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TITLE: The Development of a Macromolecular Dexamethasone Prodrug for the Treatment of Focal Segmental Glomerulosclerosis (FSGS)

PRINCIPAL INVESTIGATOR: Dong Wang

CONTRACTING ORGANIZATION: University of Nebraska Medical Center

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14. ABSTRACT

The overall purpose of the project is to develop a nephrotropic polyethylene glycol (PEG)-based dexamethasone (Dex) prodrug (PEG-Dex or ZSJ-0228) as an effective and safe therapy for focal segmental glomerulosclerosis (FSGS). During this funding period, we have identified the reason for the Adriamycin dose variation in FSGS mouse model induction as reported in the literature and have established a robust model establishment protocol. Using the Adriamycin-induced Balb/C mouse model of FSGS, we have confirmed the superior therapeutic efficacy and safety of a single dose i.v. administered PEG-Dex than dose equivalent Dex. Healthy mice and FSGS mice administered with Saline were used as the controls. Specifically, PEG-Dex treatment is significantly more effective than Dex in reducing proteinuria, ameliorating FSGS lesions, and restoring kidney functions. Multiple side effects, including abnormalities in body weight, blood glucose, liver functions, adrenal gland weight and lymphocyte counts were observed in Dex group, but not in PEG-Dex group. We have also synthesized PEG-Dex labeled with near-infrared dye IRDye 800CW and Alexa Fluor 647 for the studies planned for next year.

15. SUBJECT TERMS
FSGS, Dexamethasone, PEG, Prodrug, Micelle, Nanomedicine

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1. **INTRODUCTION:**

FSGS is a disease in which scar tissue develops on glomeruli, the tiny filtering units inside the kidney where blood is cleaned. FSGS is a serious condition that can lead to kidney failure, for which the only treatment options are dialysis or kidney transplant. Currently, there is no FDA approved drug to treat FSGS. Clinically, glucocorticoids (GCs) are widely used to control the disease progression. The long-term use of GCs, however, can cause multiple severe adverse effects, such as osteopenia, weight gain, diabetes, high blood pressure, and increased risk of infection. Therefore, there is an urgent unmet clinical need for effective GC formulations with reduced systemic toxicity. In this application, we propose to develop and optimize a novel micelle-forming polyethylene glycol (PEG)-based dexamethasone (Dex, a potent glucocorticoid) prodrug nanomedicine (ZSJ-0228 or PEG-Dex) that has been uniquely engineered to specifically target Dex to the kidney. This novel design will lead to the development of a safer, and more effective GC therapy for FSGS, while avoiding GC-associated adverse side effects.

2. **KEYWORDS:**

FSGS, Dexamethasone, PEG, Prodrug, Micelle, Nanomedicine

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

Specific Aim 1: To assess the therapeutic efficacy and safety of ZSJ-0228 in the Adriamycin-induced FSGS mouse model	**Time line**	**%completed**
Major Task 1: Assess the therapeutic efficacy and safety of ZSJ-0228 in the Adriamycin-induced FSGS mouse model	Mon	
Subtask 1: Prepare and submit documents for ACURO approvals, including all the animal studies involved in the project	1-4	100%
Milestone(s) Achieved: Obtain ACURO approval	4	100%
Subtask 2: Assess the therapeutic efficacy of ZSJ-0228 treatment in mice (BALB/c, Jackson Laboratory) [15 mice per group × 4 groups = 60 mice total]	4-9	100%
Subtask 3: Evaluate GC-associated side effects using same batch of the mice in task 1.2	7-10	100%
Milestone(s) Achieved: Confirmation of ZSJ-0228's efficacy and safety in treating Adriamycin-induced FSGS mice	10	100%
Specific Aim 2: To investigate the working mechanism of ZSJ-0228		
Major Task 2: Investigation of ZSJ-0228's working mechanism		
Subtask 1: Preparation of fluorescence-labeled ZSJ-0228	11-12	100%
Subtask 2: Determination of *in vivo* biodistribution of ZSJ-0228 by in mice (BALB/c, Jackson Laboratory) [10 mice per group × 5 groups = 50 mice total]	13-15	
Subtask 3: Explore PK/BD parameters of the Dex released from ZSJ-0228	16-20	
Subtask 4: Assessment of ZSJ-0228's cellular sequestration and transcriptomic mechanisms of action (BALA/c mice, Jackson Laboratory) [5 mice per group × 10 groups = 50 mice total]	21-26	
Milestone(s) Achieved: Fully understand ZSJ-0228's working mechanism	26	

