Cell Calcium xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Cell Calcium

journal homepage: www.elsevier.com/locate/ceca

Review

Ca^{2+} signalling underlying pancreatitis

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ARTICLE INFO

Article history:
Received 19 April 2017
Received in revised form 16 May 2017
Accepted 16 May 2017
Available online xxx

Keywords:
Asparaginase
Pancreatic acinar cells
Pancreatic stellate cells
Acute pancreatitis
Bradykinin

ABSTRACT

In spite of significant scientific progress in recent years, acute pancreatitis (AP) is still a dangerous and in up to 5% of cases deadly disease with no specific cure. It is self-resolved in the majority of cases, but could result in chronic pancreatitis (CP) and increased risk of pancreatic cancer (PC). One of the early events in AP is premature activation of digestive pro-enzymes, including trypsinogen, inside pancreatic acinar cells (PACs) due to an excessive rise in the cytosolic Ca^{2+} concentration, which is the result of Ca^{2+} release from internal stores followed by Ca^{2+} entry through the store operated Ca^{2+} channels in the plasma membrane. The leading causes of AP are high alcohol intake and biliary disease with gallstones obstruction leading to bile reflux into the pancreatic duct. Recently attention in this area of research turned to another cause of AP – Asparaginase based drugs – which have been used quite successfully in treatments of childhood acute lymphoblastic leukaemia (ALL). Unfortunately, Asparaginase is implicated in triggering AP in 5–10% of cases as a side effect of the anti-cancer therapy. The main features of Asparaginase-elicited AP (AAP) were found to be remarkably similar to AP induced by alcohol metabolites and bile acids. Several potential therapeutic avenues in counteracting AAP have been suggested and could also be useful for dealing with AP induced by other causes. Another interesting development in this field includes recent research related to pancreatic stellate cells (PSCs) that are much less studied in their natural environment but nevertheless critically involved in AP, CP and PC. This review will attempt to evaluate developments, approaches and potential therapies for AP and discuss links to other relevant diseases.

Contents

1. Introduction

Acute pancreatitis (AP) is a disease usually caused by alcohol abuse or bile reflux due to gallstones. Other causes include some type of antibiotics, chemotherapy, infections, certain rare conditions and others [1]. One of the complications that can result from AP is chronic pancreatitis (CP) that significantly increases risk of pancreatic cancer [2–5]. AP is a severe disease and has a significant mortality of about 5% [1,3]. However, in severe cases, the mortality rate can rise to 30% [6], with significant pancreatic acinar cell (PAC) necrosis followed by a damaging inflammatory response. The leading causes of AP have been identified as gallstone biliary disease and high alcohol intake, while abnormality in calcium signalling in PACs was found to be one of the first events in the initiation of AP [3].

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http://dx.doi.org/10.1016/j.ceca.2017.05.010
0143-4160/

2. Physiological calcium signalling in PACs

Calcium signalling plays a fundamental role in regulation of digestive enzymes and fluids secretion by the exocrine pancreas [7]. A range of endogenous stimuli such as the neurotransmitter acetylcholine (ACh) [8] produced by the vagal nerve endings and the hormone cholecystokinin (CCK) secreted by enteroendocrine I cells of the small intestine [9,10] serve as triggers in the activation of the calcium signalling machinery. Binding of ACh to the muscarinic receptor type 3, which is a G-protein-coupled receptor, results in activation of phospholipase C and production of the second messenger inositol trisphosphate (IP_3) that induces Ca^{2+} release from the internal stores through IP_3 receptors [11]. In fact, in the absence of IP_3 receptors or type 2 and 3 (double-knockout), carbachol −induced secretion is completely abolished in PACs [12]. Similarly, Ca^{2+} release in PACs elicited by physiological doses of CCK, also in the human pancreas [13,14], is primarily mediated by nicotinic acid adenine dinucleotide phosphate (NAADP). In this case the action depends on functional ryanodine receptors (RyRs) and two-pore channels (TPCs) and also involves acid Ca^{2+} stores [15–18]. Calcium response then further amplified by calcium induced calcium release (CICR) from the acid stores and the ER [17,18]. While the exact mechanism of the NAADP production and action remains elusive, in PACs NAADP-induced calcium responses are linked to both ER and acidic stores, and is highly depend on RyR type 1 and TPC type 2 [18]. A hypothesis that takes into account practically all published data suggests a mechanism involving 3 stores: an initial, virtually undetectable, NAADP-elicited Ca^{2+}release via TPCs from endosomes/lysosomes triggers the detectable Ca^{2+}-induced Ca^{2+} release via RyR1 and RyR3 occurring from the granules and the ER. Both pathways result in feeding of Ca^{2+} to the cytosol and subsequent exocytosis of zymogen granule content into the acinar lumen that, together with fluid supplemented with bicarbonate, is transported via the pancreatic duct system to the duodenum under physiological conditions [17,18].

