

Original Article

Changes of amount and ultrastructure of interstitial cells of Cajal in rats with severe acute pancreatitis and their importance

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Abstract: Objective: To investigate the changes in the amount and ultrastructure of interstitial cells of Cajal (ICC) in rats with severe acute pancreatitis (SAP) and their importance. Methods: A modified Aho method was employed to establish a SAP rat model. Laser scanning confocal immunofluorescence microscopy was employed to detect the amount of ICC, and transmission electron microscopy was employed for observation of ultrastructure of ICC and nerve-ICC-smooth muscle network. Results: The amount of ICC reduced, the intercellular space of ICC was enlarged, cell processes reduced or were absent, cell morphology was irregular, and ICC had unclear borderline, reduced organelles, impaired organelles, nuclear shrinkage and deformation. The junctions between ICC and between ICC and smooth muscle cell/nerve reduced, and the network-like structure was disrupted. Conclusion: In SAP, ICC reduces, with disrupted ultrastructure, and the integrity of network among intestinal nerve, ICC and smooth muscle is impaired, both of which may affect the intestinal functions.

Keywords: Severe acute pancreatitis, intestinal cells of Cajal, c-kit positive cells, ultrastructure

Introduction

Severe acute pancreatitis (SAP) is a disease significantly threatening the human health. It usually presents rapid progression and has a high mortality. It is shown that gastrointestinal motility disorder plays important roles in the whole process and prognosis of SAP and is crucial for the progression of SAP [1]. Interstitial cells of Cajal (ICC) are pivotal in the occurrence and regulation of gastrointestinal motility disorder. In the intestine, c-kit positive cells are mainly mast cells and ICC [2]. To date, c-kit has been used as a marker of ICC to investigate the distribution and density of ICC, as well as the relationship between ICC and other intestinal cells [3]. In the present study, the amount and ultrastructure of c-kit cells (ICC) were investigated, aiming to explore the role of ICC in the gastrointestinal motility disorder of SAP rats.

Materials and methods

Animals

Healthy adult Sprague-Dawley (SD) rats (specific pathogen free) aged 2-2.5 months and

weighing 160-200 g were purchased from the Experimental Animal Center of the First Affiliated Hospital of Shanghai Jiaotong University.

Establishment of SAP animal model

The modified Aho method was employed to establish the SAP animal model. A total of 12 healthy adult SD rats were used in the present study with 8 rats in SAP group and 4 in control group. Animals received food deprivation for 12 h, but were given ad libitum access to water before surgery. About 15 min before surgery, animals were intraperitoneally anesthetized with 0.2% sodium pentobarbital. Then, a mid-line incision (4 cm) was made at the abdomen, and the outlet of bile duct was identified along the inner side of the duodenum. Two clamps were used to clamp the bile duct at the hepatic hilum and the duodenal papilla, respectively, and 5.5-gauge needle was retrogradely inserted into pancreatic duct, followed by injection of 50 g/L sodium taurocholate (1 mL/kg) at a rate of 0.1 mL/min. The pancreas was observed for 8-10 min, and then the clamps were released.

