

16. Kasum, C.M., C.K. Blair, A.R. Folsom, J.A. Ross. 2003. Non-steroidal Anti-inflammatory Drug Use and Risk of Adult Leukemia**.** *Cancer Epidemiol Biomarkers Prev.* 12:534–537.

17. Rajakarjar, R., M. Hilliard, T. Lawrence, S. Trivedi, P. Colville-Nash, G. Bellingan, D. Fitzgerald, M. M. Yaqoob, and D. W. Gilroy. 2007. Hematopoietic prostaglandin D_2 synthase controls the onset and resolution of acute inflammation through PGD_2 and 15-deoxy$\Delta^{12–14}$ PGJ_2. *Proc Natl Acad Sci U S A*. 104(52):20979-84.

ACADEMIC VITA
Jocelyn Stoudt

Education

The Pennsylvania State University, University Park, PA

Schreyer Honors College — *Expected Graduation: May 2016*
College of Agricultural Sciences
'Immunology and Infectious Disease' (BS)

Eastern Lebanon County High School, Myerstown, PA

High School Diploma — *Graduation: June 2012*
Salutatorian

Employment, Research and Community Service

The Pennsylvania State University -University Park, PA- *August 2013-present*

Undergraduate Researcher in Dr. K Sandeep Prabhu's Laboratory

- Conduct research on NSAID and selenium impact on chronic myeloid leukemia through various procedures and assays including: protein isolation, Western blots, RNA isolation, dissections, cell culture and flow cytometry
- Received Undergraduate Research grants from the College of Agricultural Sciences for pursuing an independent research project for Fall 2014, Spring 2015 and Fall 2015

Mid-State Literacy Council -State College, PA- *February 2015-May 2015*

English as a Second Language Volunteer Tutor

- Meet with my student twice a week to help her English speaking, reading and writing skills

Franklin House Tavern -Schaefferstown, PA- *June 2014-December 2014*

Server and Hostess

Elmcroft Senior Living -State College, PA- *May 2015-present*

Resident Assistant

- Assist residents with toileting, showering, activities of daily living, laundry, feeding, and ambulation

Skills

Laboratory: Scientific approach and understanding; mouse methodology; pure culture method; Western blotting; protein purification; various other laboratory procedures

Computer: MS Office; PowerPoint

Activities and Awards

Alpha Epsilon Delta- National Health Preprofessional Honors Society: *Secretary (2015-present); Distinguished Member (2014-Present); Blood Cup Subchair (2014)* — 2013-Present

Dean's List — 2013-2015

Alliance Christian Fellowship: *Food Deaconess (2013-2014)* — 2012-2015

Penn State SHO Time Orientation Leader — 2013-2015

Dancer in Penn State Dance Marathon — 2016