Specific Aim 3: To evaluate the long-term efficacy and safety of the optimized ZSJ-0228		
Major Task 3: Optimization of ZSJ-0228's structure		
Subtask 1: Design and synthesize nine different ZSJ-0228 structures	27-32	
Subtask 2: Screen the above ZSJ-0228 candidates	33-36	
Milestone(s) Achieved: Obtain optimized ZSJ-0228	36	
Major Task 4: Evaluation of the long-term efficacy and safety of the optimized ZSJ-0228		
Subtask 1: Assess the long-term therapeutic efficacy of optimized ZSJ-0228 in PAN-induced FSGS rats (Sprague-Dawley, Taconic Biosciences) [8 mice per group × 8 groups = 64 rats total]	37-45	
Subtask 2: Assess the long-term safety of optimized ZSJ-0228	46-48	
Milestone(s) Achieved: Better understanding of the long-term efficacy and safety of optimized ZSJ-0228 in treating PAN-induced FSGS rat model	48	

What was accomplished under these goals?

- Prepare and submit documents for ACURO approvals, including all the animal studies involved in the whole project. Months 1-4.

  The animal protocols have been approved by ACURO.

- Assess the therapeutic efficacy of ZSJ-0228 treatment in mice (BALB/c, Jackson Laboratory) Months 4-9.

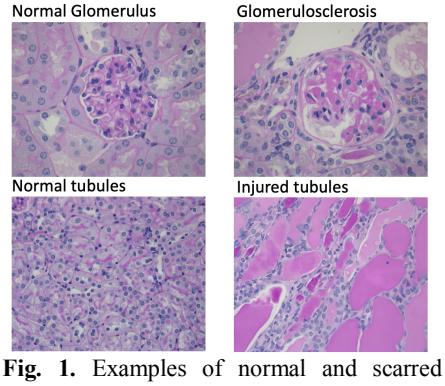

Fig. 1. Examples of normal and scarred glomerulus; normal and injured tubules

Balb/c mice (10-weeks old, Jackson laboratory) were acclimated for 1 week prior to the administration of Adriamycin (10.5 mg/kg, tail vein injection). The mice were monitored for urine protein levels using Albustix strips (SIEMENS). At 1-week post induction, the mice were randomized into three groups (15 mice/group), including PEG-Dex (Dex equivalent = 28 mg/kg, monthly i.v.), dexamethasone sodium phosphate (Dex equivalent = 1 mg/kg/day, daily i.p.), and Saline control (monthly i.v.), respectively. An additional 15 mice without Adriamycin induction were be used as the Healthy control. Renal disease progression (proteinuria) was monitored weekly after the Adriamycin induction and throughout the entire treatment course. At the end of week 6, the mice were euthanized with all major organs/tissues (including blood) isolated for organ weight, gross assessment, and histological analysis. The kidney samples were scored histologically by a nephropathologist blinded to the experimental design for percentage of injured glomeruli and tubulointerstitial injury. Examples of normal vs glomerulosclerosis and tubular injury are shown in **Fig.1**. Blood glucose levels were measured using OneTouch Blood Glucose Meter (LifeScan, Malvern) monthly to evaluate the impact of different treatments on glucose metabolism. Lymphocyte counts was assessed using VetScan HM5 Hematology Analyzer. Blood chemistries were

analyzed using the VetScan VS2 chemistry analyzer with comprehensive diagnosis rotors.

At the end point of the study, 11 out of 13 mice (84.6%) in the Dex group and 10 out of 13 (76.9%) mice in Saline group had developed nephritis, characterized by a proteinuria reading ≥2. Two mice from each of these two groups had to be euthanized prematurely due to FSGS symptom-associated animal welfare concerns (lose of body weight > 25%). In contrast, only 7 out of 15 mice (46.7 %) treated with PEG-Dex developed nephritis. The average proteinuria levels of the PEG-Dex group were significantly lower than that of the Dex and the Saline groups (1.46 vs. 2.27 and 2.31, $P < 0.05$). Histological analysis of kidney sections revealed that the glomerulonephritis percentage for Dex and Saline groups were both significantly higher than that of the Healthy control (1.4 and 6.3 vs. 0%, $P < 0.05$), while the PEG-Dex-treated mice (0.86%) was not statistically different from the Healthy group. According to the blood chemistry analysis, Dex-treated FSGS mice showed significantly higher levels of total protein level (51.62 vs. 47.4 g/L, $P < 0.001$) than the Healthy group. No significant difference was detected between PEG-Dex group and Healthy control (**Fig.2**).