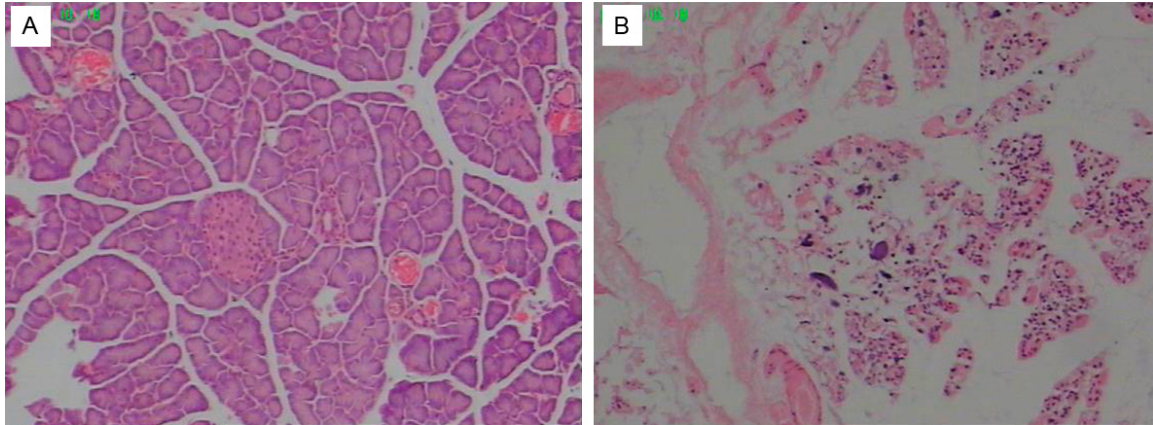


Figure 1. A HE staining shows the pancreas massive hemorrhage and necrosis, absence of normal acini and ducts and infiltration of inflammatory cells in SAP group. A. Control group; B. SAP group.

When active bleeding was not observed, the wound was closed. In control group, the pancreas was touched, without injection of sodium taurocholate.

Detection of c-kit positive cells by Laser scanning confocal immunofluorescence microscopy

1) Preparation of intestine samples: Animals (8 rats with SAP and 4 normal rats) were sacrificed by cervical dislocation. The stomach (upper 1/3), intestine (5 cm away from Treitz ligament) and colon (5 cm away from the ileocecal valve) were collected and washed in normal saline, and 30- μ m frozen sections were prepared. These sections were fixed in acetone for 0.5 h, washed in PBS and then stored at 4°C for use.

2) Laser scanning confocal microscopy: Sections at 4°C were allowed to stay at room temperature for 10 min, blocked with 1% bovine serum albumin, treated with primary antibody (rabbit anti-c-kit polyclonal antibody) and then with secondary antibody (FITC conjugated goat anti-rabbit IgG) in dark, washing in PBS and mounted with glycerin in PBS. These sections were immediately observed under a laser scanning confocal microscope (LSCM, Carl Zeiss LSM-510, Jena, Germany). In negative control group, the primary antibody was replaced with primary antibody. Rats in control group served as controls.

Detection of ICC ultrastructure and intestinal nerve-ICC-smooth muscle network by transmission electron microscopy

1) Sample collection and fixation: Animals (8 rats with SAP and 4 normal rats) were sacri-

ficed by cervical dislocation. The stomach (upper 1/3), intestine (5 cm away from Treitz ligament) and colon (5 cm away from the ileocecal valve) were collected and washed in normal saline. These tissues were placed on a filter and then fixed in 2.5% glutaraldehyde (pH 7.4) over night.

2) Transmission electron microscopy (TEM): After fixation, tissues were cut into blocks (0.2 cm \times 0.5 cm) and washed with 0.1 M PBS, followed by fixation in 1% osmic acid for 1.5 h. After dehydration in a series of ethanol solution, tissues were embedded in EPON812, and cut into 50-60 nm sections with a microtome (LKB-I). Sections were subjected to double staining with 3% uranyl acetate and lead citrate. Finally, these sections were observed under a transmission electron microscope (PHILIPS CM-120, Holland), and representative photographs were captured.

Statistical analysis

Statistical analysis was carried out using SPSS 16.0 (IBM, Chicago, USA). Data were expressed as means \pm standard deviation. Difference of the fluorescence intensity between SAP and control group was assessed for significance using the independent-sample t-test. A *P* value of less than 0.05 was considered statistically significant.

Results

Pancreatic pathology of SAP rats

When compared with normal rats (**Figure 1A**), the pancreas showed massive hemorrhage and necrosis, absence of normal acini and ducts

