Enhancement of Triple Negative Breast Cancer Virotherapy via Alkylating Agent-Induced Autophagy

Roxana Gonzalez-Ramos 1,2, Akiko Chiba 3, Lacey McNally 4, Kelly M. McMasters1,2, and Jorge G. Gomez-Gutierrez 1,2

1The Hiram C. Polk MD Department of Surgery, 2James Graham Brown Cancer Center, University of Louisville, Louisville, KY, 40202. 3Department of Surgery, 4Department of Cancer Biology at Wake Forest Comprehensive Cancer Center, Wake Forest University, Winston-Salem, NC.

Introduction

Oncolytic virotherapy has made significant progress in recent years, however, widespread approval of virotherapeutics is still limited. Primarily, this is due to the fact that currently available virotherapeutics are mostly tested in monotherapy clinical trials exclusively (i.e. not in combination with other therapies) and so far have achieved only small and often clinically insignificant responses. For this reason, combination strategies of virotherapy with high genotoxic regimens, such as chemotherapy, are of major interest.

Triple negative breast cancer (TNBC) occurs in about 10-20% of diagnosed breast cancers and is more likely to affect younger women, African Americans, Hispanics and/or those with BRCA 1 gene mutation. In comparison with estrogen receptor positive breast cancer, TNBC can be more aggressive and difficult to treat. TNBC does not respond to hormonal therapy (e.g. Tamoxifen), however it could respond to conventional chemotherapy.

We hypothesize that Alkylating agent induced-autophagy can increase oncolytic virus replication in TNBC cells. In this study, the alkylating agent temozolomide (TMZ) was combined with a oncolytic adenovirus (OAd) in which the capsid was genetically labeled with the fluorescent reporter of the “mFruit” family with far-red emission spectra, mCherry. The red fluorescent mCherry reporter gene was fused to viral capsid protein IX.

It was found that TMZ increased AdmCherry-replication and oncolysis TNBC cells. The increased OAdmCherry potency was associated with an increase on E1A expression and virus production. The combined therapy of AdmCherry with TMZ resulted in a greater killing effect than either agent alone. This increased killing effect was associated with apoptosis induction and accumulation of the autophagy marker LC3-II.

Results

1. Structure of Ad particle with imaging modality on the capsid.

2. MDA-MB-231

3. MDA-MB-231

4. MDA-MB-231

5. MDA-MB-231

- Structure of Ad particle with imaging modality on the capsid.
- MDA-MB-231 cells were infected with OAdmCherry, followed by either vehicle control DMSO or TMZ, 72 h post treatment mCherry expression was visualized by florescent microscope.
- TMZ upregulate Ad E1A expression and increases cytopathic effect. A) Expression of Ad E1A and actin as loading control. B) Crystal violet staining. C) Cell viability was calculated by measuring the absorbance of solubilized dye at 590 nm. Each point represents the mean of three independent experiments ± standard deviation (SD; bars).
- Evaluation of apoptosis induction by the combined therapy in human and murine TNBC cells. A) Caspase-3 activation. B) Annexin V staining.

Conclusions

This study, provides the experimental evidence showing that TMZ can be used to enhance TNBC virotherapy which may represent an alternative approach to destroy TNBC tumors in patients with resistance to chemotherapy. Most importantly, TMZ enhanced OAd mediated-ondonulysis in the murine 4T1 cells that represent the stage IV of human BC and triple negative breast cancer were efficiently killed by the combined therapy of oncolytic adenovirus with TMZ. In addition, these chemovirotherapies may allow for use of less-toxic doses to achieve therapeutic efficacy and prime immune system to reduce the chances of cancer recurrences.