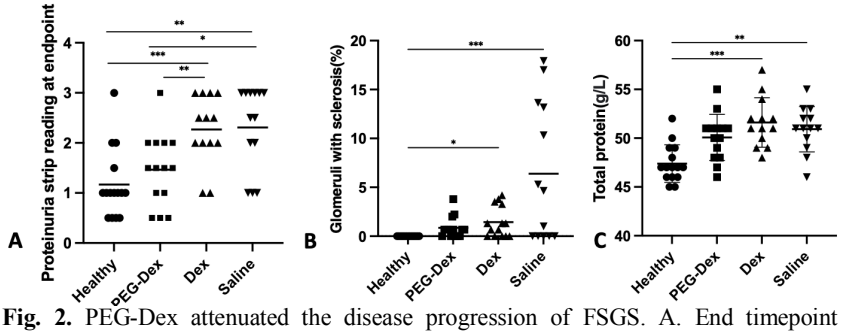

Fig. 2. PEG-Dex attenuated the disease progression of FSGS. A. End timepoint proteinuria comparison among different groups B. Percentage of scarred glomeruli versus total glomeruli counts. C. Level of total protein Data are presented as mean ± SEM. Mann-Whitney *U* test, *$P < 0.05$, ***$P < 0.001$, ****$P < 0.0001$.

- Evaluate GC-associated side effects using same batch of the mice in task 1.2 Months 7-10.

For safety assessment, it was found that the Dex-treated mice had a significantly lower body weight than that the Saline, PEG-Dex and Healthy groups (21 vs. 26, 25 and 27 g, $P < 0.05$). While Dex-treated mice showed significantly lower blood glucose levels than Healthy ($P < 0.05$) and Saline group (99 vs. 125 and 166 mg/dL, $P < 0.0001$), PEG-Dex-treated mice (121 mg/dL) showed no significant difference in blood glucose with the Healthy control. Dex-treated mice showed significantly higher ALT than the Healthy, PEG-Dex and Saline groups (89 vs. 40, 49 and 41 U/L,

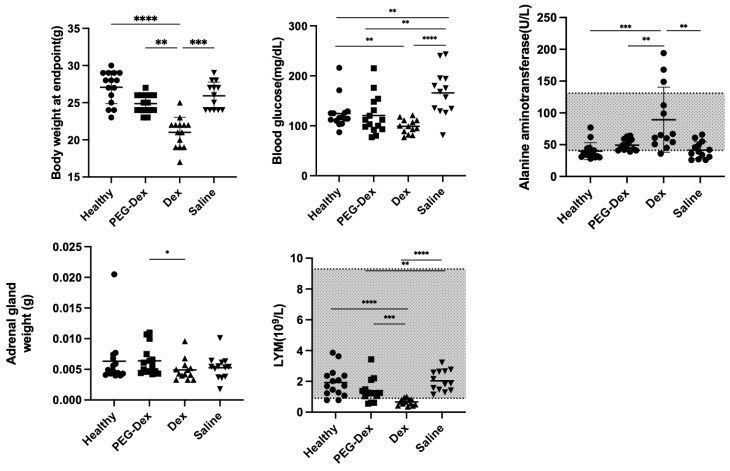

Fig. 3. Assessment of GC-associated toxicities induced by different treatments. A. End timepoint body weight comparison among different groups B. Blood glucose level. C. Level of alanine aminotransferase. D. Adrenal gland weight. E. Lymphocytes counts. Data are presented as mean ± SEM. Mann-Whitney *U* test, *$P < 0.05$, ***$P < 0.001$, ****$P < 0.0001$.

$P < 0.05$). Its serum calcium level was also significantly higher than Healthy control (2.564 vs. 2.440 mmol/L, $P < 0.05$). Both Dex and PEG-Dex-treated mice showed significantly higher serum sodium levels than Healthy (148 and 146.9 vs. 144 mmol/L, $P < 0.05$). Lymphocyte counts in Dex group was significantly lower than that of the PEG-Dex, Healthy and Saline groups (0.6592×10^9 vs. 1.389×10^9, 1.925×10^9 and 2.039×10^9/L, $P < 0.001$). Additionally, the adrenal gland weight in the Dex-treated mice was significantly lower than that of the PEG-Dex-treated mice (0.0049 vs. 0.0064 g, $P < 0.05$). No difference was observed in adrenal gland weight among PEG-Dex, Saline and Healthy groups **(Fig.3)**.

In conclusion, using an Adriamycin-induced FSGS mouse model, we have demonstrated the potent and superior therapeutic efficacy of PEG-Dex in reducing proteinuria levels, ameliorating FSGS lesions, and restoring kidney functions than the Dex treatment. In addition, PEG-Dex treatment also showed a much-improved safety profile than Dex with minimal adverse event detected. Collectively, these data suggest that PEG-Dex may be established as a promising drug candidate for more effective and safe treatment of FSGS.

- Preparation of fluorescence-labeled ZSJ-0228 Preparation of fluorescence-labeled ZSJ-0228. Months 11-12.