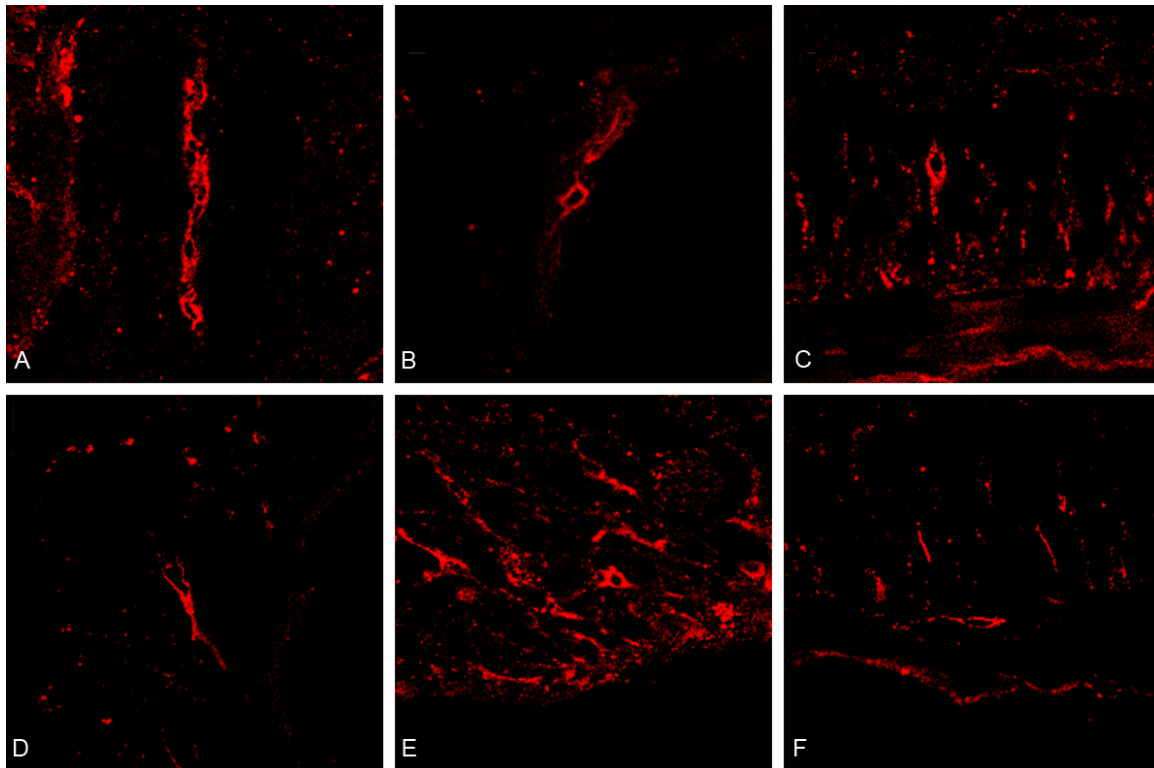


Figure 2. Distribution and morphology of ICC. A, C, E. control group. ICC formed a network; B, D, F. SAP group, the ICC network was absent and the amount of ICC reduced significantly.

and infiltration of inflammatory cells in SAP group (**Figure 1B**), suggesting that the animal SAP model was successfully established.

Detection of ICC by laser scanning confocal microscopy

c-kit was used as a marker of ICC to identify ICC in rats. Laser scanning confocal microscopy showed ICCs in control group formed a network and were distributed from mucosa to myometrium, some of ICCs were fusiform and had plump cell body, thin processes or multi-level projections were observed at both ends of ICCs; some ICCs showed spot-like and/or filamentous distribution (**Figure 2A, 2C and 2E**). The fluorescence intensity can reflect the network-like structure and amount of c-kit positive cells. So, we found that, when compared with control group, the amount of c-kit positive cells reduced significantly and network-like structure was also disrupted in SAP group (21.05 ± 7.86 vs. 3.895 ± 7.86 $t=3.163$, $P<0.05$) (**Figure 2B, 2D and 2F**).

ICC ultrastructure in control group

TEM scan for ICC ultrastructure shows: ICCs were largely spindle-shaped and had long and

abundant processes. The nucleus was large and irregular, had a large amount of chromatin and accounted for a large part of the cell. The cytoplasm was relatively less, and the cytoplasm and processes were rich in mitochondria, well-developed Golgi complexes, free ribosomes, rough endoplasmic reticulum and smooth endoplasmic reticulum. There were a lot of intermediate filaments and filaments, and there was no myofilament, which was different from smooth muscle cells. The hole-like depression in the cell membrane of ICC was a characteristic different from other fibroblasts, macrophages and glial cells (**Figure 3A**).

