CRACking pancreatitis

By Lev Osherovich, Senior Writer

The handful of companies pursuing CRAC inhibitors for immunological disorders have a new indication to consider—acute pancreatitis. New findings from **Cardiff University**¹ show that the target plays a central role in the painful and potentially life-threatening condition for which there are no FDA-approved, disease-modifying therapies.

Binge drinking can trigger acute pancreatitis, in which excessive release of pancreatic enzymes leads to pain, inflammation and damage to pancreatic tissue.

Treatment focuses on alleviating pain and inflammation or on managing rare metabolic conditions that bring on bouts of the disorder independently of binge drinking. For example, Glybera alipogene tiparvovec (AMT-011), a gene therapy from **uniQure B.V.**, is approved in the EU to treat lipoprotein lipase (LPL) deficiency, a hereditary condition that leads to acute, recurring pancreatitis.

The Cardiff team, led by professor and director of biosciences Ole Petersen, now has evidence in cell culture that blocking the CRAC (calcium release-activated calcium channel) can prevent the toxicity and cell death that lead to tissue damage in pancreatitis.

CRAC is a plasma membrane complex that opens up to recharge intracellular calcium when calcium levels in the secretory system fall after release of digestive enzymes (see **Figure 1**, “CRACKdown on pancreatitis”). The complex consists of transmembrane protein 142A (ORAI1; TMEM142A; CRACM1), which forms the channel’s pore, and stromal interaction molecule 1 (STIM1), a regulatory subunit associated with the endoplasmic reticulum.

CRAC previously was thought to be expressed primarily in the immune system. Thus, companies including **Synta Pharmaceuticals Corp.** and **CalciMedica Inc.** are developing CRAC inhibitors for immunological disorders.

“To date, CRAC has been detected in numerous different tissues, but since most tissues outside of the immune system possess other channels that can provide intracellular calcium at suitable levels, CRAC has only been [thought to be] essential for the function of lymphocytes,” said Christian Cortis, head of business development at Synta. “These results are novel in that they appear to identify another cell type with significant dependence on CRAC.”

“This paper provides good evidence for a role for CRAC in acute pancreatitis. There is evidence that GSK-7975A works, so it looks and smells like CRAC, but it would be good to test other compounds [that block other calcium channels].”

**—Kenneth Stauderman,
CalciMedica Inc.**

CRACKdown

Petersen said his team had suspected for some time that deranged calcium release played a role in acute pancreatitis,² but it was not clear until now which types of calcium channels were involved.

“We made the original hypothesis in the early 1990s that pancreatitis might be a calcium toxicity disease,” said Petersen. “There’s no doubt that an early step in pancreatitis is calcium channel release from internal stores in response to high concentrations of bile acids or alcohol-related compounds.”

In the new study, Petersen’s team used *in vitro* pharmacology and electrophysiology to show that CRAC was involved in normal regulation of calcium in pancreatic cells. GSK-7975A, a CRAC antagonist, blocked the influx of calcium into cultured murine acinar cells depleted of intracellular calcium stores.

GSK-7975A was originally developed by **GlaxoSmithKline plc**’s respiratory disease unit as a research tool.

The Cardiff team then used an *in vitro* assay of acinar cell activity in which exposure to fatty acid ethyl esters, a toxic metabolite of fat and alcohol, led to high calcium spikes, excessive enzyme release and cell death.

“We expose cells isolated from mice to a combination of fatty acids and alcohol, a scenario that we think the cells would experience in an alcoholic binge,” said Petersen.

In the fatty acid ethyl ester-treated cells, GSK-7975A prevented calcium spiking, proteolytic enzyme activation and necrosis.

Results were reported in the *Proceedings of the National Academy of Sciences* and were not patented.

CRAC pipeline

Petersen’s electrophysiological studies, together with previous genetic studies of hereditary mutations in components of the CRAC complex, suggest the target is likely not required for normal pancreatic secretion and becomes activated only in extreme situations such as alcohol overexposure.

“The absolute dependence of the pancreatic acinar cells on CRAC *in vivo* has yet to be established,” said Cortis. “The absence of digestive problems in children with genetically defective CRACM1 protein may suggest that at least CRACM1 is not essential for normal functioning of the human pancreas.” Petersen’s laboratory is undertaking animal studies to establish whether blocking CRAC can prevent acute pancreatitis *in vivo*.

Petersen said current animal models of pancreatitis do not respond to alcohol exposure, making it difficult to develop therapies for alcohol-induced pancreatitis.