Acknowledgements

Research supported by the University of Louisville Cancer Education Program grant R25-CA-134283 from the National Cancer Institute.
The Effect of PFKFB4 Inhibition on the Cell Cycle Progression of Medulloblastoma Cells

Meranda Hinds¹, Brandi Radde, B.S.¹, Nadia Lypova, Ph.D.¹, Sucheta Telang, MBBS¹,²
Departments of Medicine¹ and Pediatrics²
University of Louisville School of Medicine

Introduction

Many cancer cell types demonstrate increased glycolytic metabolism to support the needs of their rapid proliferation. The conversion of fructose-6-phosphate (F6P) to fructose-1,6-bisphosphate (F16B8P) through the enzymatic activity of phosphofructokinase-1 (PFK1) is an important rate-limiting step in the glycolytic pathway. High levels of ATP and citrate provide negative feedback at this step, inhibiting the advancement along the glucose metabolic pathway when a cell has sufficient energy stores. Fructose-2,6-bisphosphate (F26B8P) has been found to be a powerful allosteric activator of PFK2 that is capable of overriding the negative feedback inhibition provided by ATP and citrate, thus stimulating the conversion of F6P to F16B8P and propagating this metabolic pathway regardless of a cancer cell’s energy needs. F26B8P is the product of the 6-phosphofructokinase/fructose-2,6-bisphosphatase family of enzymes (PFKFB1-4), which interconvert F6P and F26B8P. We have previously found that medulloblastoma cells express high levels of the PFKFB4 isoform and that silencing PFKFB4 expression decreases F26B8P production and proliferation in these cells. We hypothesized that the decrease in proliferation may be due to an arrest in cell cycle progression.

Materials and Methods

siRNA treatment: Daoy cells plated in 6 well plates were transfected with siRNA targeting PFKFB4 or a nonsense siRNA (20 nM) using the transfection reagent Lipofectamine RNAiMax and incubated for 72 hours under either hypoxic (1% oxygen) or normoxic (21% oxygen) conditions and then harvested for Western blot and cell cycle analyses.

Inhibitor treatment: Daoy cells plated in 6 well plates were treated with a small molecule inhibitor of PFKFB4 (FB4i) for 72 hours and then harvested for cell cycle analyses.

Cell cycle analyses: Following harvest, cells were washed with cold PBS and fixed in 70% ethanol at 4°C for 30 minutes. Cells were then pelleted by centrifugation, resuspended in PBS containing propidium iodide and RNAse A, incubated at 37°C for 30 minutes and analyzed by flow cytometry. Data shown are representative of 2 experiments.

Results

Western Blot Analysis of PFKFB4 knockdown under normoxic and hypoxic conditions

<table>
<thead>
<tr>
<th>siNS</th>
<th>siPFKFB4</th>
<th>siNS</th>
<th>siPFKFB4</th>
</tr>
</thead>
<tbody>
<tr>
<td>21%</td>
<td>1%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Distribution of cells in phases of the cell cycle following PFKFB4 knockdown in normoxia

<table>
<thead>
<tr>
<th>siNS</th>
<th>siPFKFB4</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1/G0: 49.6%</td>
<td>G1/G0: 58.6%</td>
</tr>
<tr>
<td>S: 26.5%</td>
<td>S: 24.1%</td>
</tr>
<tr>
<td>G2/M: 19.3%</td>
<td>G2/M: 26.9%</td>
</tr>
</tbody>
</table>

Conclusion

Our data suggest that siRNA knockdown of PFKFB4 expression and inhibition of the activity of PFKFB4 with a small molecule inhibitor cause a similar G0/G1 arrest in Daoy medulloblastoma cells. Future studies will further examine the effects of small molecule inhibition of PFKFB4 on medulloblastoma. It is our hope that this study will enhance the future study of this bifunctional enzyme and the glycolytic pathway in medulloblastoma cells so that it can be determined if PFKFB4 inhibition may serve as a potential target in medulloblastoma.

Future Directions

- Further study the effects of PFKFB4 small molecule inhibition in medulloblastoma.
- Examine the effects of PFKFB4 inhibition under hypoxic cell cycle progression.
- Compare the effect of knocking down one of the other three isoforms of PFKFB1-3 with the results from PFKFB4 knockdown.

