Pancreatology xxx (2014) 1–5

Contents lists available at ScienceDirect

Pancreatology

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

Original article

Electron-microscopic evidence of mitochondriae containing macroautophagy in experimental acute pancreatitis: Implications for cell death

Tony George Jacob [a], Vipin Iyani Sreekumar [b], Tara Sankar Roy [a], Pramod Kumar Garg [c, *]

[a] Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India
[b] Department of Gastrointestinal Surgery, All India Institute of Medical Sciences, New Delhi 110029, India
[c] Department of Gastroenterology, All India Institute of Medical Sciences, New Delhi 110029, India

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Acute pancreatitis
Autophagy
Mitochondria
Necrosis
Electron microscopy
Caerulein

ABSTRACT

Background: Dysfunctional autophagy and necrosis are characteristic features of severe acute pancreatitis.
Objective: To unravel the cellular mechanisms underlying the pathogenesis of acute pancreatitis.
Methods: We studied the ultrastructural pancreatic morphology using electron microscopy in experimental acute pancreatitis. The control group of animals received intraperitoneal injections of normal saline. Different severity of acute pancreatitis was induced by low and high doses of caerulein in Swiss albino mice. In the low dose group, pancreatitis was induced by 4 injections of caerulein given hourly [50 μg/kg/dose − total of 200 μg/kg] and in the high dose group by 8 injections given hourly (total of 400 μg/kg). The experiments were repeated in Na-taurocholate model of acute pancreatitis in rats. The pancreatic tissue was processed and studied by transmission electron microscopy for ultrastructural changes.
Results: The acinar cells of the pancreatitis animals revealed autophagosomes that contained cellular organelles, including mitochondria. The animals that received a higher dose of caerulein had numerous cells showing a necrotic morphology, whereas the animals in the low dose group showed a predominantly apoptotic cell morphology. The Na-taurocholate model in rats also showed similar features of severe pancreatitis with cellular necrosis and macroautophagy.
Conclusions: Dysfunctional mitochondria in the injured pancreatic acinar cells are degraded by macroautophagy. These observations are not model specific. Mitochondrial dysfunction and consequent energy deficit in the cells might be causally related to cellular necrosis.

Introduction

Acute pancreatitis [AP] is an acute inflammatory condition of the pancreas associated with significant morbidity and mortality [1]. Inflammation, edema, and pancreatic cell injury are common features in the pathogenesis of AP. Cellular injury can be either self limiting or severe leading to cell death. Usually, cell death is due either to apoptosis or necrosis. The mechanism of cellular necrosis is not well understood. Activation of intracellular pancreatic enzymes by itself is unlikely to cause cellular necrosis. Mitochondrium plays an important role in cellular homeostasis and energy metabolism. It has been elegantly shown in experimental models that there is mitochondrial dysfunction in the pancreatic acinar cells during severe acute pancreatitis [2]. The consequent deficit of energy in the cell can lead to necrosis [3,4]. Mitochondrial permeability transition pore [MPTP] and its role in maintaining the mitochondrial membrane potential for the generation of the energy molecule ATP has been extensively studied in the pancreatic cell [5]; however, so far no group has shown morphological evidence of the mitochondria being degraded within a pancreatic acinar cell that concomitantly reveals either a necrotic or apoptotic morphology. Here, we provide electron microscopic evidence of mitochondrial degradation within autophagosomes of the injured pancreatic acinar cells as a part of a general macroautophagic process.

* Corresponding author. Tel.: +91 1126593556; fax: +91 1126588663.
E-mail addresses: pkgarg@aiims.ac.in, pgarg10@gmail.com, pgarg10@hotmail.com (P.K. Garg).