Fmoc-NH-PEG-COOH (150 mg, 0.075 mmol) and DCC (154 mg, 0.75 mmol) were dissolved in anhydrous DCM at 0 °C. The solution was stirred for 10min and then Dex dimer (378 mg, 0.3 mmol) and HOBt (153 mg, 1 mmol) were added. The solution was stirred at room temperature for 16 h. The mixture was separated by LH-20 to remove the small molecules. The product solution was collected, and the solvent was removed to get the crude product. The crude product was further purified by preparative HPLC to give 105 mg of Fmoc-NH-PEG-Dex-TBS.

The Fmoc-PEG-Dex was then dissolved in THF (3 mL). TBAF (0.2 mL, 1 M) was added and stirred for 2 h, then piperidine (0.5 mL) was added. The solution was stirred at 21 °C for 2 h, the solution was purified by LH-20 to give NH_2-PEG-Dex (80 mg).

NH_2-PEG-Dex (40 mg) was dissolved in DMF (2 mL), IRDye800-NHS (0.5 mg) and triethylamine (50 mg) were added. The solution was stirred overnight and then purified with LH-20. After lyophilization, IRDye800-labeled PEG-Dex was obtained (38 mg).

NH2-PEG-Dex (40 mg) was dissolved in DMF, Alexa 647-NHS (0.5mg) and triethylamine (50 mg) were added. The solution was stirred overnight and then purified with LH-20. After lyophilization, IRDye800-labeled PEG-Dex was obtained (37 mg).

ACURO report

Protocol (1 of 2 total)
Protocol: 18-138-01-FC
Target required for statistical significance: 60
Target approved for statistical significance: 60
Total subjects to date: 60
Approval date: 09/29/2022

What opportunities for training and professional development have the project provided?

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

During the next funding period, we will focus on experiments proposed in Task 2. Using fluorescence-labeled PEG-Dex, we will determine *in vivo* biodistribution of ZSJ-0228 by in mice and explore PK/BD parameters of the Dex released from ZSJ-0228. We will also start assessment of ZSJ-0228's cellular sequestration and transcriptomic mechanisms of action.

4. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project?

Without proper management, FSGS may progress to end-stage kidney disease (ESKD), at which point patients require dialysis or kidney transplantation. It has been estimated that approximately 30-40% of FSGS patients will receive kidney transplants. In the United States, around 90,000 people will die annually due to kidney disease, which is more than breast and prostate cancers combined. Furthermore, veterans have approximately 34% higher chronic kidney disease (CKD) prevalence than the general population, which has been attributed to significant pre-existing comorbidities and higher mean age in this group. Thus, more research is urgently needed to develop more effective therapies for FSGS to halt progression of the kidney disease. Currently, there is no FDA approved medication for FSGS. While glucocorticoids are widely used in the clinical management of FSGS, they are associated with severe adverse side effects. In this project, the investigation of PEG-Dex or ZSJ-0228 that is highly effective in ameliorating FSGS pathology but is devoid of the typical adverse side effects associated with glucocorticoids, could provide a new direction of designing novel glucocorticoid prodrug for this disease, utilizing passive targeting ELVIS mechanism (Extravasation of the polymeric prodrugs through Leaky Vasculature and its' subsequent Inflammatory cell-mediated Sequestration).

Animal models are essential parts for the development of new drug. Genetic engineering in animal models reveal the possibility of studying functions of many novel genes and proteins. However, so far, mutations can be identified only in a minority of patients with FSGS. Renal ablation model is the first animal model for FSGS, but it is more acute and drastic than human disease. Comparing pro and cons of all animal models, Adriamycin-induced animal model remains to be the most widely used one. However, dose of induction agent and optimal duration of the animal study were not consistent in the current literature. Also details about animal model establishment were not reported. Therefore, we investigated into these details very carefully before we officially start our efficacy study. Through titration of the induction agent dose depending on the doses previously reported, we confirmed that the optimal Adriamycin dose is 10.5 mg/kg. We also found the instability of Adriamycin in solution may have contributed to the wide range of Adriamycin induction dose in the literature, which can be misleading to scientists in the field of study. We also thoroughly examined the kidney

histopathology development time course of this FSGS mouse model to identify the optimal window for therapeutic evaluation. We plan to publish this detailed characterization and description of FSGS mouse model establishment. We believe will benefit all who are interested in studying FSGS.

