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TITLE: Optimizing a Novel Intraductal Delivery of Calcineurin Inhibitors as a Radiocontrast Infusion Formulation to Prevent Post-ERCP Pancreatitis

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14. ABSTRACT

An endoscopic retrograde cholangiopancreatography (ERCP) is a common and life-saving gastrointestinal (GI) procedure that is performed in almost half a million American each year. It was found that about 3-15% of patients underwent ERCP developed post-ERCP pancreatitis (PEP), the most common adverse effect of ERCP. However, and the efficacy and practical use of the current preventative modalities against PEP are still debated.

The present project funded by Award W81XWH-19-1-0683 was proposed based on our recent discovery of an important signaling pathway for PEP mediated via the activation of the calcium-activated phosphatase calcineurin (Cn). It aims to optimize a novel intraductal delivery of Cn inhibitors as a radiocontrast infusion formulation to prevent post-ERCP pancreatitis. We designed to perform two specific aims, (1) determining the safety profile of the Cn inhibitor-RC formulations delivered via an intraductal route and (2) evaluating the efficacy of the intraductal Cn inhibitor-RC formulations to test our hypothesis that the Cn inhibitor-RC formulations were safe for use in ERCP and would prevent PEP in both clinical situations of ERCP with pancreatic duct injection as well as biliary duct injection.

Our proposed project has been progressing smoothly. Due to the COVID-19 pandemic and related social distancing policy, we postponed rabbit safety testing (which usually requires two or more researchers working together) and efficacy studies to the second and third project years. Instead, we have conducted systemic safety studies in mice, which were originally proposed for the second and third project years in the grant proposal. In this reporting period (the first project year), therefore, we have conducted the mouse experiments designed for Aims 1a and 1b: (1) we have examined the influence of intraductal administration of the Cn inhibitor-RC formulations in glucose tolerance in mice, and found that neither Tacrolimus (Tac)-RC nor Cyclosporin A (CsA)-RC formulation caused in endocrine toxicity and (2) we have also determined whether there was potential for systemic toxicity with the intraductal administration of the novel Cn inhibitor Tac-RC formulation, and found that intraductal Tac-RC formulation did not cause acute and subacute systemic toxicity. Taken together, these data suggest that Cn inhibitor-RC formulations can move forward to next steps efficacy studies and limited GLP testing.

We plan to continue to perform the rest of safety testing as well as efficacy studies as proposed in the “Summary and timeline for the proposed work”. We think that the information generated in our studies will broaden our knowledge about the safety and efficacy of the application of Cn inhibitor-RC formulations in the ERCP procedure to prevent the development of post-ERCP pancreatitis.

15. SUBJECT TERMS

-None listed.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
					USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**	Unclassified	13	**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified			

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1. INTRODUCTION:

Post-ERCP pancreatitis (PEP) is a significant iatrogenic challenge. We discovered that PEP is dependent upon calcineurin (Cn) signaling within the pancreatic acinar cell, and is prevented by intraductal pharmacological administration of the Cn inhibitor tacrolimus or cyclosporin A, along with ERCP radiocontrast (RC) dye. However, and the efficacy of the current preventative modalities against PEP are still debated. Our research aims to optimize a novel intraductal delivery of Cn inhibitors as a RC infusion formulation to prevent PEP. We designed to perform two specific aims, (1) determining the safety profile of the Cn inhibitor-RC formulations delivered via an intraductal route and (2) evaluating the efficacy of the intraductal Cn inhibitor-RC formulations to test our hypothesis that these formulations were safe for use in ERCP and would prevent PEP in both clinical situations of ERCP with pancreatic duct injection as well as biliary duct injection.

2. KEYWORDS:

ERCP, mice, pancreas, PEP, inhibitor, Tacrolimus, Tac, islet, systemic, safety, toxicity, intraductal, Cyclosporin A, CsA, RC, radiocontrast, calcineurin, Cn

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Due to covid-19, we postponed rabbit safety testing and efficacy studies to the second and third project years. Instead, we have conducted systemic safety studies in mice, which were originally proposed for the second and third project years in the grant proposal. In this reporting period (the first project year), therefore, we have conducted the mouse experiments designed for Aims 1a and 1b:

1. We have examined the influence of intraductal administration of the Cn inhibitor-RC formulations in glucose tolerance in mice, and found that neither Tacrolimus (Tac)-RC nor Cyclosporin A (CsA)-RC formulation caused in endocrine toxicity. (70% finished)
2. We have also determined whether there was potential for systemic toxicity with the intraductal administration of the novel Cn inhibitor Tac-RC formulation, and found that intraductal Tac-RC formulation did not cause acute and subacute systemic toxicity. (50% finished)

Regulatory work:

1. Pre-IND meeting preparation. (35% finished)

What was accomplished under these goals?