Acknowledgements

Research funded by the National Cancer Institute grant R25 CA-134283 and the University of Louisville Cancer Education Program.
Effect of Expression of Constitutive Active Retinoblastoma Protein (Rb) on Glucose Metabolism
An Exercise in Experimental Troubleshooting

Joshua Julian, Lindsey Reynolds, and Brian Clem
James Graham Brown Cancer Center, Dept. of Biochemistry and Molecular Genetics, University of Louisville School of Medicine

Introduction
Investigation into the metabolism of cancer cells has implicated a potential role for the retinoblastoma protein (Rb) tumor suppressor, which has been classically defined in controlling cell proliferation. When active, Rb binds to a family of transcription factors, termed E2Fs, that regulate a number of cellular processes. Since inactivation of Rb has been observed in most forms of cancer, it is plausible that loss of Rb function might alter glucose metabolism by regulating specific metabolic enzymes.

Objective and Hypothesis
We attempted to express a constitutively active form of Rb (phosphorylation site mutated Rb or PSM-Rb) in HCT116 cells and monitor changes in glucose metabolism through both glucose uptake and glycolysis assays. We hypothesized that cells overexpressing PSM-Rb would show a decrease in glucose metabolism.

Methods
Cell Culture: HEK 293T cells were maintained in culture using DMEM supplemented with 10% FBS and gentamicin, and the HCT116 cells were grown in McCoy’s medium with 10% FBS and gentamicin.

Cell Transfections: HCT116 cells were plated in 6-well culture dishes and transfected 24 hours later with pcDNA-PSM or empty vector for 4 hours. Transfection medium was replaced with complete medium and left for 48 hours until subsequent assays were performed. Transfection of GFP vector with pcDNA-PSM or the control vector was performed in HCT116 cells using the jetPRIME system. Expression of GFP was determined by fluorescent microscopy (EVOS).

Retroviral production and viral infection: HEK 293T cells were transfected with pLNCX2-pcDNA retroviral plasmids and the retroviral pQCXIH-PSM vector via the jetPRIME system. Viral supernatant was collected 48 hours later and used in varying amounts to infect HCT116 cells. Infected cells were compared to untreated samples 48 hours later.

Cell sorting: Flow cytometry (MolBLO) was used to sort the GFP/PSM- and GFP/control-cotransfected cells collected in 1mL of medium. Glucose uptake assay was immediately performed on pellet cells.

Western blotting: Protein lysates from transfected or retrovirus infected cells were separated by SDS-PAGE. Proteins were then transferred to PVDF membrane and probed for both PSM-Rb and actin expression.

Glucose uptake assay: Glucose-starved transfected/infected cells were incubated with 14C-2-deoxy-glucose. Cells were washed, lysed with SDS, and intracellular glucose was measured via scintillation counting normalized to protein content.

Glycolysis assay: Transfected/infected cells were incubated with 3H-glucose. 3H2O release into the media was then assessed by scintillation counting after equilibration with dH2O in evaporation chambers for 48-72 hours.

Conclusion/Future Direction
These preliminary results suggest a lack of direct metabolic influence by PSM-Rb, but there remains a need for optimization for PSM-Rb expression. This includes protein analysis by Western blotting to assess the expression of PSM in the GFP-sorting cells. In addition, the GFP and PSM pcDNA should also be encapsulated in the same vector to ensure any cell that expresses GFP will also express PSM. Finally, all the metabolic assays should be repeated on each of the methods as each assay was performed once per method. Further evaluation of the results found during this program will hopefully produce an optimized method of transfecting PSM-Rb so the investigation of the effects of constitutively Rb can be elucidated.

Acknowledgements/Grant Support
R25 Program U11L CEP through James Graham Brown Cancer Center NHINCT (R25-CA144283).
Mechanisms by which rapamycin protects from liver damage caused by VC metabolites in mice.

Austin M. Krueger1, Anna L. Lang2, Brenna R. Kaelin3, and Juliane I. Beier1
1 Department of Pharmacology and Toxicology, University of Louisville, Louisville, KY 40202, USA

ABSTRACT

Background: Vinyl chloride (VC) is a persistent, carcinogenic, co-carcinogenic tumor initiator by a co-carcinogen, widely ordered gas at room temperature. The majority of the production of VC is used in the manufacturing of polyvinyl chloride (PVC), PVC is predominantly used in the production of a variety of plastic container materials. VC has been known to cause liver damage at high occupational doses, although low exposure to VC is not yet fully understood.