What was the impact on other disciplines?
Nothing to Report

What was the impact on technology transfer?
Nothing to Report

What was the impact on society beyond science and technology?
Nothing to Report

5. **CHANGES/PROBLEMS:**
Nothing to Report

6. **PRODUCTS:**
October 2022 The Globalization of Pharmaceutics Education Network
- Oral presentation *Dose Optimization and Time-Course Histology Study for Adriamycin-induced FSGS mouse model*

August 2023 Military Health System Research Symposium (abstract submitted)
- Oral presentation *A Dexamethasone Prodrug Effectively Attenuated Symptoms of Focal Segmental Glomerulosclerosis (FSGS) in an Adriamycin-induced Mouse Model with Minimal Adverse Effects*

July 2023 Controlled release society annual meeting (abstract accepted)
- Oral presentation *Polymeric Dexamethasone Prodrug Attenuates Adriamycin-induced Focal Segmental Glomerulosclerosis without Apparent Glucocorticoid Side Effects*

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Dong Wang
Project Role:	Principle investigator
Person month worked:	3 calendar months
Contribution to Project:	Dr. Wang is responsible for overseeing the entire project, especially the synthesis and characterization of the various PEG copolymers, and PEG copolymer-drug conjugates used in the study and the animal experiment.
Funding Support:	N/A

Name:	Troy J. Plumb
Project Role:	Co-Investigator
Person month worked:	0.6 calendar month
Contribution to Project:	Dr. Plumb works closely with Dr. Wang and provide the team with guidance, clinical insight and understanding of

	pathophysiology of FSGS. He meets with Dr. Wang regularly to review, analyze, and interpret the data generated. He also supports the team's effort in preparing reports, writing manuscripts and presenting the research progress at scientific meetings.
Funding Support:	N/A
Name:	Kirk W. Foster
Project Role:	Co-investigator
Person month worked:	0.6 calendar month
Contribution to Project:	Dr. Foster works closely with Dr. Wang and his team to provide histological analysis for all the kidney tissue samples. In addition, he also contributes to the preparation of annual reports and other scientific presentations.
Funding Support:	N/A
Name:	Fang Yu
Project Role:	Biostatistician
Person month worked:	0.6 calendar month
Contribution to Project:	She works closely with all the team members in providing biostatistics support for experimental design and data analyses of the entire project.
Funding Support:	N/A
Name:	Zhenshan Jia
Project Role:	Researcher
Person month worked:	1.5 calendar months
Contribution to Project:	He focuses his effort on the design, synthesis, purification, characterization, and formulation of all the proposed ZSJ-0228 analogues. He meets with Dr. Wang weekly to discuss the progress and the compounds synthesized.
Funding Support:	N/A
Name:	Shahnaz Rahimi
Project Role:	Postdoc with Dr. Wang
Person month worked:	2.75 calendar months
Contribution to Project:	She cooperates with Dr. Jia for the design, synthesis, purification, characterization, and formulation of all the proposed ZSJ-0228 analogues. She is responsible for synthesis of fluorescence dye labeled ZSJ-0228.
Funding Support:	N/A
Name:	Xin Wei
Project Role:	Researcher
Person month worked:	2 calendar months
Contribution to Project:	Under the guidance/direction of Dr. Wang and in collaboration with other team members, she contributed to

	the *in vivo* treatment efficacy and safety study of ZSJ-0228 in the Adriamycin-induced FSGS mouse model.
Funding Support:	N/A

Name:	Haochen Jiang
Project Role:	Graduate student
Person month worked:	3 calendar months
Contribution to Project:	Ms. Jiang contributed the assessment of the therapeutic efficacy and safety of ZSJ-0228 in the Adriamycin-induced FSGS mouse model.
Funding Support:	N/A

Name:	Xiaoke Xu
Project Role:	Graduate student
Person month worked:	2 calendar months
Contribution to Project:	She supported Haochen Jiang and Dr. Wei in the FSGS animal experiments and contributed to the ZSJ-0228 synthesis.
Funding Support:	N/A

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
This is our first reporting. Since we received the award, Dr. Dong Wang has received the following grants from NIH:

- NIAMS/NIH 1R01 AR080500-01. PI: Dong Wang. “Nonaddictive opioid prodrug nanomedicine for musculoskeletal pain”. 08/01/2022 - 07/31/2027.
- NIAMS/NIH 1R01 AR082148-01. PI: Dong Wang. “Effective local delivery of bone anabolic agent to accelerate the healing of delayed fracture union”. 01/20/2023 - 12/31/2027

There are in no conflict with the DoD award and would not affect Dr. Wang’s effect committed to the FSGS project.
No other senior/key personnel have reported any change to their individual Other Support.

What other organizations were involved as partners?
Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS: None

9. APPENDICES:

1. Abstract for 2022 The Globalization of Pharmaceutics Education Network Conference (oral presentation).

Title	Dosage Escalation and Time-Course Histology Study for Adriamycin-induced FSGS animal model

