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

PRINCIPAL INVESTIGATOR: Sohail Husain, MD

CONTRACTING ORGANIZATION: Stanford University, Stanford, A

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Sohail Husain, MD

E-Mail: sohail.husain@stanford.edu

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Stanford University School of Medicine
1701 Page Mill Road
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14. ABSTRACT

An endoscopic retrograde cholangiopancreatography (ERCP) procedure is a common and life-saving gastrointestinal procedure that is performed in about half a million American each year, among which about 3-15% of patients were found to develop post-ERCP pancreatitis (PEP), the most common adverse effect of ERCP without effective preventative modalities.

The current project funded by Award W81XWH-19-1-0683 was proposed based on our recent discovery showing the critical role of calcineurin (Cn) signaling pathway in the development of PEP. Its overarching goal is to optimize the delivery of calcineurin inhibitors (CnIs) to prevent PEP. In the first and second project years, we conducted mouse experiments designed for Aim 1, including endocrine and systemic safety testing of novel formulations of CnIs. We also developed the conceptual model to evaluate the preventive effect of rectal delivery of CnI tacrolimus (Tac) against post-ERCP pancreatitis.

Our proposed project has been progressing smoothly. Our request of one-year non-cost extension was approved which allows us to schedule experiments to the fourth (next) project year. In the third year, we have (1) performed safety testing of infusion with CnI formulations, (2) analyzed the pharmacokinetic features of rectal suppository delivery of Tac versus intravenous or intragastric administration, and (3) confirmed the preventive efficacy of rectal suppository administration of Tac in mouse models of post-ERCP pancreatitis and cerulein-induced pancreatitis. Our results demonstrate that (1) Tac or Cyclosporin A (CsA) formulation can decrease pancreatic injury and inflammation in mouse model of post-ERCP pancreatitis, (2) rectal suppository delivery of Tac retains a higher blood Tac level compared with intravenous or intragastric administration, and (3) rectal Tac administration significantly decreases pancreatic injury and inflammation in both mouse models of pancreatitis. Taken together, these data suggest that rectal suppository delivery of Cn inhibitor is a promising strategy in the prophylaxis of pancreatitis including post-ERCP pancreatitis.

In the next project year, we plan to implement the rest of the experiments as proposed in the "Summary and timeline for the proposed work". In summary, our preclinical IND-enabling studies are able to determine the most effective formulations and dosing, as well as the optimal routes for calcineurin inhibitor delivery in the prevention and/or treatment against pancreatic disorders including post-ERCP pancreatitis.

15. SUBJECT TERMS
None listed.

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1. INTRODUCTION:

Post-ERCP pancreatitis (PEP) remains a significant iatrogenic challenge. Employing mouse models, we identified that calcineurin (Cn) signaling plays a critical role in the development of PEP, suggesting that Cn inhibitors (CnIs, including tacrolimus (Tac) and cyclosporin A [CsA]) can be use in the prophylaxis of PEP. Given the unmet clinical needs for effective preventative modalities against PEP, our study aims to develop novel CnI formulations and the optimal routes of administration to prevent PEP. Specifically, we will (1) evaluate the safety profile of CnI formulations in the setting of ERCP models, (2) further explore the optimal delivery (e.g., via the rectum) and CnI formulations that may include non-steroidal anti-inflammatory drugs (NSAIDs). This preclinical IND-enabling research project will suggest the most effective CnI formulations and the optimal routes of administration in the prevention of post-ERCP pancreatitis.

2. KEYWORDS:

Calcineurin, calcineurin inhibitor, ERCP, pancreatitis, post-ERCP pancreatitis, tacrolimus, cyclosporin A, endocrine toxicity, systemic toxicity, rectal suppository

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The Covid-19 pandemic delayed some of our experiments, and we now arrange safety and efficacy experiments to the fourth project year based on the approval of our non-cost extension request. Therefore, mouse studies we have performed the following during this reporting period (the third project year):

Aim 1: We have confirmed that administration of the Cn inhibitor formulations in mice are safe and do not specifically cause endocrine or systemic toxicity (Aim 1, 90% completed during project years 1-3)

Aim 2: We have performed efficacy testing of the rectal administration of the Cn inhibitor formulations (Tac or CsA) in mice, and found that both formulations could decrease pancreatic injury and inflammation in our mouse models of post-ERCP pancreatitis (Aim 2, 90% completed during project years 1-3)

In addition, we have analyzed the pharmacokinetic features of rectal suppository delivery of Tac versus intravenous or intragastric administration and confirmed the preventive efficacy of rectal suppository administration of tacrolimus in mouse models of post-ERCP pancreatitis and cerulein-induced pancreatitis.
Regulatory work:

Pre-IND meeting preparation (80% finished).