Methods: C57BL/6J mice were administrated chloroethene (CE), VC, metabolites, then rapamycin one hour following the CE administration. 24 hours following the CE exposure, the mice were administrated rapamycin (RAPA), an immunosuppressive. The mice were then sacrificed 4 or 24 hours following US administrations.

Results: It was found that the control mice were exhibit normal liver morphology and function. Those administrated with CE alone showed no liver pathology. The altered metabolic profile, US alone increased inflammatory damage. CE and US significantly enhanced inflammation, necrosis, death, and significant pathologies. The addition of rapamycin significantly dampened the inflammatory response of CE+US mice via mTOR inhibition and oxidative stress pathways.

Conclusion: This study highlights that the consumption of CE through US exposure contribute to liver injury. It was found that the presence of rapamycin may dampen the impact that CE+US exert on oxidative stress, inflammation, and cell death.

MATERIALS AND METHODS

Animals. Eight-week-old male (C57BL/6J) mice were administrated chloroethene (CE), VC metabolites, then rapamycin one hour following the CE administration. 24 hours following the CE exposure, the mice were administrated rapamycin (RAPA), an immunosuppressive. The mice were then sacrificed 4 hours following the US administration.

Immunoblot. Proteins were extracted from hepatic tissue. 10 µg of total protein was loaded onto SDS-polyacrylamide gels followed by electrophoresis and Western blotted onto PVDF membranes. Antibodies were used at the dilutions recommended by the suppliers. Immunoreactive coupled secondary antibodies and chemiluminescence detection reagents were from Pierce (Rockford, IL, USA). The bands were detected using the Chemi-Doc™ Typhoon™ (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Immunoblot data were analyzed using the GE Healthcare’s Image Studio software (version 6.0) and normalized to GAPDH. Densitometric analysis was performed with ImageJ (NIH, Bethesda, MD, USA). Statistics. Summary data represent means ± SEM (n = 6). ANOVA and Bonferroni’s post-hoc test was used to determine statistical significance among treatment groups, as appropriate. In vivo; p < 0.05 compared to vehicle, p < 0.05 compared to animals exposed LPS alone, p < 0.05 compared to animals exposed to CE + LPS.

RESULTS

Figure 1: Previous hypothesis. Increased activation of mTOR in V凯blastic cells. Autophagy has been shown to negatively regulate inflammatory autophagy. However, inhibition of mTORC1 restores autophagy and depleting rapamycin for proper regulation of inflammatory processes, including blunted inflammatory autophagy.

Figure 2: Serum transaminase levels. Mice were treated as described in Material and Methods. Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined in plasma samples collected 48 hours after CE and 24 hours after US administrations.

Neither CE nor LPS with rapamycin altered serum transaminase levels. US significantly increased both AST and ALT levels. CE further enhanced this effect. However, rapamycin significantly decreased these levels.

Figure 3: Effect of CE and rapamycin on liver health. Mice were treated and sacrificed as described in Material and Methods. Paraffin-embedded liver sections were stained with hematoxylin and eosin (H&E) and counterstained with Masson’s trichrome. A blinded investigator evaluated the tissue slides. Control samples show no signs of hepatic damage or inflammation. US increased necrosis and positive staining for both neutrophil activation and oxidative stress. CE further enhanced this damage. Rapamycin blunted cytokine activation and oxidative stress. Rapamycin blunted necrosis, inflammation, and increased mTOR positive staining.

Figure 4: Effect of rapamycin on mTOR activation and Akt inactivation in vivo. Mice were treated, hepatic samples were removed, and Western Blots were performed as described in Material and Methods.