Please see the appendix A.

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

The project was not intended to provide training or professional development opportunities. Therefore, we have nothing to report.

How were the results disseminated to communities of interest?

The results were disseminated to communities of interest through presenations at conferences and talks at department and division levels. We are alo working on a manuscript that will describe in details the scope and the impact of our research findings.

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

We hope to accomplish the following goals for the next reporting period:

- Perform safety and dose efficacy testing in preclinical models, in anticipation of IND-filing and the next steps of a clinical trial
- Identify CROs for assistance with drug formulations, drug levels and regulations associated with the new formulations.
- Perform pharmacokinetic/pharmacodynamic studies.
- Perform experiments using oral and rectal indomethacin in addition to the Calcinuerin inhibitors and the radiocontract dye.
- Perform the ERCP model in rabbits.

4. IMPACT:

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

We established that intraductal administration of tacrolimus and cyclosporin A with radiocontrast dye do not cause any systemic or endocrine toxicities. We also discovered that radiocontrast dye may play a protective role in regulation of insulin secretion post-ERCP. With these findings, we can now move on to the next steps of the clinicl trials. The products that may result from this research could lead to gains in the field of gasteroenterology, hepatology and nutrition by providing an efficacious and safe preventative for post-ERCP pancreatitis.

What was the impact on other disciplines?

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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:

Changes in approach and reasons for change

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

- Our lab moved from University of Pittsburgh, Pittsburgh PA to Stanford University, Palo Alo CA in 2019. The move caused a delay in initiating the project as it took about a year to get all compliance protocols set up and to recruit lab members to work on the project.
- Stanford University shut down in March 2020 due to covid-19, and no research was allowed. Although we were able to come back after two months, we could only perform the very basic research, and no more than two people were allowed at a time in the lab. Additionally, research core facilities were also shut down.
- We're now back in the lab and fully functional. We're working on the project at max speed and employing CROs when possible to make up for the lost time.

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to Report

Books or other non-periodical, one-time publications.

Nothing to Report

Other publications, conference papers and presentations.

We will be presenting our research findings in form of a poster at the the upcoming Americn Pancreatic Associaton (APA) and North American Society of Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) comferences in November 2020.

- **Website(s) or other Internet site(s)**

www.husainlab.org: The Husain Lab website gives an overview of our research work and our accomplishments. The home page has a video for a poster presentation that dissemninates the some of the work we have accomplished on this project We will be presenting this video at the upcoming Americn Pancreatic Associaton (APA) and North American Society of Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) comferences. The direct links for the videos relating to the abstracts for the current work at Link1 (https://stanford.zoom.us/rec/share/oD5GtWg1sJ0FInXnpJ9fnLmWewA_swYNEGctylE1YJtEc0hfrddwVZQgJ2oLJZTw.pH_oCziHRYcldKtT) and Link2 (https://office365stanford-my.sharepoint.com/:v:/g/personal/szh_stanford_edu/EVP4gykUoTlIsGPumn944IwBoL1LrUi8pQJ7WsCZjaJOYg?e=i1rLtl)

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

We filed the following provisional patent for rectal administration of indomethacin:
U.S. Provisional Patent Application No. 63/027,541
Filed: May 20, 2020
For: COMPOSITIONS AND METHODS FOR PREVENTING POST-ERCP PANCREATITIS
Stanford Ref.: S20-149
KTS Ref.: 079445-1190749-005800US

- **Other Products**

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

1. Sohail Husain, MD
Project Role: Principal Investigator
Nearest person month worked: 12
Contribution to Project: Dr. Husain oversees the overall scientific and administrative leadership of the project. He has worked with Dr. Yu to construct the experimental plans and has reviewed each of the data outcomes. He presides over the main meetings of the projects and directly interfaces with the CROs and other collaborators.

2. Mang Yu, MD, PhD
Project Role: Senior Scientist
Nearest person month worked: 12
Contribution to Project: Dr. Yu assisted Dr. Husain in overseeing and implementing the project. He also performed IPGTT in mice.