What was accomplished under these goals?

Please see the appendix A.

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

Nothing to report
This project was not intended to provide training or professional development opportunities.

How were the results disseminated to communities of interest?

The results from our study were disseminated to communities of interest through presenations at conferences and talks at department and division levels. We are also working on a manuscript that will describe in detail the scope and the impact of our research findings. For example, we presented our results as posters at the 6th Annual Frontiers in Diabetes Research Symposium (April 2022, in Palo Alto, CA) and at 2022 Digestive Disease Week (May 2022, in San Diego, CA).

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

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

- Complete the experiments of safety testing of the Cn inhibitor formulation in support of IND filing.
- Further optimize the dosing and rectal delivery of Cn inhibitor formulations including non-steroidal anti-inflammatory drugs (NSAIDs) and analyze the pharmacokinetic features in blood and tissues.
- Validate findings in mouse studies using post-ERCP pancreatitis model.

4. IMPACT:

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

We have established that calcineurin inhibitor formulations are safe (without endocrine and systemic toxicities) and effective (with the ability to significantly decrease pancreatic injury and inflammation in mouse models of post-ERCP pancreatitis [PEP]). We also defined that rectal suppository delivery can retain a higher level of blood tacrolimus compared with intragastric or intravenous administration, in addition to its efficacy in the prophylaxis of pancreatitis including PEP. Our findings significantly support the next step IND filing and the ensuing clinicl trials. Products that may result from this research could provide an efficacious and safe preventative for pancreatitis including PEP.

What was the impact on other disciplines?

` Nothing to Report

What was the impact on technology transfer?

Upon the completion of this preclinical project, we will be able to put together a strategic plan with multiple partners move our novel calcineurin inhibitor formulations from concept to initial FDA approval, necessary clinical trials, final FDA approval, and finally to the commercial market.

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

Nothing to Report

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

- The relocation of our lab from University of Pittsburgh, Pittsburgh, PA to Stanford University, Palo Alto, CA in 2019 as well as the relocation from Stanford University main campus to Stanford Research Park significantly delayed and interfered with the implementation of the proposed work.
- COVID-19 pandemic-related policy and safety measures also slowed down our research activities.
- However, barring more obstacles, we believe we should be able to complete the rest of the proposed work in the fourth project year.

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 presented our results as posters at the 6th Annual Frontiers in Diabetes Research Symposium (April 2022, Palo Alto, CA) and at 2022 Digestive Disease Week (May 2022, San Diego, CA).

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

The Husain Lab website (www.husainlab.org) 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 presented this video at 2020 Americn Pancreatic Associaton (APA) annual conference and North American Society of Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) annual conference.

- **Technologies or techniques**

Nothing to Report

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

We filed the following utility patent:
For: Compositions and Methods for Preventing Post-ERCP Pancreatitis
Incentor: Sohail Z. Husain, Monique T. Barakat
U.S. PCT Patent Application No. PCT/US2021/023144
Date Filed: March 19, 2021
Stanford Ref.: S20-149
KTS Ref.: 079445-1234339-005810PC
Declaration with the World Intellectual Property Organization: July 16, 2021

- **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 oversaw the experimental plans and 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 Research Scientist
Nearest person month worked: 12
Contribution to Project: Dr. Yu assisted Dr. Husain in overseeing and implementing the project. He also conducted mouse studies proposed in the proposal.

3. Rejowana Majid, MD
Project Role: Visiting Scholar
Nearest person month worked: 12
Contribution to Project: Dr. Majid performed surgical procedures to generate post-ERCP pancreatitis models.

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?

In this project year, we continued working with RPI (San Francisco, CA), a regulatory CRO company, which offers strategic and tactical regulatory expertise in all phases of drug development. We would like to take advantage of their comprehensive experience to strategically guide us to achieve our drug development goals.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES:

Appendix A

1) Major activities:
(1) Preclinical efficacy testing of the calcineurin (Cn) inhibitor formulations in mouse models of post-ERCP pancreatitis.
(2) Pharmacokinetic analysis of rectal suppository versus intragastric or intravenous administration of calcineurin inhibitor tacrolimus (Tac).
(3) Efficacy assessment of rectal suppository administration of calcineurin inhibitor Tac in the presence or absence of non-steroidal anti-inflammatory drug (NSAIDs) using mouse models of post-ERCP pancreatitis or cerulein-induced pancreatitis.