Normal SMC-ICC-NF network in control group

ICC and NF (**Figure 3C**): Nerve endings and nerve bundles were usually accompanied, the cell body was close to nerve fibers, and the long and abundant processes wrapped and stretched into nerve bundles, were close to nerve endings and formed junctions with cells. ICC and SMC (**Figure 3D**): ICCs were close to SMCs. Processes of ICC formed junctions with SMCs, and tight junctions were also observed between SMCs. ICCs were close to neurons,

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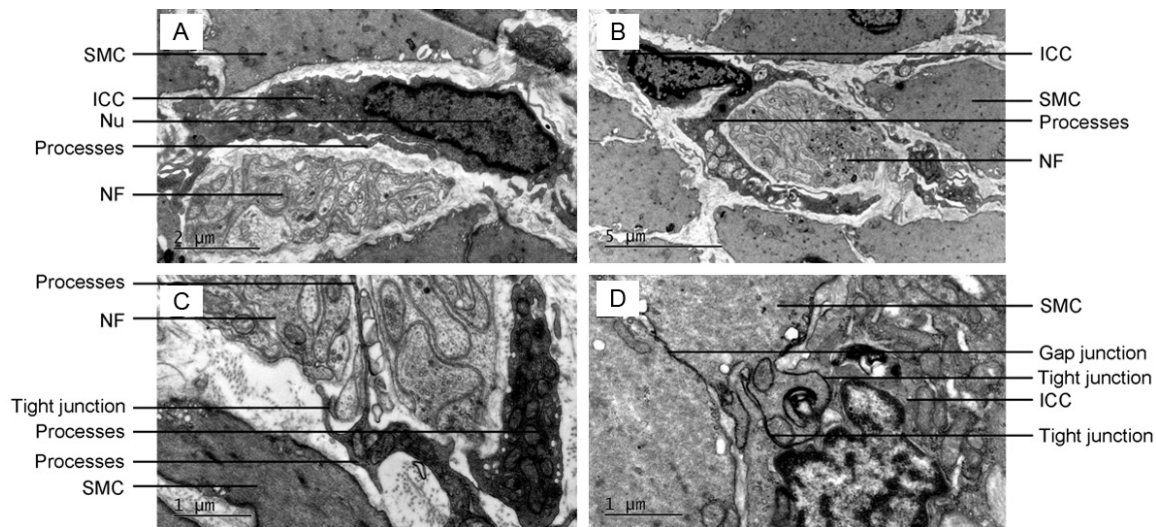


Figure 3. ICC ultrastructure in control group. A. ICCs were Fusiform, had long and abundant processes and large nucleus which was largely irregular, accounted for a large proportion of a cell and had non-solidified chromatin; the cytoplasm and processes were rich in mitochondria, well-developed Golgi complexes, free ribosomes, rough endoplasmic reticulum and smooth endoplasmic reticulum (stomach); B. SMC wrapped ICC and NF, and processes of ICC wrapped NF and were close to SMC (stomach); C. Long processes of ICC stretched into NF (colon) and cell junction was observed between ICC and NF (colon); D. Cell junction between SMC and SMC as well as SMC and ICC (intestine). Smooth muscle cells, SMC; interstitial cells of Cajal, ICC; nerve varicosities; nerve fiber, NF; mitochondrion, M; Golgi apparatus, G; rough endoplasmic reticulum, RER; smooth endoplasmic reticulum, SER; ribosome, R; nucleus, Nu.