Keywords (up to 5)	Focal segmental glomerulosclerosis, FSGS, Adriamycin, Histology, Mouse model
Authors	Haochen JIANG[1], Zhifeng ZHAO[1], Kirk FOSTER[2], Xin WEI[1], Zhenshan JIA[1], Dong WANG[1] *[1]Department of Pharmaceutical Sciences and [2]Department of Pathology and Microbiology, University of Nebraska Medical Center, 986125 Nebraska Medical Center, PDD 3020, Omaha, Nebraska 68198-6125, United States*
Abstract	Focal segmental glomerulosclerosis (FSGS) is a rare chronic renal injury marked proteinuria and podocyte injury with glomerulus scarring and tubulointerstitial fibrosis.[1] Glucocorticoids (GCs) are the first line treatment for FSGS, but their long-term utility and therapeutic effects are limited by the significant toxicities associated with GCs. Therefore, significant efforts have been invested to develop novel therapeutic agents for FSGS.[2] Animal models are essential to the new drug development process. Adriamycin (ADR)-induced mouse model is the most widely used animal model for FSGS. However, different ADR doses have been used in FSGS model induction according to literature, ranging from 10-17 mg/kg, with the duration of the model being chosen from 3 - 6 weeks after ADR injection. [4-7] Through a series of *in vitro* experiments, we have found that the stability of ADR is poor, especially after its dissolution in aqueous solution. Therefore, the ADR solution to be used for FSGS induction must be freshly prepared and be used immediately. This observation may partially explain the diverse ADR doses used in the literature. To establish a reproducible ADR-induced FSGS mouse model, we proposed to perform a ADR dosage escalation experiment. Through this study, we found that 10.5mg/kg ADR is the most suitable dosing level for the FSGS model establishment, with low mortality and sustained proteinuria. Furthermore, we have investigated the changes kidney histology at different time points post ADR-induction, which include glomerulosclerosis, tubulointerstitial injury and podocyte effacement over a 8-week duration. This data provides an in-depth understanding of the ADR-induced FSGS animal model and will help to establish a firm foundation for our FSGS drug development program.
References	1. Fogo AB. Causes and pathogenesis of focal segmental glomerulosclerosis. Nat Rev Nephrol. 2015 Feb;11(2):76-87. doi: 10.1038/nrneph.2014.216. Epub 2014 Dec 2. PMID: 25447132; PMCID: PMC4772430. 2. Ponticelli C, Locatelli F. Glucocorticoids in the Treatment of Glomerular Diseases: Pitfalls and Pearls. Clin J Am Soc Nephrol. 2018 May 7;13(5):815-822. doi: 10.2215/CJN.12991117. Epub 2018 Feb 23. PMID: 29475991; PMCID: PMC5969489. 3. Yang JW, Dettmar AK, Kronbichler A, Gee HY, Saleem M, Kim SH, Shin JI. Recent advances of animal model of focal segmental glomerulosclerosis. Clin Exp Nephrol. 2018 Aug;22(4):752-763. doi: 10.1007/s10157-018-1552-8. Epub 2018 Mar 20. PMID: 29556761. 4. Liu G, Shi Y, Peng X, Liu H, Peng Y, He L. Astaxanthin attenuates adriamycin-induced focal segmental glomerulosclerosis. Pharmacology. 2015;95(3-4):193-200. doi: 10.1159/000381314. Epub 2015 Apr 22. PMID: 25924598. 5. Vielhauer V, Berning E, Eis V, Kretzler M, Segerer S, Strutz F, Horuk R, Gröne HJ, Schlöndorff D, Anders HJ. CCR1 blockade reduces interstitial inflammation and fibrosis in mice with glomerulosclerosis and nephrotic syndrome. Kidney Int. 2004 Dec;66(6):2264-78. doi: 10.1111/j.1523-1755.2004.66038.x. PMID: 15569315. 6. Zhuang Q, Li F, Liu J, Wang H, Tian Y, Zhang Z, Wang F, Zhao Z, Chen J, Wu H. Nuclear exclusion of YAP exacerbates podocyte apoptosis and disease progression in Adriamycin-induced focal segmental glomerulosclerosis. Lab Invest. 2021 Feb;101(2):258-270. doi: 10.1038/s41374-020-00503-3. Epub 2020 Nov 17. PMID: 33203894; PMCID: PMC7815513. 7. Li L, Zhang T, Diao W, Jin F, Shi L, Meng J, Liu H, Zhang J, Zeng CH, Zhang MC, Liang S, Liu Y, Zhang CY, Liu Z, Zen K. Role of Myeloid-Derived Suppressor Cells in Glucocorticoid-

	Mediated Amelioration of FSGS. J Am Soc Nephrol. 2015 Sep;26(9):2183-97. doi: 10.1681/ASN.2014050468. Epub 2015 Jan 7. PMID: 25568177; PMCID: PMC4552109.