Jianbo Ni, MD, PhD
Project Role: Visiting Scholar
Nearest person month worked: 12
Contribution to Project: Dr. Ni performed the intraductal ERCP surgeries

3. Asna Khalid, B.Sc
Project Role: Research Associate
Nearest person month worked: 12
Contribution to Project: Ms. Khalid worked on obtaining IACUC, ACURO and IRB approvals needed to carry out the project related experiments. She also performed islet safety studies.

4. Yu Chu Daisy Li, M.S.
Project Role: Research Technician
Nearest person month worked: 1
Contribution to Project: Ms. Lin performed IPGTT, and is also observing/learning the intraductal model of ERCP.

5. Jing Wang, MD, PhD
Project Role: Senior Scientist
Nearest person month worked: 1
Contribution to Project: Dr. Wang provided trainings for islet isolation, and islet hand picking and plating.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report

What other organizations were involved as partners?

Dr. Rita Bottino at the Allegheny Singer Research Institute in Pittssburgh, PA. Dr. Rita participated in overall coordination and supervision of islet function studies. She assisted with the interpretation of the data from glucose tolerance tests.
Moving forward, we will no longer be working with Dr. Bottino as she has left the Allegheny Singer Research Institute. Instaed, we will be working with Stanford Diabetes Research Center (SDRC), an organization internal to Stanford, to help us procure human islets and conduct islet function studies.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES:

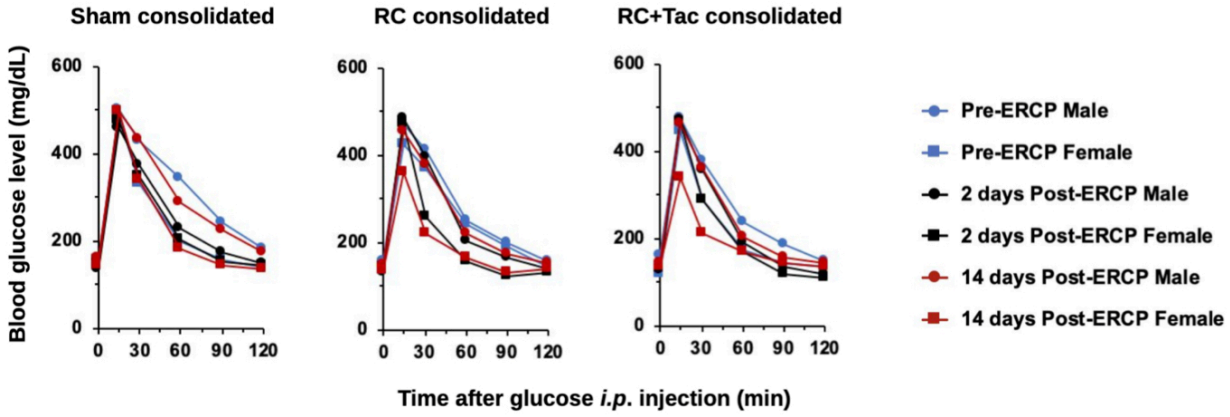
Appendix A

1) Major activities:
(1) Examining the influence of intraductal administration of the Cn inhibitor-RC formulations in glucose tolerance in mice.
(2) Examining whether there was potential for systemic toxicity with the intraductal administration of the novel Cn inhibitor-RC formulation
Regulatory work:
1. Pre-IND meeting preparation.

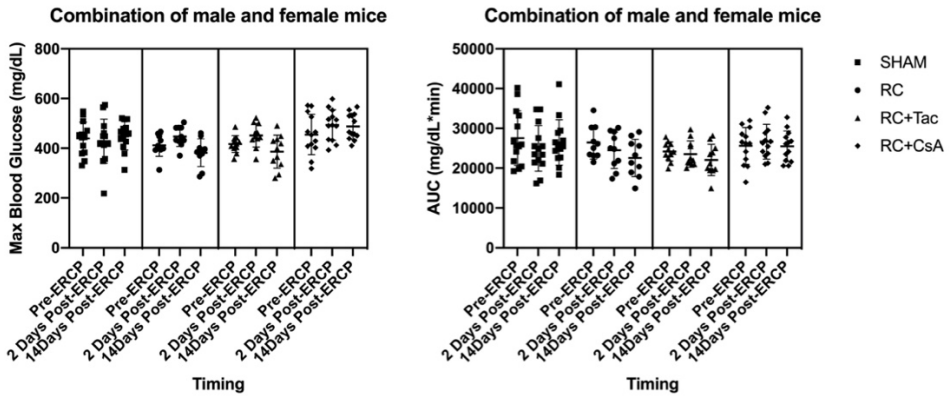
2) Specific objectives:
(1) Performing intraductal infusion of mice with calcineurin inhibitor-radiocontrast (RC) formulations, including Tacrolimus (Tac)-RC formulation and Cyclosporin A (CsA)-RC formulation;
(2) Performing glucose tolerance testing (GTT, a standardized method for testing the ability of the body to handle a glucose load) in mice via intraperitoneal (IP) administration of glucose (IPGTT).
(3) Performing chemistry analysis of indices associated to potential nephrotoxicity and hepatotoxicity.