Regulatory work:
Pre-IND meeting preparation.

2) Specific objectives:
(1) Performing efficacy testing of Tac or cyclosporin A (CsA) formulations in mouse models of post-ERCP pancreatitis,
(2) Analyzing the pharmacokinetic features of rectal suppository versus intragastric or intravenous administration of Tac in mice, and
(3) Evaluating the efficacy of rectal Tac suppository in mouse model of post-ERCP pancreatitis and cerulein-induced pancreatitis.

Regulatory work:
Working with RPI, a regulatory CRO company on the strategic and tactical regulatory issues guiding our project from pre-IND meeting preparation to IND application, as well as the following steps towards new drug application (NDA).

3) Significant results or key outcomes:

Specific Aim 1. Determine the safety profile of the novel Cn inhibitor formulation via a rectal route.

Major Task 1 (Aim 1a) Determine whether the novel rectal route, which provides direct exposure of the Cn inhibitors to the pancreas, specifically poses a risk of acute pancreatic islet or exocrine toxicity.

Key outcomes: Tac or CsA formulations can significantly decrease pancreatic injury and inflammation in mouse model of post-ERCP pancreatitis (**Figs. 1** and **2**). Importantly, they fail to induce pancreatic injury or inflammation during the acute or subacute period after ERCP.

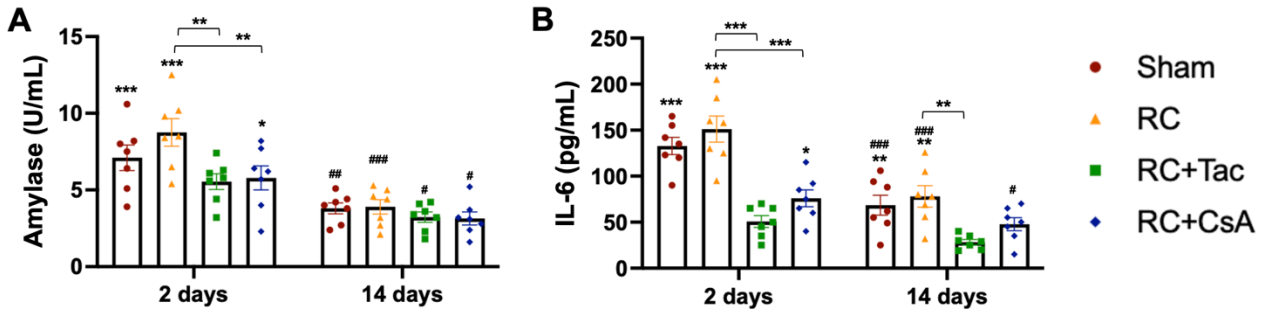


Fig. 1. Influence of the calcineurin inhibitor-radiocontrast (RC+CnI) formulations on the levels of plasma amylase and interleukin-6 (IL-6). Plasma levels of amylase (**A**) and IL-6 (**B**) were measured 2 and 14 days after the infusion or sham procedures. *, **, or *** $P < 0.05$, 0.01, or 0.001 versus the baseline levels of amylase (3.2 +/- 0.46 U/mL) and IL-6 (18.4 +/- 2.5 pg/mL) measured from naïve mice, respectively, or versus the group indicated. $^{\#}$, $^{\#\#}$, or $^{\#\#\#}P < 0.05$, 0.01, or 0.001 versus the corresponding value measured 2 days post procedure. N = 7 mice per group. Sham mice underwent cannulation without infusion; RC, RC+Tac, or RC+CsA mice underwent infusion with radiocontrast dye alone, radiocontrast dye plus tacrolimus, or radiocontrast dye plus cyclosporin A respectively.