FUNDING SUPPORT

This research was supported by National Cancer Institute grant R25 CA104488, M01 RR000055 (UNMC, UNMC Institutional Research Support Grant), and the Veterans Administration (SM, GVR).
Genotypic Analysis of Mammary Carcinoma Susceptibility 3 Nominated Gene Expression Levels in Rat Mammary Glands

Sarah McQuaide, Emily Duderstadt, and David J. Samuelson
Department of Biochemistry and Molecular Genetics, University of Louisville School of Medicine

Abstract

In previous studies, it has been concluded that mammary cancer susceptibility in rats is strongly influenced by the Mammary carcinoma susceptibility 3 quantitative trait locus (Mcs3 QTL). This was tested using the cancer susceptible Wistar Furth (WF) strain and the cancer resistant Copenhagen (Cop) strain in congenic studies. Exploration into this topic, using WF.Cop congenic strains, narrowed the Mcs3 QTL region, and provided framework for further genetic testing of genes that reside in the Mcs3 region. Mammary gland transcript levels of select Mcs3 nominated genes, Ilk, Pak1, Rsf1, and Il18bp, were measured in order to determine if there were differences between Cop and WF strains or environment. It was our hypothesis that there is significant difference in the expression of these genes between these two strains. Rat Ilk, Pak1, Rsf1, and Il18bp expression was measured using Taqman quantitative PCR. The Mcs3 nominated genes in this study were chosen because they have been shown to potentially have a role in breast cancer. Rat Rplp2 was used as an endogenous control gene. Rat Ilk, Rsf1, and Il18bp expression was not significantly different between Copenhagen or Wistar Furth strains or in those that received DMBA vs those that did not. Rat Pak1, however, had different expression depending on environmental exposure to DMBA. An effect of genotype on rat Pak1 was not detected. Another published study established a positive relationship between the expression of mouse Pak1 and mammary cancer susceptibility. The results of our study confirms this relationship, and suggests that endogenous expression of rat Pak1 is increased following exposure to DMBA, a mammary carcinogen; therefore, suggesting that environmental exposures may influence Pak1 expression phenotype more than genotype. These findings have important relevance to female breast cancer as they suggest environmental expression is important to Pak1 expression.

Introduction

• Wistar Furth (WF) rats demonstrate a mammary cancer susceptible phenotype while Copenhagen (Cop) demonstrates a resistant phenotype.
• Previously, our lab conducted WF.Cop congenic rat studies to narrow the Mammary cancer susceptibility 3 quantitative trait locus (Mcs3 QTL).
• Using information from our lab’s congenic studies, we selected four Mcs3-nominated candidate genes, rat Ilk, Pak1, Rsf1, and Il18bp, from 310 possible annotated genes to test for differences in expression between Cop and WF strains.

Hypothesis

• We hypothesize one or more Mcs3-nominated gene transcripts are differentially regulated between susceptible Wistar Furth and resistant Copenhagen rat strains.

Methods

We selected four Mcs3 nominated genes (rat Ilk, Pak1, Rsf1, and Il18bp) that have known associations with breast cancer.
• Ingual mammary tissue was harvested from Wistar Furth (WF) and Copenhagen (Cop) rat congenic strains (WF.Cop) with or without exposure to 7,12-Dimethylbenz[a]anthracene (DMBA).
• Tissues were homogenized in Tri-reagent for RNA extraction. RNA samples with 260/230 ratios less than 1.2 were not used.
• This left 46 samples from 46 rats (WF rats with DMBA = 12; WF rats without DMBA = 13; WF.Cop without DMBA = 10; WF.Cop with DMBA = 11).
• cDNA was made from the extracted RNA using SuperScript II, a reverse transcriptase, and re-suspended in DEPC water.
• 5’ FAM labeled Taqman probes for rat Ilk, Pak1, Rsf1, Il18bp, and Rplp2 were mixed with the diluted cDNA for qPCR. Each sample had three replicates.
• Absolute quantification was used to measure transcript levels.
• Standard curves were based on pooled cDNA from eight WF rats without DMBA.
• The average sample quantity was divided by its corresponding Rplp2 quantity and converted to log2.

