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Original Article

Interstitial cells of Cajal in rats with severe acute pancreatitis

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Abstract: Many investigations have established the role of interstitial cells of Cajal (ICC) in normal functions of the gastrointestinal tract. ICC formed network structure with each other, with enteric motor nerve terminals and surrounding smooth muscle cells. ICC network is the structural foundation in providing pacemaker activity, propagation pathways for slow waves, transduction of inputs from motor neuron, and mechanosensitivity. Loss of interstitial cells has been associated with motor disorders of the gastrointestinal tract. This study aims to observe the changes of ICC in rats with severe acute pancreatitis (SAP), speculate the role of ICC in the pathogenesis of gastrointestinal dysmotility in SAP, and provide new insights into pathological mechanisms of gastrointestinal motility disturbances in SAP, suggesting that the repairing of reduced ICC and damaged ICC network maybe a potential therapeutic method.

Keywords: Interstitial cells of Cajal, severe acute pancreatitis, gastrointestinal tract, immunohistochemitry, ultrastructure

Introduction

Interstitial cells of Cajal (ICC) were first described by the Spanish neuroanatomist Santiago Ramony Cajal in the muscle coat of the gut in 1893. Since then, a number of studies have focused on the nature of ICC. Especially in the most recent three decades, technological advances have resulted in a multitude of studies confirming the distribution and normal functions of ICC in gastrointestinal tract [1-3]. Nowadays we have found ICC in all parts of digestive systems (from esophagus to rectum). Several subpopulations of ICC throughout the GI tract have been identified (based on their anatomical location in the muscle wall): subserosal ICC (ICC-SS); myenteric ICC (ICC-MY); intramuscular ICC (ICC-IM); ICC of the deep muscular plexus (ICC-DMP); septal ICC (ICC-SEP) and submucosal ICC (ICC-SM). Some compelling evidences have suggested that ICC is pacemaker cells, mediate enteric motor neurotransmission and provide mechanosensitive regulation of pacemaker activity [4-9]. Absence, reduction in number or altered integrity of ICC network result in gastrointestinal motility dysfunctions, which lead to a number of gastrointestinal diseases [4], including severe acute pancreatitis (SAP). Furthermore, investigators have found that damage of ICC occurred in the small intestine in experimental acute pancreatitis [10].

Despite the association of SAP with gastrointestinal motility disturbances on the basis of evidence acquired through both clinical [11] and experimental investigations [12, 13], the detailed mechanisms of the changes in gastrointestinal motility in SAP are not clear. Thus, we hypothesized that ICC might play an important role in the pathogenesis of gastrointestinal dysmotility in SAP. In the present study we tested our hypothesis in a rat model of SAP.

Materials and methods

Animal model establishment

Sixteen adult male Sprague-Dawley (SD) rats with body weight between 200 g and 250 g were purchased from the animal research cen-

ter of the Central Hospital of Songjiang District, Shanghai and randomly divided into two groups of equal number (n = 8 each): the control (C) group and the severe acute pancreatitis (SAP) group. To establish the SAP rat model, freshly prepared 5% sodium taurocholate solution was injected at a volume of 1.0 mL/kg from the duodenal papilla into the pancreatic duct. In the C group, the duodenum and pancreas of animals were manually manipulated and scratched lightly by blunt a few times after laparotomy. All procedures took place under sterile conditions and all animals were housed under pathogen-free conditions in the animal facility with a 12-h light/dark cycle and free access to food and water. The study protocol was approved by the Central Hospital of Songjiang District, Shanghai.

Histopathologic examination of the pancreas and the jejunum

Both the pancreas and the jejunum were removed at the time of harvest of the small intestine described above. Four segments of the jejunum 15 cm distal to the ligament of Truiz, approximately 10 mm each in length, were collected for the following study. One segment of the jejunum was opened, cleaned, and inspected macroscopically along with the pancreas that was transversely sectioned, for visible pathologic changes. After gross examination, both organs were fixed with 10% buffered neutral formalin solution for 24 h. The tissue from both organs was sectioned at 3 μm in thickness. Histology sections were stained with hematoxylin and eosin and evaluated microscopically by experienced pathologists.