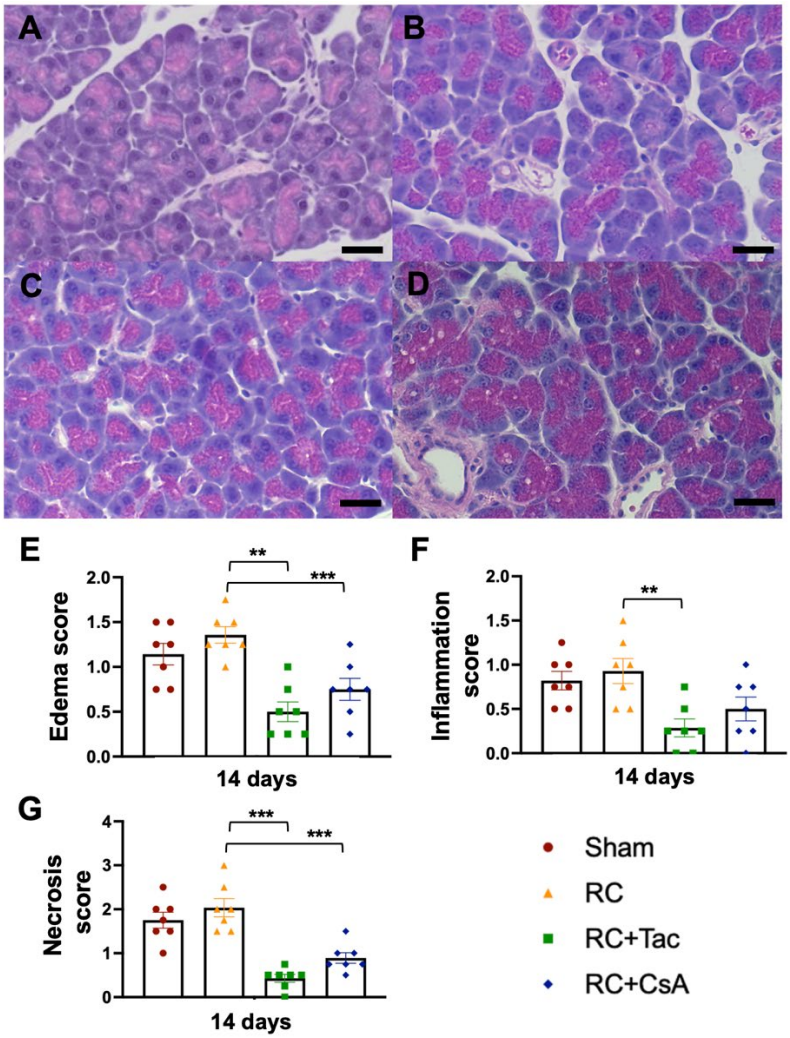


Fig. 2. Influence of the calcineurin inhibitor-radiocontrast (RC+CnI) formulations on the histopathological scoring of pancreatic tissue samples. Representative micrographs of H&E-stained pancreatic tissue samples (**A**-**D**) were taken from mice 14 days after the procedure of cannulation without infusion (Sham), or infusion with radiocontrast dye alone (RC), radiocontrast dye plus tacrolimus (RC+Tac), or radiocontrast dye plus cyclosporin A (RC+CsA). The edema scores (**E**), inflammation scores (**F**), and necrosis scores (**G**) were determined with H&E-stained tissue sections accordingly. ** or ***$P < 0.01$ or 0.001 versus the group indicated. N = 7 mice per group. Scale bars = 20 mm (**A**-**D**)

Major Task 2 (Aim 1b) Determine whether there is potential for systemic toxicity with the rectal administration of the novel Cn inhibitor formulations and determine blood Cn inhibitor drug levels with rectal administration of the novel Cn inhibitor formulations.

Key outcomes: Rectal suppository delivery retains a higher blood Tac level compared with intragastric or intravenous administration in C57BL/6J mice (**Fig. 3**).