Results

• Our results suggest that environmental exposure of DMBA had an effect on Pak1 expression, suggesting that expression phenotype is linked more to environmental exposure than to genotype.
• Pak1 was expressed more in rats that received DMBA.
• The results of this experiment suggest that there is no difference in expression of rat Ilk, Rsf1, and Il18bp regardless of treatment or strain.

Conclusion

• Other genes within the Mcs3 QTL should be explored to determine if they follow a similar pattern or if there is a genotypic effect on expression.
• Expression of Mcs3 nominated genes should also be tested in other rat tissues (i.e. ovaries).

Future Directions

• Funding by the R25-CA134283 grant from the National Cancer Institute and the Department of Biochemistry and Molecular Genetics, University of Louisville School of Medicine.
• Dr. Corey Watson for his assistance in statistical analysis.

Acknowledgments

Figure 1 depicts the congenic map of the Mcs3 QTL
Figure 2 is an ideogram of showing the location of known rat mammary carcinoma susceptibility QTLs.
Figure 3 is a map depicting human orthology in relation to the Mcs3 QTL.
Figures 4 and 5 depict results of expression vs. treatment for rat Rsf1 and Pak1.
Figures 7 - 10 are box and whisker plots for each gene with results by congenic strain and environmental exposure plotted.

Figure 6: P-values from 2-way ANOVA. Note that ps 0.05 for rat Pak1.
ABSTRACT

Kupffer cells are specialized macrophages located in the liver and constitute the largest resident mononuclear phagocyte population of the body. They are critical for the clearance of bacteria, viruses, and damaged cells. In our study, a group has been generated in which the Kupffer cell population is targeted for the expression of a membrane-inducible reporter. This allows for the non-invasive visualization of Kupffer cells in a live animal model, which may enable pulmonary inflammation? Such an assay will allow for the evaluation of new or existing drugs that target Kupffer cells.

Materials and Methods: Generation of Transgenic Mice: Male mice were housed in a 12:12 hour light:dark cycle in a temperature- and humidity-controlled environment. All experiments were performed in accordance with the animal care guidelines. CD31 mice were crossed with a Cre recombinase-driven reporter strain to achieve a CD31-GFP reporter line. The resulting CD31-GFP mice were then bred with mice expressing a membrane-inducible reporter cassette (mGpCA) to generate the CD31-GFP/mGpCA line. The membrane-inducible reporter cassette is a fusion of the membrane-inducible promoter (PIK3CA) and a fluorescent protein reporter gene (GFP).

Intrasplenic Tamoxifen-Loaded Nanoparticles: The mice were injected with tamoxifen-loaded nanoparticles (n.p.) at a dose of 0.75 mg n.p. or 1.5 mg n.p. via intrasplenic injection. The nanoparticles were prepared by mixing tamoxifen with a mixture of lipids and polymers and then injecting into the liver via intrasplenic injection.

Flow Cytometry: The mice were sacrificed 7 days after the injection of tamoxifen-loaded nanoparticles. The liver was removed and frozen in liquid nitrogen. The liver tissue was then homogenized and stained with anti-CD31 and anti-GFP antibodies. The samples were analyzed on a FlowJo flow cytometer.

RESULTS

The mice injected with tamoxifen-loaded nanoparticles showed a significant increase in the number of GFP-positive cells in the liver compared to control mice. The membrane-inducible reporter was activated in the Kupffer cells, allowing for the visualization of the Kupffer cell population in real-time.

CONCLUSIONS

The membrane-inducible reporter cassette allows for the non-invasive visualization of Kupffer cells in a live animal model. This will enable the evaluation of new or existing drugs that target Kupffer cells.

MATERIALS AND METHODS

Generation of Transgenic Mice: Male mice were housed in a 12:12 hour light:dark cycle in a temperature- and humidity-controlled environment. All experiments were performed in accordance with the animal care guidelines. CD31 mice were crossed with a Cre recombinase-driven reporter strain to achieve a CD31-GFP reporter line. The resulting CD31-GFP mice were then bred with mice expressing a membrane-inducible reporter cassette (mGpCA) to generate the CD31-GFP/mGpCA line. The membrane-inducible reporter cassette is a fusion of the membrane-inducible promoter (PIK3CA) and a fluorescent protein reporter gene (GFP).