Immunohistochemical staining

The segments of jejunum harvested previously were immersed in a fixative containing 4% paraformaldehyde for 6 h at 4°C. Then the segments were embedded with the optimum cutting temperature compound and sectioned at 10 μm in thickness. The tissue section was mounted on glass slides. For the c-kit staining, tissue sections were incubated with 0.3% Triton X in 10% normal rabbit serum for 60 min and then incubated with the goat anti-c-kit polyclonal antibody (clone sc-1494; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, United States) at 4°C overnight. Next, we applied a biotin-free polymeric horseradish peroxidase (HRP)-linked antibody conjugate system for 20 min followed by DAB condensed chromogen for 5 min. Tissue sections were counterstained with hematoxylin and eosin (HE). For negative control experiments, the primary antibody was omitted. The positive cell density was assessed with the Image-Pro plus 6.0 software (Media Cybernetics, Bethesda, MD, United States).

Electron microscopy

Immediately after resection, blocks of jejunal tissue were cut and immersed into a fixative containing 5% glutaraldehyde and stored at 20°C for at least 2 h. Following fixation, tissues were cut into small pieces (1 mm × 2 mm) and further fixed in 5% glutaraldehyde overnight, and then rinsed for 60 min in 0.1 mol/L phosphate buffer, pH 7.3, and postfixed in 2% OsO_4 in 0.1 mol/L phosphate buffer for 2 h. The tissue specimens were subsequently dehydrated and embedded. Thin sections were cut at 1 μm in thickness and stained with toluidine blue for light microscopy to select suitable areas for ultra-thin sectioning. Ultrathin sections were cut at 70-80 nm, mounted onto copper grids, and stained with lead citrate for electron microscopy with a Philips Morgagni 261 EM microscope.

Statistical analysis

The data obtained were expressed as mean ± SD. Comparison between the two groups was performed by using the Student t-test, and the differences with $P<0.05$ were considered as statistically significant. All data were analyzed with SPSS 13.0 software (SPSS Inc., Chicago, IL, United States).

Results

Pathological changes

Under gross examination, the pancreas and jejunum in the SAP group appeared edematous at 24 h. The jejunum was full of yellow intestinal juice and ascites, and adhesions of organs were observed in 1 rats of the SAP group. Under light microscope examination, the pancreas from the C group exhibited no signs of pancreatitis (**Figure 1A**). Histological evaluation of the pancreas in rats with SAP revealed widespread parenchymal cell necrosis accompanied by edema, visible hemorrhage and inflammatory cell infiltrate (**Figure 1B**). In the C group, the