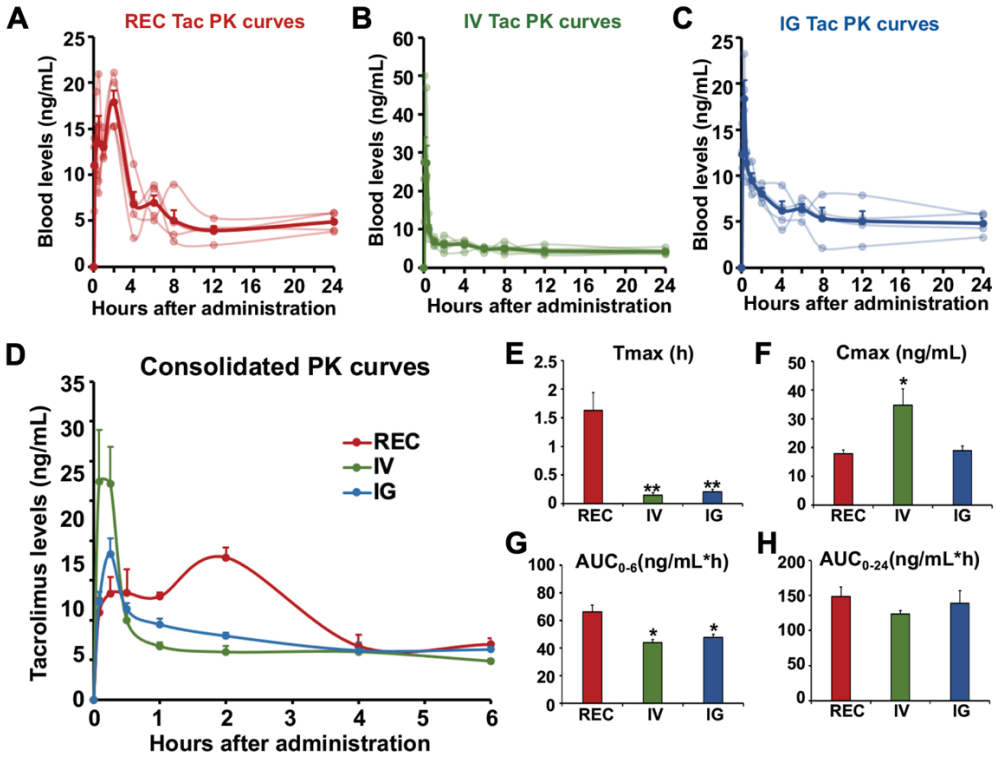


Fig. 3. Pharmacokinetic features of rectal suppository (REC) versus intravenous (IV) or intragastric (IG) administration of tacrolimus (Tac). Blood tacrolimus concentration curves over time following rectal, intravenous, or intragastric administration of tacrolimus (**A**-**D**). Tmax (time to reach blood peak concentration, **E**), Cmax (peak blood concentration, **F**), as well as $AUC_{0\text{-}6}$ (the area under the concentration curve within 6 hours, **G**) and $AUC_{0\text{-}24}$ (area under the concentration curve within 24 hours, **H**) were also determined accordingly. *, **$P < 0.05$ or < 0.01 versus the REC group. N = 3-5 mice per group.

Specific Aim 2. Evaluate the efficacy of the rectal Cn inhibitor formulations.

Major Task 1 (Aim 2a) Determine the optimal Cn inhibitor dosing, as well as sterile preparation, of the novel rectal Cn inhibitor formulations that is effective in preventing PEP.

Key outcomes: Rectal suppository delivery of Tac significantly decreases pancreatic injury and inflammation in mouse models of post-ERCP pancreatitis and cerulein-induced pancreatitis (**Figs. 4** and **5**).