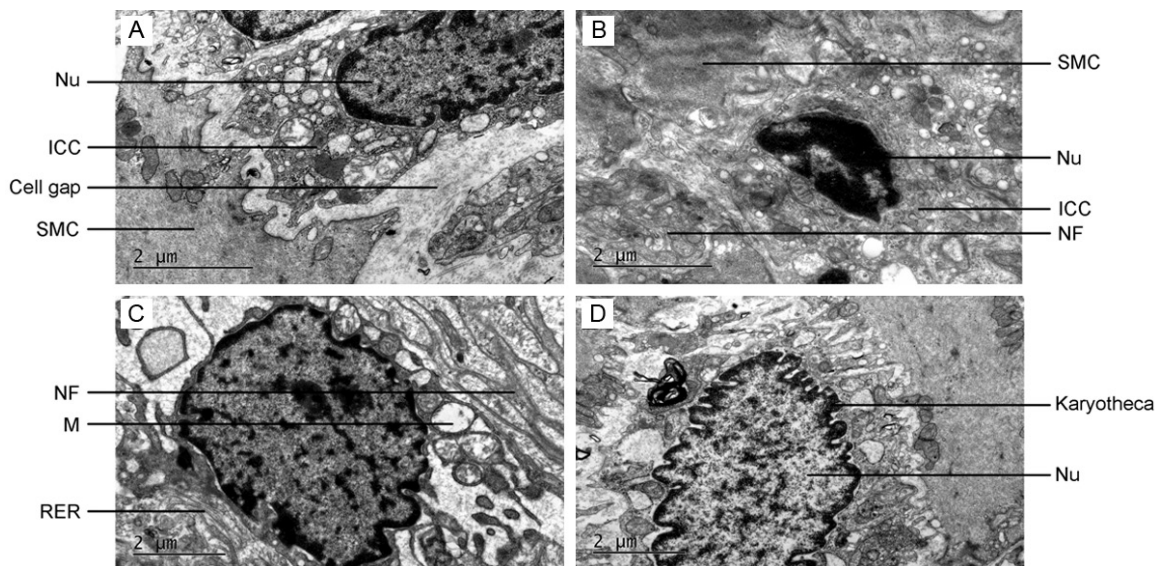


Figure 4. Ultrastructure and network-like structure of ICC in SAP group. ICCs were irregular, had no processes, organelles reduced, and cell structure was damaged. Junctions among ICC, SMC and NF reduced, and the network-like structure was damaged. A. Colon; B. Intestine; C. Stomach; D. Colon. Smooth muscle cells, SMC; interstitial cells of Cajal, ICC; nerve varicosities; nerve fiber, NF; mitochondrion, M; Golgi apparatus, G; rough endoplasmic reticulum, RER; smooth endoplasmic reticulum, SER; ribosome, R; nucleus, Nu.

and gap junctions were observed between nerve fibers and ICCs. SMC-ICC-NF (**Figure 3B**): A lot of SMCs wrapped ICCs and NF, and junctions were observed between SMCs.

ICC ultrastructure of SAP rats

ICC ultrastructure Cell gaps were enlarged; cell processes reduced or were absent, a lot of

peripheral projections were ruptured and had no cytoplasmic contents. Cells were irregular and had no clear borderline, cell membrane became bubble-like, vacuoles formed in the cytoplasm, some ultrastructure was unclear, and lysosomes increased. Organelles significantly reduced and became blurred, their structure was damaged, and mitochondria were swelling, reduced, formed vacuoles or even ruptured. Endoplasmic reticulum was dilated and showed degranulation, some filaments and intermediate filaments were expelled and large lipid droplets were present. Nucleus became shrinkage, showed irregular borderline and had jagged chromatin solidification (**Figure 4A-D**).

SMC-ICC-NF network in SAP group

A large amount of tabular bodies were present between ICCs, there were a lot of liquid substances and disordered collagens in the intercellular gaps. The processes of ICCs were damaged and reduced significantly or were absent. Gap junctions reduced among ICCs, SMCs and NF, intercellular gap was obvious, and network-like structure was damaged (**Figure 4A**).

Discussion

Gastrointestinal motility disorder plays important roles in the progression and prognosis of SAP. However, the mechanism underlying the relationship between SAP and gastrointestinal motility disorder is still poorly understood.

ICCs are a special type of mesenchymal cells in the gastrointestinal system and were first identified by Cajal (a Spanish neuroanatomist) in 1893. To date, it is known that ICCs have following activities: acting as pacemaker cells of gastrointestinal motility, to promote gastrointestinal electrical activity propagation and regulate neurotransmitters.