2. Abstract of oral presentation for 2023 Military Health System Research Symposium (Submitted)

Abstract ID: MHSRS-23-09025

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Abstract Details:

Breakout Session: Polytrauma and Multi-Organ Dysfunction
Submission Category: Oral Presentation

Title: A Dexamethasone Prodrug Effectively Attenuated Symptoms of Focal Segmental Glomerulosclerosis (FSGS) in an Adriamycin-induced Mouse Model with Minimal Adverse Effects

Abstract:

Introduction
Focal segmental glomerulosclerosis (FSGS) is chronic renal injury characterized by proteinuria and podocyte injury with glomerulus scarring and tubulointerstitial fibrosis. Glucocorticoids (GCs) are the current first-line treatment. Long-term use of GCs, however, is associated with numerous off-target adverse effects. Therefore, there is an urgent unmet clinical need for novel FSGS therapies. Recognizing GCs' potent efficacy in managing FSGS, we have developed a polyethylene glycol (PEG)-based nephrotropic dexamethasone (Dex) prodrug (ZSJ-0228 or PEG-Dex) to mitigate the side effects of GCs. Previously, PEG-Dex has demonstrated potent therapeutic efficacy and exceptional safety in lupus nephritis mice. The focus of the present study is to assess its therapeutic efficacy and safety in an Adriamycin-induced BALB/c mouse model of FSGS.

Materials and Methods
Balb/c mice (10-weeks old, Jackson laboratory) were acclimated for 1 week prior to the administration of Adriamycin (10.5 mg/kg, tail vein injection). The mice were monitored for urine protein levels using Albustix strips (SIEMENS). At 1-week post induction, the mice were randomized into three groups (15 mice/group), including PEG-Dex (Dex equivalent = 28 mg/kg, monthly i.v.), dexamethasone sodium phosphate (Dex equivalent = 1 mg/kg/day, daily i.p.), and Saline control (monthly i.v.), respectively. An additional 15 mice without Adriamycin induction were be used as the Healthy control. Renal disease progression (proteinuria) was monitored weekly after the Adriamycin induction and throughout the entire treatment course. At the end of week 6, the mice were euthanized with all major organs/tissues (including blood) isolated for organ weight, gross assessment and histological analyses. The kidney samples were scored histologically by a nephropathologist blinded to the experimental design for percentage of injured glomeruli and tubulointerstitial injury. Blood glucose levels were measured using OneTouch Blood Glucose Meter (LifeScan, Malvern) monthly to evaluate the impact of different treatments on glucose metabolism. Lymphocyte counts was assessed using VetScan HM5 Hematology Analyzer. Blood chemistries were analyzed using the VetScan VS2 chemistry analyzer with comprehensive diagnosis rotors.

Results
At the end point of the study, 11 out of 13 mice (84.6%) in the Dex group and 10 out of 13 (76.9%) mice in Saline group had developed nephritis, characterized by a proteinuria reading ≥2. Two mice from each of these two groups had to be euthanized prematurely due to FSGS symptom-associated animal welfare concerns (lose of body weight > 25%). In contrast, only 7 out of 15 mice (46.7 %) treated with PEG-Dex developed nephritis. The average proteinuria levels of the PEG-Dex group were significantly lower than that of the Dex and the Saline groups (1.46 vs. 2.27 and 2.31, $P < 0.05$). Histological analysis of kidney sections revealed that the glomerulonephritis percentage for Dex and Saline groups were both significantly higher than that of the Healthy control (1.4 and 6.3 vs. 0%, $P < 0.05$), while the PEG-Dex-treated mice (0.86%) was not statistically different from the Healthy group. According to the blood chemistry analysis, Dex-treated FSGS mice showed significantly higher levels of total protein level (51.62 vs. 47.4 g/L, $P < 0.001$) than the Healthy group. No significant difference was detected between PEG-Dex group and Healthy control.
For safety assessment, it was found that the Dex-treated mice had a significantly lower body weight than that the Saline, PEG-Dex and Healthy groups (21 vs. 26, 25 and 27 g, $P < 0.05$). While Dex-treated mice showed significantly lower blood glucose levels than Healthy ($P < 0.05$) and Saline group (99 vs. 125 and 166 mg/dL, $P < 0.0001$), PEG-Dex-treated mice (121 mg/dL) showed no significant difference in blood glucose with the Healthy control. Dex-treated mice showed significantly higher ALT than the Healthy, PEG-Dex and Saline groups (89 vs. 40, 49 and 41 U/L, $P < 0.05$). Its serum calcium level was also significantly higher than Healthy control (2.564 vs. 2.440 mmol/L, $P < 0.05$). Both Dex and PEG-Dex-treated mice showed significantly higher serum sodium levels than Healthy (148 and 146.9 vs. 144 mmol/L, $P < 0.05$). Lymphocyte counts in Dex group was significantly lower than that of the PEG-Dex, Healthy and Saline groups (0.6592×109 vs. 1.389×109, 1.925×109 and 2.039×109/L, $P < 0.001$). Additionally, the adrenal gland weight in the Dex-treated mice was significantly lower than that of the PEG-Dex-treated mice (0.0049 vs. 0.0064 g, $P < 0.05$). No difference was observed in adrenal gland weight among PEG-Dex, Saline and Healthy groups.