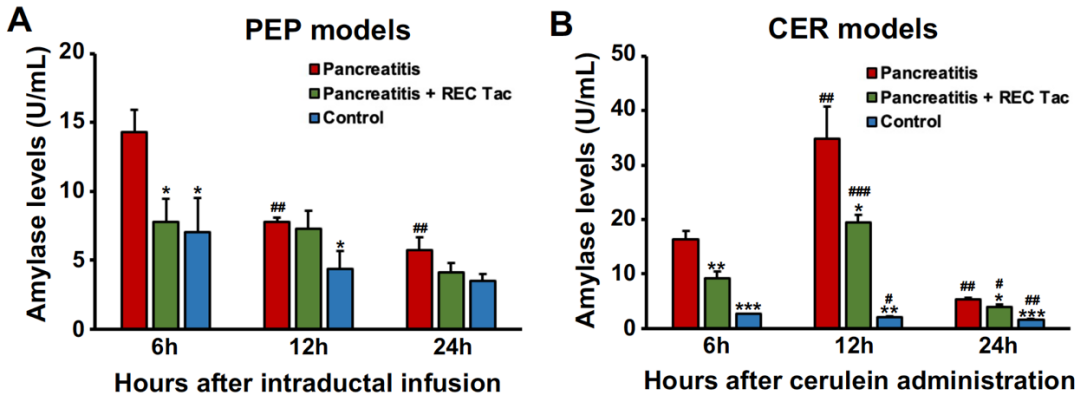


Fig. 4. Influence of rectal suppository delivery of tacrolimus (REC Tac) on the levels of plasma amylase in mouse models of pancreatitis. (**A**) Plasma levels of amylase measured 6, 12, and 24 hours after the induction of hyperpressure in mouse model of post-ERCP pancreatitis (PEP models), (B) plasma levels of amylase measured 6, 12, and 24 hours after the initial cerulein injection in mouse model of cerulein-induced pancreatitis (CER models). *, **, or ***$P < 0.05$, 0.01, or 0.001 versus the corresponding Pancreatitis Group. $^{\#}$, $^{\#\#}$, or $^{\#\#\#}P < 0.05$, 0.01, or 0.001 versus the amylase level measured 6 hours after the procedure or cerulein injection in the same experimental group. N = 3-5 mice per group.

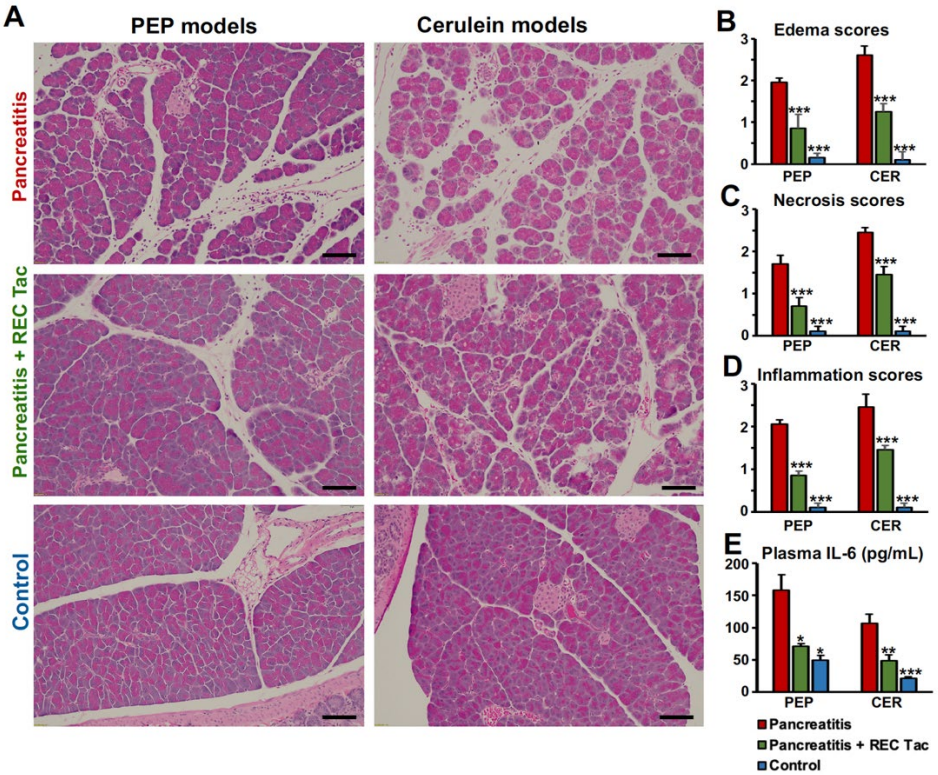


Fig. 5. Influence of rectal suppository delivery of tacrolimus (REC Tac) on the histopathological scoring of pancreatic tissue samples and plasma IL-6 levels. Representative micrographs of H&E-stained pancreatic tissue samples (**A**) were taken from mice 24 hours after the procedure to induce hyperpressure or the initial cerulein injection. The edema scores (**B**), necrosis scores (**C**), and inflammation scores (**D**) were determined with H&E-stained tissue sections accordingly, and the plasma levels of IL-6 (**E**) were determined with blood

samples collected immediately before tissue collection. *, ** or ***$P < 0.05$, 0.01, or 0.001 versus the corresponding Pancreatitis Group. N = 3-5 mice per group. Scale bars = 40 mm (in **A**)

Supplemental information: Research Methods

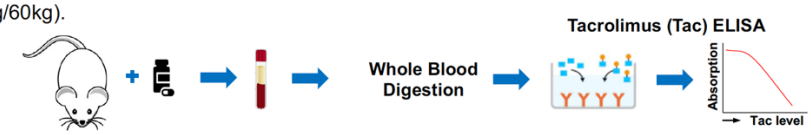
(1) Table 1: The method of histology scoring of pancreatitis