Ward and Sander [4] found that the intracellular Ca^{2+} concentration controlled by the endoplasmic reticulum and mitochondria is essential for the instantaneous pacemaker current of ICCs. They investigated ICCs with or without SMCs, and found that Ca^{2+} induced pacemaker current was controlled by inositol triphosphate (IP3). That is, IP3 mediates the release of Ca^{2+} into mitochondria and also the re-uptake of Ca^{2+} , which periodically increases the intracellular Ca^{2+} and results in Ca^{2+} oscillation. This

oscillation is crucial for the generation of slow mode wave. The increase in the intracellular Ca^{2+} may activate Ca^{2+} dependent inward current, resulting in membrane depolarization, which further induce the IP3 generation and activates the voltage-dependent Ca^{2+} channel, causing obvious Ca^{2+} influx and subsequent action potential [5]. On the basis of above findings, the spontaneous rhythmic contraction and periodic slow mode wave in gastrointestinal smooth muscles are as a result of Ca^{2+} oscillation induced ICC pacing. Thus, the integrity of ICC structure, especially the ultrastructure, is crucial for their normal functions (such as pacing). ICCs form network-like structure among them, and there are network function units among motor nerve terminals of enteric nervous system (ENS), ICCs of myenteric plexus and smooth muscle cells (SMCs), which are the structural basis of slow wave propagation and neurotransmitter-mediated effects and in which gap junctions play important roles [6-13].

Li et al [14, 15] found that there was intestinal disorder in SAP guinea pigs and they proposed that the reduction in ICCs of intestinal myenteric plexus was an important factor causing intestinal motility suppression. Li et al [16] found that the interstitial nerve-ICC network structure was disrupted in MODS rats, and they proposed that the intestinal motility disorder was related to the injury of intestinal nerve-ICC network in MODS rats.

Whether there is other structural abnormalities in SAP rats, except for the reduction in ICCs and whether there is disruption of nerve-ICC-SMC network are still unclear.

Our results showed the amount of c-kit positive cells reduced significantly and the network like structure was also markedly disrupted in SAP rats when compared with normal rats. In addition, results also revealed that ICCs in normal rats were largely spindle-shaped, and had long and abundant processes, large nucleus, relatively less cytoplasm and a lot of organelles. ICCs were close to neurons and usually existed accompanied by nerve endings and nerve bundles, the cell body was close to nerve fibers, cells had long and abundant processes which wrapped and stretched into nerve bundle, were close to nerve endings and formed cell junctions. ICCs were close to SMCs, processes of ICCs formed junctions with SMCs, and tight

junctions were also observed between SMCs. These findings showed intestinal nerve, ICC and SMCs formed junctions and had integrate network-like structure.

Our results also revealed that the ICC ultra-structure was significantly disrupted in SAP rats, cell gaps were enlarged, and cell processes reduced significantly or disappeared. Cells were irregular and had unclear borderline. Organelles were significantly injured and blur, mitochondria became swelling, reduced, formed vacuoles or even ruptured. Endoplasmic reticulum was dilated and showed degranulation. Nucleus became shrinkage, had irregular borderline and were serrated. Moreover, gap junctions among ICCs, SMC and intestine nerves reduced, intercellular gaps were obvious, and the network structure was significantly disrupted. The damage to organelles of ICCs (such as mitochondria, Golgi complexes and endoplasmic reticulum, especially the mitochondria) may affect the Ca^{2+} oscillation and finally influence the pacing of ICCs. Network structure of ICCs was injured, and gap junctions were also damaged or absent, which also disrupted the network function units among intestinal nerves-ICC-SMCs, affecting the electrical propagation of ICCs and the neurotransmitter-mediated signal transduction in the intestine. These changes finally caused intestinal motility disorder in SAP rats.

Taken together, ICCs reduce, their ultrastructure is significantly injured, and the integrity of network structure among intestinal nerves, ICC and SMCs is also disrupted. Our study preliminarily elucidates the role of ICCs in the intestinal motility disorder in case of SAP. To further explore the potential mechanism underlying the ICCs mediated intestinal motility disorder in case of SAP may enrich the theory on the pathogenesis of intestinal motility disorder in SAP and provide evidence for the investigation and treatment of intestinal motility disorder in SAP. In future studies, the repair of ICC and relevant network structure may be one of important strategies for the treatment of intestinal motility disorder in SAP.

Disclosure of conflict of interest

None.

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