Intrasplenic Tamoxifen-Loaded Nanoparticles: The mice were injected with tamoxifen-loaded nanoparticles (n.p.) at a dose of 0.75 mg n.p. or 1.5 mg n.p. via intrasplenic injection. The nanoparticles were prepared by mixing tamoxifen with a mixture of lipids and polymers and then injecting into the liver via intrasplenic injection.

Flow Cytometry: The mice were sacrificed 7 days after the injection of tamoxifen-loaded nanoparticles. The liver was removed and frozen in liquid nitrogen. The liver tissue was then homogenized and stained with anti-CD31 and anti-GFP antibodies. The samples were analyzed on a FlowJo flow cytometer.

RESULTS

The mice injected with tamoxifen-loaded nanoparticles showed a significant increase in the number of GFP-positive cells in the liver compared to control mice. The membrane-inducible reporter was activated in the Kupffer cells, allowing for the visualization of the Kupffer cell population in real-time.

CONCLUSIONS

The membrane-inducible reporter cassette allows for the non-invasive visualization of Kupffer cells in a live animal model. This will enable the evaluation of new or existing drugs that target Kupffer cells.
**Introduction**

- Cisplatin is a potent chemotherapeutic used to treat a multitude of solid cancers.
- The dose-limiting side effect of this drug is nephrotoxicity, causing acute kidney injury (AKI) in 30% of adult patients.
- Patients with cisplatin-induced AKI are more likely to develop end stage renal diseases, particularly chronic kidney disease (CKD), which is marked by the development of fibrosis.
- Currently, there are no therapeutic interventions for cisplatin-induced kidney injury, which may be due to limitations in the current mouse model used to study this type of injury.

**Hypothesis**

- C57BL/6J mice, a strain commonly used in renal studies that is resistant to some forms of fibrosis, will require a higher dose of cisplatin in order to develop interstitial fibrosis that occurs with repeated dosing of cisplatin.

**Methods**

- Five male C57BL/6J mice were treated with vehicle saline once a week for four weeks and ten male C57BL/6J mice (8 weeks old) were treated with 9 mg/kg of cisplatin once a week for four weeks; both groups were sacrificed three days after the last injection. The same protocol was conducted with FVB/n mice but treated with 7 mg/kg of cisplatin.
- G4TPCR, IHC, and Western blot analysis were utilized to determine the presence of fibrosis in these mice, as well as compare fibrotic and inflammatory markers to FVB/n mice treated with cisplatin.

**Rationale for this Study**

- C57BL/6J mice is a strain frequently used in renal studies.
- This strain is resistant to some forms of renal fibrosis, such as glomerular fibrosis.

**Results**

**Clinical Impact**

- Human patients diagnosed with cancer are administered low doses of cisplatin over an extended period of time in order to limit nephrotoxicity while maintaining therapeutic efficacy.
- Genetic factors may play a role in human fibrosis. By studying different strains of mice, we will better understand whether genetic susceptibility plays a role in fibrogenesis.
- This information could be translated to the differential effects of cisplatin in humans and susceptibility to drug toxicities in order to determine a patient’s optimal administration and dosage.

**Conclusions**

- C57BL/6J mice are susceptible to developing renal fibrosis when treated with 9 mg/kg cisplatin once a week for 4 weeks.
- Strain differences indicate that C57BL/6J mice need a higher dose of cisplatin in order to develop fibrosis in comparison to FVB/n mice.

**Future Directions**

- Current studies are underway for FVB/n mice treated with 9 mg/kg cisplatin once a week for 4 weeks.
- Repeat the repeated dosing regimen in other strains of mice in order to fully study genetic variability.

**Acknowledgments**

- This research was supported by the NIH: National Cancer Institute Grant R25-CA134283.

**References**