Table 1. Histology scoring of pancreatitis

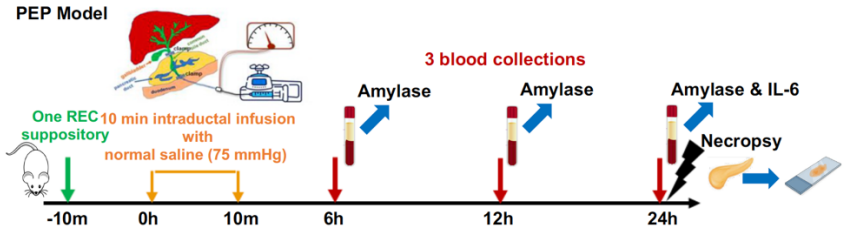
Phenotype	Score	Morphological changes
Edema	0	absent
	1	focally increased between lobules
	2	diffusely increased between lobules
	3	acini disrupted and separated
Inflammation (leukocyte Infiltration)	0	absent
	1	rare or around ductal margins
	2	diffusely increased between lobules
	3	acini disrupted and separated
Necrosis	0	absent
	1	architectural changes, pyknotic nuclei
	2	focal necrosis (<10% of the parenchyma)
	3	Diffuse parenchymal necrosis (>10% of the parenchyma)

(2) Supplemental Figure: methods to measure blood levels of tacrolimus and to generate mouse models of post-ERCP pancreatitis and cerulein-induced pancreatitis.

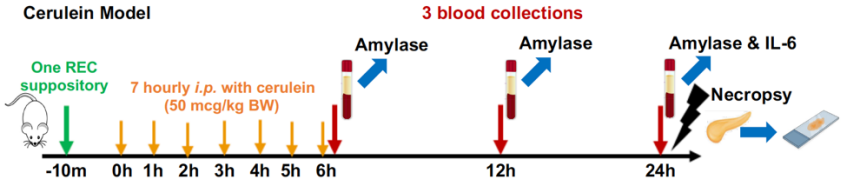
1. ELISA Measurement of Whole Blood Tacrolimus (Tac) Levels: To assess the pharmacokinetic (**PK**) characteristics of different routes of administration: rectal suppository (**REC**), intravenous (**IV**), or intragastric (**IG**) delivery of a single dose of **Tac** (1 mcg/kg body weight, equal to 1 human dose of 5 mg/60kg).



2. Generation of post-ERCP pancreatitis models (PEP models): To subject C57BL/6J mice to intrapancreatic ductal infusion with normal saline for 10 minutes at a constant pressure of 75 mmHg. In the **Pancreatitis Group**, PEP models underwent rectal suppository delivery of excipient (Witepsol® H-15 suppository base), while in the **Pancreatitis+REC Tac Group**, PEP models received 1 rectal Tac suppository (1 mcg Tac/kg). Mice in the **Control Group** underwent surgical procedures without an intrapancreatic ductal infusion of normal saline.



3. Generation of cerulein (CER) hyperstimulation-elicited chemical pancreatitis models (CER models): To subject C57BL/6J mice to 7 hourly *i.p.* injection of cerulein (50 mcg/kg body weight). CER models in the **Pancreatitis Group** or **Pancreatitis+REC Tac Group** underwent the identical rectal administration as the corresponding PEP models did. Mice in the **Control Group** received *i.p.* injection with normal saline without a rectal procedure.



Conclusions:

(1) The calcineurin inhibitor formulations are safe and can protect against pancreatic injury and inflammation in mouse model of post-ERCP pancreatitis.

(2) Rectal suppository delivery of tacrolimus can retain a higher level of blood tacrolimus compared with intravenous or intragastric administration.

(3) Prophylactic rectal suppository delivery of tacrolimus can significantly decrease pancreatic injury and inflammation in mouse models of post-ERCP pancreatitis and cerulein-induced pancreatitis.

Regulatory work:

Pre-IND meeting preparation: We continued working with RPI, a regulatory contract research organization (CRO) and a division of Premier Research, on the strategic and tactical regulatory issues that guide our project from pre-IND meeting preparation to IND application, as well as the following steps towards new drug application (NDA).