http://dx.doi.org/10.1016/j.pan.2014.08.009

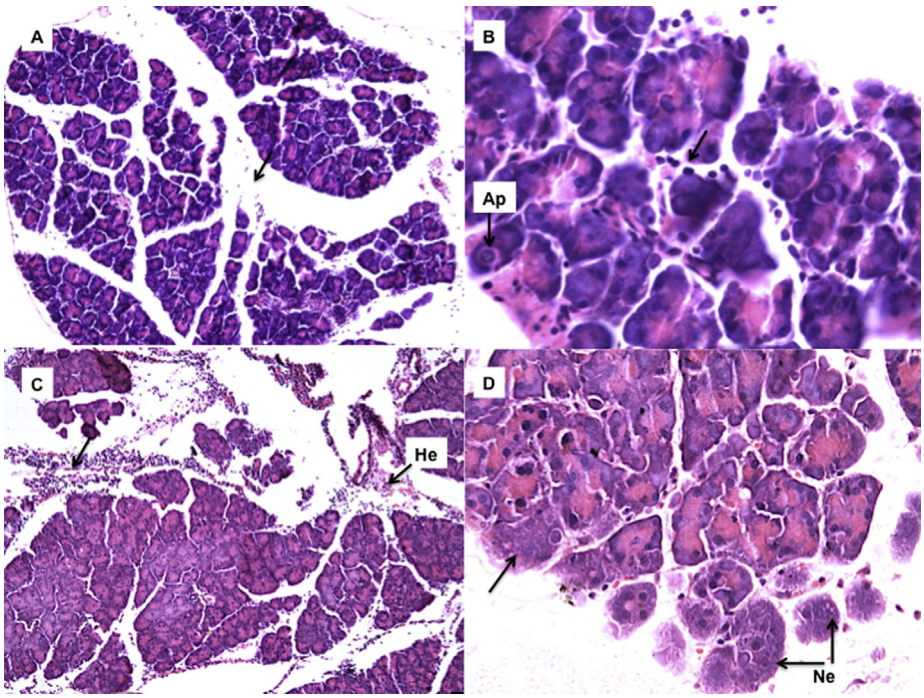

Fig. 1. Photomicrographs of H&E stained, 5μ thick sections of the pancreas derived from mice of Groups 2 ('A' and 'B') and 3 ('C' and 'D'). 'C' when compared to 'A' shows more inflammatory infiltration (arrows) and even sites of microhemorrhages (He). G2 also shows more apoptosis (Ap) than necrosis (Ne).

Methods

Caerulein induced acute pancreatitis in mice

Experiments were performed according to the protocol approved by Animal Ethics Committee of the All India Institute of Medical Sciences, New Delhi. We induced AP in adult, 4–6 weeks old, male Swiss albino mice by giving hourly injections of caerulein [Sigma, St Louis, Missouri, USA], dissolved in normal saline, intra-peritoneally at a dose of 50 μg/kg/hour. The mice were divided into three groups [G]- G1 were controls and received injections of equivalent volumes of normal saline. G2 received 4 injections of caerulein given hourly and were sacrificed 1 h after the last injection i.e. at 5th hour (low dose of 200 μg/kg). G3 received 8 injections of caerulein given hourly (high dose of 400 μg/kg) and were sacrificed 1 h after the last injection i.e. at 9th hour, as described before [6]. The animals were sacrificed by CO_2 asphyxiation. The pancreatic tissue was fixed in 4% buffered paraformaldehyde (phosphate buffer, pH 7.4) and processed for paraffin embedding. The blocks were sectioned at 5 μm thickness and stained with hematoxylin and eosin. Simultaneously, for transmission electron microscopy [TEM], 1 mm^3 blocks of the pancreas were immersed in modified Karnovsky's solution [1% glutaraldehyde, 2% paraformaldehyde in phosphate buffer, pH 7.2]. The tissues were post-fixed in 1% OsO_4, dehydrated in ascending grades of acetone, embedded and blocked in araldite CY212. After determining the regions of interest on toluidine blue stained sections, 50–60 nm thick sections were cut on a Reichert-Jung [Leica, Massachussetts, USA] ultracut microtome and collected on 300 mesh copper grids. The sections were stained with uranyl acetate and lead citrate and viewed under Philips Morgagni 268 D TEM [Field Emission Inc., Netherlands]. A MegaView III CCD camera that was integrated with the iTEM software [Olympus Soft Imaging Solutions, Münster, Germany] acquired the photographs, with instrument-calibrated scale bars.

Na-taurocholate model of pancreatitis in rats

The Na-taurocholate model of acute pancreatitis was established in adult, male, Sprague Dawley rats that weighed about 250 g. On the night prior to the surgery the animals were given free access to water, but no chow. The animals were anesthetized by ketamine (5 mg/kg) and midazolam (50 mg/kg). Under aseptic conditions, the duodenum was exposed through a small (1.5 cm) incision, inferior to the right costal margin. The common bile duct (CBD) was clamped and then saline (controls) or 2% Na-taurocholate was slowly infused into the main pancreatic duct (MPD), using a 28G needle and syringe. The abdomen was closed

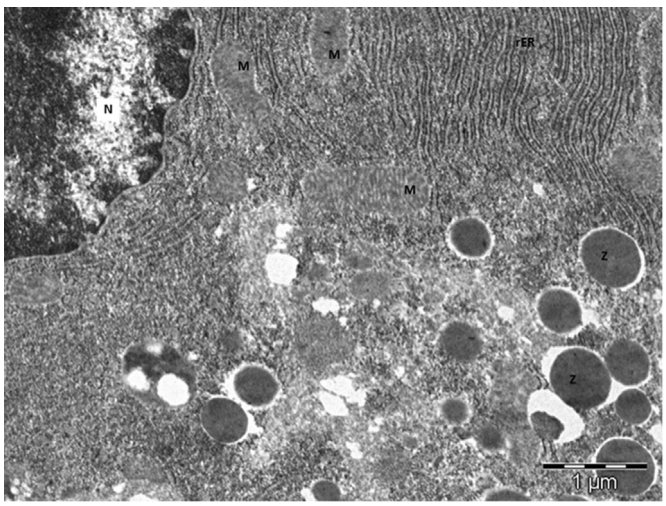

Fig. 2. Electron micrograph of a normal pancreatic acinar cell showing normal mitochondria [M], nucleus [N], zymogen granules [Z] and rough endoplasmic reticulum [rER]. Scale bar = 1 μm.

Please cite this article in press as: Jacob TG, et al., Electron-microscopic evidence of mitochondriae containing macroautophagy in experimental acute pancreatitis: Implications for cell death, Pancreatology (2014), http://dx.doi.org/10.1016/j.pan.2014.08.009