3. Pathological calcium signalling in PACs

In AP, the pancreatic proenzymes such as trypsinogen become prematurely activated intracellularly, resulting in the molecular cannibalism that digests pancreas and its surroundings [3,6]. There is overwhelming evidence, collected during last two decades, implicating abnormal cytosolic Ca^{2+} overload in the initiation and development of AP [3,19–23]. The most damaging are the sustained elevations in [Ca2 +], from high concentrations of some secretagogues, as well as from ductal hypertension, alcohol, hypoxia, hypercalcaemia, hyperlipidaemia, viral infection, and various drugs—all factors known to precipitate acute pancreatitis [19,24]. These factors cause either excessive release of acinar [Ca2 +]i, or damage to the integrity of mechanisms that restore low resting levels of [Ca2 +]i, and the consequent calcium toxicity become the key trigger of acute pancreatitis [19,20].

Aberrant calcium signalling, as the main initiation event in AP, has been proposed more than two decades ago and became the widely accepted mechanistic explanation [19,20,25]. Pathological stimuli, such as bile and alcohol, are capable of triggering massive Ca^{2+} release from intracellular stores through IP_3Rs and RyRs followed by excessive Ca^{2+} entry through Ca^{2+} release activated Ca^{2+} (CRAC) channels that are the most important mechanisms of Ca^{2+} overload in pancreatic acinar cells [3,26].

It has been shown that long lasting Ca^{2+} responses with a development of a sustained elevated cytosolic Ca^{2+} plateau component result in destabilisation of the secretory zymogen granules and conversion of them into empty looking vacuoles [27]. Intracellular vacuolisation [4], causes another calcium-dependent process, intracellular protease (trypsin) activation [5]. As a result, the inactive pancreatic pro-enzymes stored in zymogen granules (ZG) become active enzymes inside the PACs [21,27], inducing mitochondrial malfunction [25,28–30], cell necrosis, digestion of pancreas and its surroundings [19–21].AP is known to involve reactive oxygen (ROS) and reactive nitrogen (RNS) species, that together with calcium overload leads to abnormal mitochondrial Ca^{2+} uptake [25,30] and opening of the mitochondrial permeability transition pore (MPTP) [32,33] resulting in reduction of ATP production. The lack of ATP together with calcium overload and protease activation leads to acinar cells necrosis generating the damaging inflammatory response [5].

4. Asparaginase-induced pancreatitis

Recently another form of AP has been studied in detail, a well-known complication of the treatment of childhood acute lymphoblastic leukaemia (ALL). The incidence of AP following childhood ALL treatment is between 7- 18% [34]. Whilst numerous anti-leukemic medications have been reported, the most important are based on Asparaginase [34]. The development of AP is one of the commonest causes for stopping Asparaginase treatment, because re-exposure is associated with recurrence of pancreatitis [35]. However, stopping the scheduled Asparaginase treatment because of previous pancreatitis has been linked to an increased relapse rate [36]. While significant progress has been made in characterizing the effects of alcohol and bile acids on pancreas [5,23,37,38], the Asparaginase-induced pancreatic pathology was largely unknown (Fig. 1).

Findings presented recently [39] provide the first mechanistic insight into the process by which Asparaginase treatment of ALL may cause Asparaginase-induced AP (AAP). Pancreatic acinar cells can respond to a very low dose (0.1IU/ml, Fig. 2A) and in practically all cases to higher doses of Asparaginase (Fig. 2B). The most accessible therapeutic target in Asparaginase-elicited toxicity is the Ca^{2+} release activated Ca^{2+} (CRAC) channel in pancreatic acinar cells (PACs) [23,40]. The Asparaginase-induced Ca^{2+} elevations (plateau) depend on CRAC channels and were markedly diminished by the inhibitor GSK-7975A [39]. Consequently, Asparaginase-induced necrosis was dramatically reduced by GSK-7975A to near control levels (Fig. 3A). The protective effects of CRAC channel inhibitors against alcohol-induced pancreatitis in isolated pancreatic acinar cells [23,26] and in pancreatic stellate cells [41,42] have been confirmed by *in vivo* studies [43]. Therefore, this approach is also likely to succeed against AAP [39] and the next step would be to test the effectiveness of CRAC channel blockade against AAP using an *in vivo* mouse model.

The AAP mechanism is apparently fundamentally different from the therapeutic action of Asparaginase on lymphoblastic cells in ALL [44]. The Asparaginase effect on cancer cells relies on depletion of asparagine, which the malignant cells cannot produce themselves, in contrast to normal cells [44], whereas the side-effect of Asparaginase, namely AAP, is owing to activation of a signal transduction mechanism involving PAR2 (Fig. 3A,C) but independent of asparagine [39]. Hence, several potential intervention points are available for treating the side effect of Asparaginase (Fig. 3C). The key initiation site of Asparaginase action on PACs seems to be PAR2. This receptor has previously been implicated in AP, although its exact role is still debated [45,46]. Blocking PAR2 has inhibited both the pathological $[Ca^{2+}]_i$ elevations and the Asparaginase-induced necrosis (Fig. 3A) [39], suggesting that PAR2 inhibitors could be a useful tool to supplement Asparaginase ALL treatment in AAP cases. Both Ca^{2+} entry and extrusion were significantly affected by Asparaginase while sustained elevation of $[Ca^{2+}]$i is responsible for the necrosis [39]. The simplest explanation for this is the

Please cite this article in press as: J.V. Gerasimenko, et al., Ca^{2+} signalling underlying pancreatitis, Cell Calcium (2017), http://dx.doi.org/10.1016/j.ceca.2017.05.010