Regulatory work:
1. Pre-IND meeting preparation: Identify the regulatory CRO (contract research organization) to retain for regulatory services and begin gap analysis.

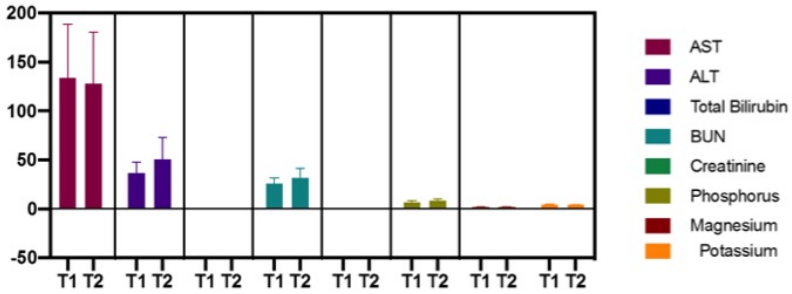
3) Significant results or key outcomes:
(1) Influence of intraductal infusion of Tac-RC or CsA-RC formulation on glucose dynamic curves in mice.



(2) Influence of intraductal infusion of Tac-RC or CsA-RC formulation on max blood glucose and area under curve (AUC) in mice.



(3) Influence of intraductal infusion of Tac-RC formulation on blood chemistry parameters.



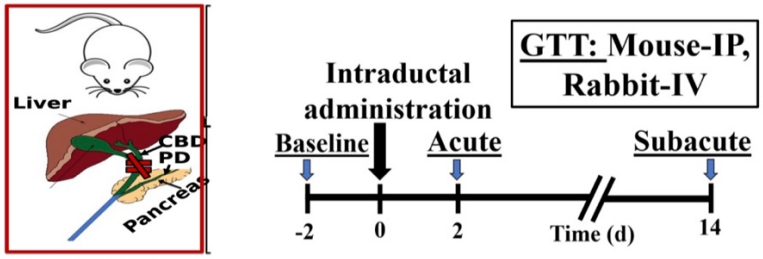
Conclusions:

(1) Neither Tacrolimus (Tac)-RC nor Cyclosporin A (CsA)-RC formulation causes in endocrine toxicity, and

(2) Intraductal Tac-RC formulation does not cause acute and subacute systemic toxicity.

These data suggest that Cn inhibitor-RC formulations can move forward to next steps efficacy studies and limited GLP testing.

General methodology:



(1) Intraductal infusion: C57BL/6J mice underwent a surgical model of ERCP with intraductal infusion of 0.1 mL RC (240 mg Iohexol/mL) alone, RC+Tac (0.8 mg/mL in RC), or RC+CsA (1.2 mg/mL in RC) into the pancreatic duct.

(2) Intraperitoneal glucose tolerance testing (IPGTT): After a 4h food fast, mice underwent a baseline glucose check via tail prick sampling using a standard clinical glucometer. Thereafter, 2 g/kg of D20NS (20g/dL dextrose in normal saline) was infused over 1 min. Additional glucose checks were obtained at 15, 30, 60, 90, and 120 min post-injection. Such IPGTT was performed 2 days before, and 2 and 14 days after the ERCP procedure, respectively.

(3) Blood chemistry analysis: Peripheral blood samples were collected 2 and 14 days after the ERCP procedure and plasma samples were sent to the Diagnostic Laboratory at Stanford University Veterinary Service Center to measure the levels of AST, ALT, total bilirubin, BUN, creatinine, phosphorus, Mg, and K for assessing the potential nephrotoxicity and hepatotoxicity.

Regulatory work:

1. Pre-IND meeting preparation

After interviewing several CROs over a two month period, we identified “RPI” as the CRO for regulatory services. They have appointed a project manager, who we are working with for the pre-IND meeting. The first task that has now begun is to perform a gap analysis, and this is underway.

4) Other achievements:

Nothing to report.