New calcium-targeting therapies for acute pancreatitis

PancreasFest: 2019

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Conflicts of Interest: Dr. Gary Desir, a collaborator, has a financial interest in Bessor Pharmaceutical, a company developing renalase as a diagnostic and therapeutic agent

Funding: NIDDK, NCI, DOD, Veterans Administration

Collaborators: Gary Desir (Yale), Larry Miller (Mayo), Markus Lerch (Greifswald)
An ideal therapy

Value given prophylactically or therapeutically

High therapeutic index

Low cost

Multiple targets in acute injury

Promotes recovery

Acute Pancreatitis
Phases and effectors

**INJURY RESPONSES**
- Acute
  - Immediate
  - Intermediate
- Stabilization
- Recovery

**EFFECTORS**
- Acinar cell
- Inflammatory cells
- Vascular
Acute Pancreatitis

Both exocrine and endocrine insufficiency develop often

- Risk DM independent of severity
- Other studies up to 40% - population dependent (male, etoh, hyperlipidemia)
- Rate up to 80% in recurrent acute
- Risk similar for pancreatic exocrine insufficiency (PEI) but severity dependent
- Implications: need to follow AP patients long-term for diabetes and PEI

Also: Das Gut 2014; 63(5):818-31; Tu BMC Gastro 2017; R. Hollemans: Pancreatolog, 2018; Petrov and Yadav, Nature Reviews, 2018; Huang DDS
Multiple cell types mediate disease

Acinar cell

- Ca²⁺ signaling
- Zymogen activation
- Inflammatory mediators
- Mechanism of cell death

Inflammatory

- Transient infiltration
- M2-inflamatory

Acinar cell

- Anti-Inflammatory
- Paracellular leak
- Thrombosis

Acute Pancreatitis
### New/rationale therapies

#### Targets: 1
- Store-operated calcium entry
- Mitochondrial transition pore
- Autophagy stimulation
- Unfolded protein response
- Inflammasome: GPR81/lactate
- Kynurenine inhibition
- Neurogenetic inflammation (alpha-2-adrenergic receptor agonist)

#### Targets: 2
- COX (prostaglandin) inhibition
- TNF synthesis and activity
- Angiopoietin inhibition
- Lipase inhibition/generation of FFA from TG
- IL6 receptor inhibitor
- Thrombin inhibition
- Microbiome (FMT)

Calcium signaling: targeting for treatment

- Calcineurin
- Intracellular calcium stores

Calcium Entry
- Orai1
- Piezo

Calcium Extrusion
- PMCA

Intracellular calcium
- RYR
- IP3R

Acute Pancreatitis

- Husain S. PNAS 2005 and AJP 2012 (dantrolene)
- Orabi Al Cell Mol Gastroenterol Hepatol 2017 (Calcineurin/NFATc)
- Gerasimenko PNAS 2013 and Wen L Gastroenterology 2015 (ORAI1)
- Mankad 2012 and Samad 2014 (JBC)
- Romac..Liddle, Nat Comm. 2018
Calcium signaling: targeting for treatment

Some calcium-targeting agents reduce both acinar cell and inflammatory cell pancreatitis responses.
Acute Pancreatitis

Calcium signaling drives activation of others pancreatitis responders

Neutrophil

Macrophage

Stellate cell

STIM1/Orai1

STIM1/Orai1

Renalase

M2-
anti-inflamatory

M1-
anti-inflamatory

Hollander, Cancer Res 76: 3884, 2016
Waldron, J Physiol. 2019 Jun;597(12):3085-3105
Renalase: (RNLS) a new survival factor

- 37 kD secretory protein (RNLS1; RNLS2 not secreted) made by the kidneys and other tissues
- NADH/FAD and catecholamine binding domains
- Pro-survival: activates plasma-membrane calcium ATPase 4B (PMCA4b) and calcium efflux; also activates ERK and AKT
- Pro-survival activity contained in 22 aa domain
- Pro-survival factor in pancreatic cancer and melanoma
- Reduced levels in renal failure

In collaboration with G. Desir, Yale University
Renalase deficiency worsens cerulein pancreatitis

KOLODECJ K
Biol Chem 2017
Exogenous renalase reduces cerulein AP* severity when given after onset (2 hrs)

*Also effective in arginine-induced pancreatitis

Kolodecik J Biol Chem 2017
Acute Pancreatitis

Renalase reduces injury responses in acinar cells and macrophages

- Cellular “pancreatitis”
  - Cerulein
  - Carbachol
  - Bile salts
  - LPS
  - ATP

Renalase reduces:
- Zymogen activation
- LDH release
- Mitochondrial function

PMCA4b

Inflammasome activation
- M1 specification

Acute Pancreatitis

**RNLS-KO mice have delayed cerulein AP recovery**

- **Edema (wet/dry)**
  - C57-WT
  - KO
  - Hours after first Cerulein injection
  - Edema % wet weight

- **Histologic Edema**
  - C57-WT
  - KO
  - Hours after first CER injection
  - Histologic Score Edema /40x field

- **Pyknotic Nuclea**
  - C57-WT
  - KO
  - Hours after first CER injection
  - Histologic Score Pyknotic nuclei /40x field

- **Vacuoles**
  - C57-WT
  - KO
  - Hours after first CER injection
  - Histologic Score Vacuoles /40x field

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Serum renalase levels decrease with the onset of acute pancreatitis

Could serum renalase levels have prognostic value in human acute pancreatitis?

Kolodecik J Biol Chem 2017
Serum renalase levels fall in clinical AP and may predict severity

With M Lerch and G Desir
Summary of renalase data:

• Secretory protein with paracrine and hormonal actions
• A broadly active survival factor
  • Acute injury
  • Select cancers (negative effects on survival- PDAC, melanoma)*
• Requires PMCA4b; possibly other co-receptors
• Blood levels decrease in experimental AP and in humans with AP – may correspond to severity
• May modulate AP recovery
• Exogenous RNLS reduces experimental AP severity

New and rationale calcium targeting therapies: 
*Suggestions for the future*

**EFFECT ON:**

SAP to MOF transition
- *Pulmonary*
- *Renal*
- *Vascular*
- *Intestinal*

- Recovery
- Short-term
  - Unresolved AP
  - Infections
- Long-term
  - Exocrine
  - Endocrine
  - Neoplasia
  - Infections