“The majority of people in this field have used a hyperstimulation model, in which you treat [rodents] with high doses of the hormones that normally elicit pancreatic secretion,” said Petersen. “The reason that people have stuck with hyperstimulation is that giving alcohol by itself doesn’t do very much.”

Petersen suspects that animals will more readily develop pancreatitis when treated with a combination of fatty acids and alcohol, which combine to form fatty acid ethyl esters. “We think it’s quite important that there also be a high-fat diet as well as alcohol,” he said.

Figure 1. CRACKdown on pancreatitis. Gerasimenko *et al.* have found that blocking calcium release-activated calcium channels (CRACs) could prevent the calcium (Ca^{2+})-mediated cytotoxicity that leads to acute pancreatitis.

CRACs consist of channel subunits such as transmembrane protein 142A (ORAI1; TMEM142A; CRACM1) and calcium-sensing subunits such as stromal interaction molecule 1 (STIM1).

Gerasimenko *et al.* propose that in pancreatic acinar cells, high levels of alcohol lead to formation of fatty acid ethyl esters [a] that cause depletion of calcium stores in the endoplasmic reticulum [b] and activate STIM1 [c]. STIM1 binds to and activates CRACM1 [d], which then opens to allow extracellular calcium to flow into the cytoplasm [e], leading to cytotoxicity, necrosis and pancreatitis [f].

GSK-7975A, a CRACM1 antagonist developed as a research reagent at **GlaxoSmithKline plc** for respiratory indications, prevented acinar cell toxicity caused by fatty acid ethyl esters.

CalciMedica Inc. and **Synta Pharmaceuticals Corp.** have CRACM1 antagonists in preclinical development for autoimmune and inflammatory indications.

An open question is whether other calcium channels besides CRAC play a role in acute pancreatitis.

"This paper provides good evidence for a role for CRAC in acute pancreatitis. There is evidence that GSK-7975A works, so it looks and smells like CRAC, but it would be good to test other compounds" that block other calcium channels, said Kenneth Stauderman, VP of research at CalciMedica.

What is also missing is evidence that the CRAC components found in rodent acinar cells are present in the human pancreas and are activated in acute pancreatitis.

Stauderman and CalciMedica president and CEO Gonul Velicelebi noted that the specific subunits of CRAC in pancreatic cells may differ from those in immune cells. In human T cells, the principal components of CRAC are CRACM1 and STIM1, but alternative variants of both proteins are known to exist.

"CRACM comes in three flavors, but CRACM1 is apparently the most important for immune cell functions. STIM exists in two forms, but STIM1 is the most relevant in T cells," said Velicelebi. "We don't know the selectivity of the GSK compound for CRACM1 versus other variants."

To establish that CRACM1 and STIM1 are the relevant players in acinar cells, she suggested knocking down either protein and testing whether it leads to an effect similar to that of GSK-7975A.

CalciMedica is developing CRACM1 inhibitors for a range of autoimmune and inflammatory diseases. Last year, the company discontinued its Phase I psoriasis candidate, cm²489, and plans to put a

more potent backup compound into Phase I testing next year.

Synta's preclinical CRACM1 antagonists, STA-12-7525 and STA-12-8336, were discovered in a now-ended collaboration with **Roche**. Cortis said Synta has full rights to the compounds, which are not undergoing further development and are available for out-licensing.

Cortis, Stauderman and Velicelebi all noted that Petersen's study suggests their respective companies' compounds could be tested in pancreatitis but said they did not currently plan to do so.

Osherovich, L. *SciBX* 6(30); doi:10.1038/scibx.2013.780

Published online Aug. 8, 2013

REFERENCES

- Gerasimenko, J.V. *et al.* *Proc. Natl. Acad. Sci. USA*; published online July 22, 2013; doi:10.1073/pnas.1300910110
Contact: Ole H. Petersen, Cardiff University, Cardiff, U.K.
e-mail: petersenoh@cardiff.ac.uk
Contact: Oleg V. Gerasimenko, same affiliation as above
e-mail: gerasimenkoov@cardiff.ac.uk
Contact: Pawel E. Ferdek, same affiliation as above
e-mail: ferdekpe@cardiff.ac.uk
- Petersen, O.H. & Sutton, R. *Trends Pharmacol. Sci.* **27**, 113–120 (2006)

COMPANIES AND INSTITUTIONS MENTIONED

CalciMedica Inc., La Jolla, Calif.
Cardiff University, Cardiff, U.K.
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
Synta Pharmaceuticals Corp. (NASDAQ:SNTA), Lexington, Mass.
uniQure B.V., Amsterdam, the Netherlands