Conclusions
Using an Adriamycin-induced FSGS mouse model, we have demonstrated the potent and superior therapeutic efficacy of PEG-Dex in reducing proteinuria levels, ameliorating FSGS lesions, and restoring kidney functions than the Dex treatment. In

addition, PEG-Dex treatment also showed a much-improved safety profile than Dex with minimal adverse event detected. Collectively, these data suggest that PEG-Dex may be established as a promising drug candidate for more effective and safe treatment of FSGS.

Disclaimer:

Learning Objectives

1. Describe the design concept of polymeric prodrug
2. Analyze the working mechanism of PEG-Dex
3. Discuss the advantages of polymeric glucocorticoid prodrugs vs. conventional glucocorticoids

Submit for Young Investigators Competition? Yes

Are you currently enrolled in a graduate medical education or graduate allied health education program? No

At the time of abstract submission, has the abstract been approved for final clearance by your organization (e.g., For DoD submitters: Public Affairs and OPSEC)? Yes

Does the research abstract being submitted have a DoD affiliation or does it represent DoD funded research? Yes

May We Publish Abstract on the MHSRS website? Yes

Conflict of Interest (COI) Disclosure: View COI Disclosure File *(uploaded: 2/16/2023 4:44 PM)*

3. Abstract for 2023 Controlled Release Society annual meeting (accepted)

CRS 2023

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CONTROL ID: 3907091

TITLE: Polymeric Dexamethasone Prodrug Attenuates Adriamycin-induced Focal Segmental Glomerulosclerosis without Apparent Glucocorticoid Side Effects

PRESENTATION TYPE: Oral

CURRENT CATEGORY: Nanomedicine and nanoscale delivery | Global health and special populations | New and emerging technologies for drug delivery

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ABSTRACT BODY:

Biography: Dong Wang is a professor in Department of Pharmaceutical Sciences at University of Nebraska Medical Center. He earned his PhD at Peking University and joined University of Utah for his postdoctoral training. Dr. Wang's research primarily focuses prodrug and nanomedicine development for inflammatory, musculoskeletal and craniofacial diseases.

Introduction: Focal segmental glomerulosclerosis (FSGS) is chronic renal injury characterized by proteinuria and podocyte injury with glomerulus scarring and tubulointerstitial fibrosis(1). Glucocorticoids (GCs) are the current first-line treatment. Long-term use of GCs, however, is associated with numerous off-target adverse effects(2). Therefore, there is an urgent unmet clinical need for novel FSGS therapies. Recognizing GCs' potent efficacy in managing FSGS, we have developed a polyethylene glycol (PEG)-based nephrotropic dexamethasone (Dex) prodrug (ZSJ-0228 or PEG-Dex) to mitigate the side effects of GCs. The focus of the present study is to assess its therapeutic efficacy and safety in an Adriamycin-induced BALB/c mouse model of FSGS.

Methods: FSGS was induced in Balb/c mice by intravenous administration of Adriamycin. Mice randomized into Healthy, Saline, Dex and PEG-Dex groups were compared. Renal disease progression (proteinuria) was monitored weekly after the Adriamycin induction and throughout the entire treatment course. At the end of week 6, the mice were euthanized with all major organs/tissues isolated. Kidney histology, especially TEM analysis was be performed to assess the therapeutic efficacy. For safety profile, blood glucose, organ weight and histology, WBC, complete metabolic panel and u-CT were also analyzed.

Results: Average proteinuria levels of the PEG-Dex group were significantly lower than that of Dex and Saline groups. Histological analysis of kidney sections revealed that glomerulonephritis percentage for Dex and Saline groups were higher than that of Healthy control, while PEG-Dex group was not statistically different from Healthy group. Dex group showed higher total protein level than Healthy group. For safety assessment, abnormal body weight, blood glucose, liver injury biomarkers, adrenal gland weight, lymphocyte counts were observed in Dex group.

Conclusions/Impact: Using an Adriamycin-induced FSGS mouse model, we have demonstrated the potent and superior therapeutic efficacy of PEG-Dex in reducing proteinuria levels, ameliorating FSGS lesions, and restoring kidney functions than the Dex treatment. In addition, PEG-Dex treatment also showed a much-improved safety profile than Dex with minimal adverse event detected. Collectively, these data suggest that PEG-Dex may be established as a promising drug candidate for more effective and safe treatment of FSGS.

Learning Objective 1: Discuss the advantages of polymeric glucocorticoid prodrugs vs. conventional glucocorticoids

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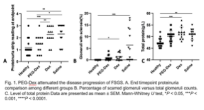

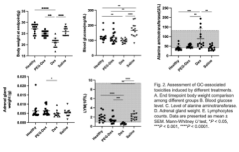

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