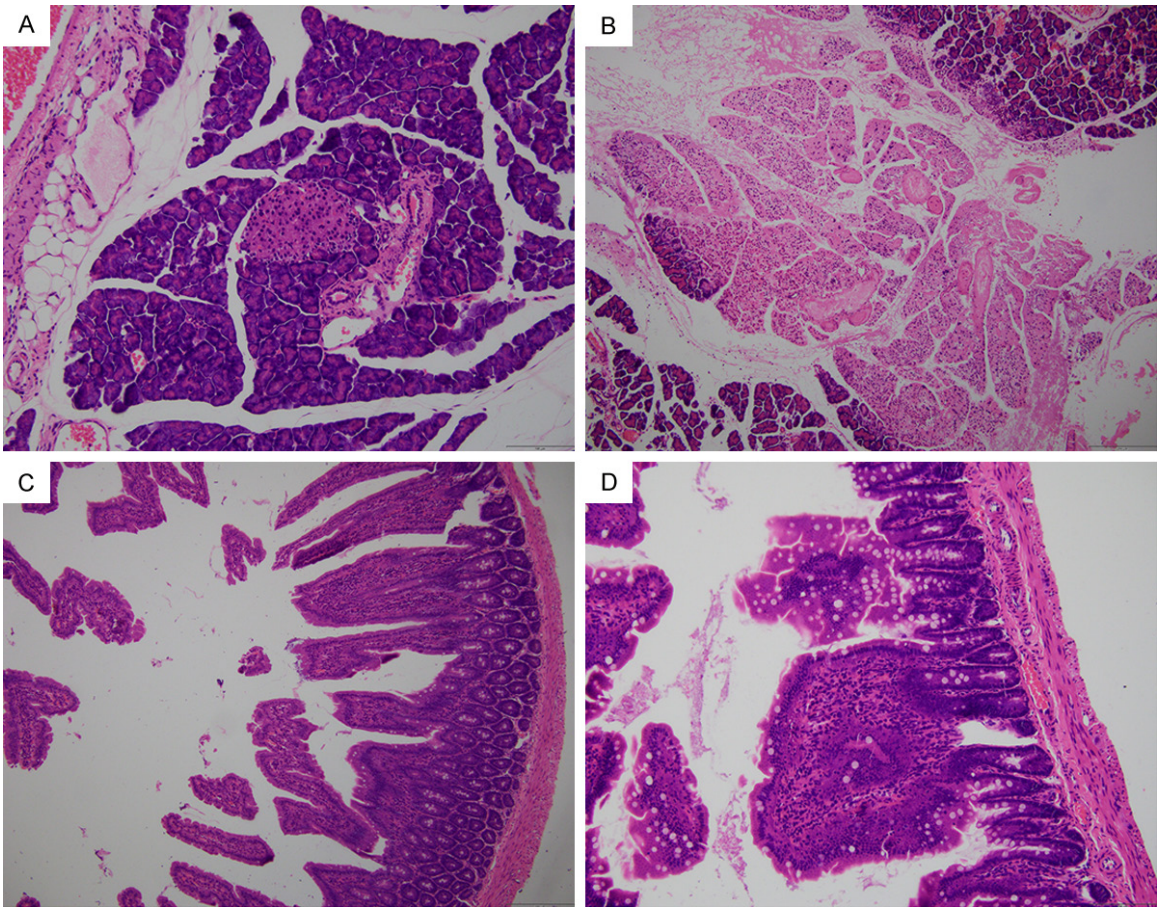

Figure 1. Histological sections from pancreas and jejunum. A: The pancreas of the control (C) group shows a normal lobular architecture, comprising of exocrine and endocrine components; B: The pancreas of severe acute pancreatitis (SAP) rats shows obvious necrosis of the parenchymal cells accompanied by edema and hemorrhage; C: The structure of jejunum in the C group is normal, villi are slender and finger-like; D: The mucosal villi were markedly shortened and low, with partially mucous epithelium absent, the submucosal and muscular layers were edematous in the SAP group. (A, D: Magnification × 200; B, C: Magnification × 100).

structure of jejunum was normal, intestinal villi are slender and finger-like (**Figure 1C**). In the SAP group, the mucosa partially denuded, villi were markedly shortened and low, with partially mucous epithelium absent, submucosal and muscular layers showed slight alteration characterized by dilated thickness (**Figure 1D**).

Immunohistochemical staining

Including interstitial cells of Cajal, mast cells within the mucosa layer also show c-kit-positivity (**Figure 2A**). Two cell populations have unequivocally different shapes. ICC predominantly located in the submucosal layer and with large oval nuclei, sparse cytoplasm and 2-5 branching processes, ICC predominantly situated in the submucosal layer (**Figure 2B**) and in the muscularis propria (**Figure 2C**). The number of c-kit-positive cells in the SAP group was significantly lower than in the C group (22.12±7.24 vs 50.23±8.16, $P<0.05$) (**Figure 2D**).

Ultrastructure of ICC

ICC profiles in control tissue are present in fusiform shapes. The nucleus of ICC is very voluminous surrounded by a small perinuclear cytoplasm that expands with 2-5 long cytoplasmic processes. The cytoplasm of these cells presents a higher electron density than the cytoplasm of the surrounding muscle cells. ICC contains mitochondria, rough and smooth endoplasmic reticulum, thin and intermediate filaments, caveolae, Golgi apparatus, free ribosomes and cytoplasmic vesicles. They are closely associated with each other and surrouding cells, some of them are intercalated

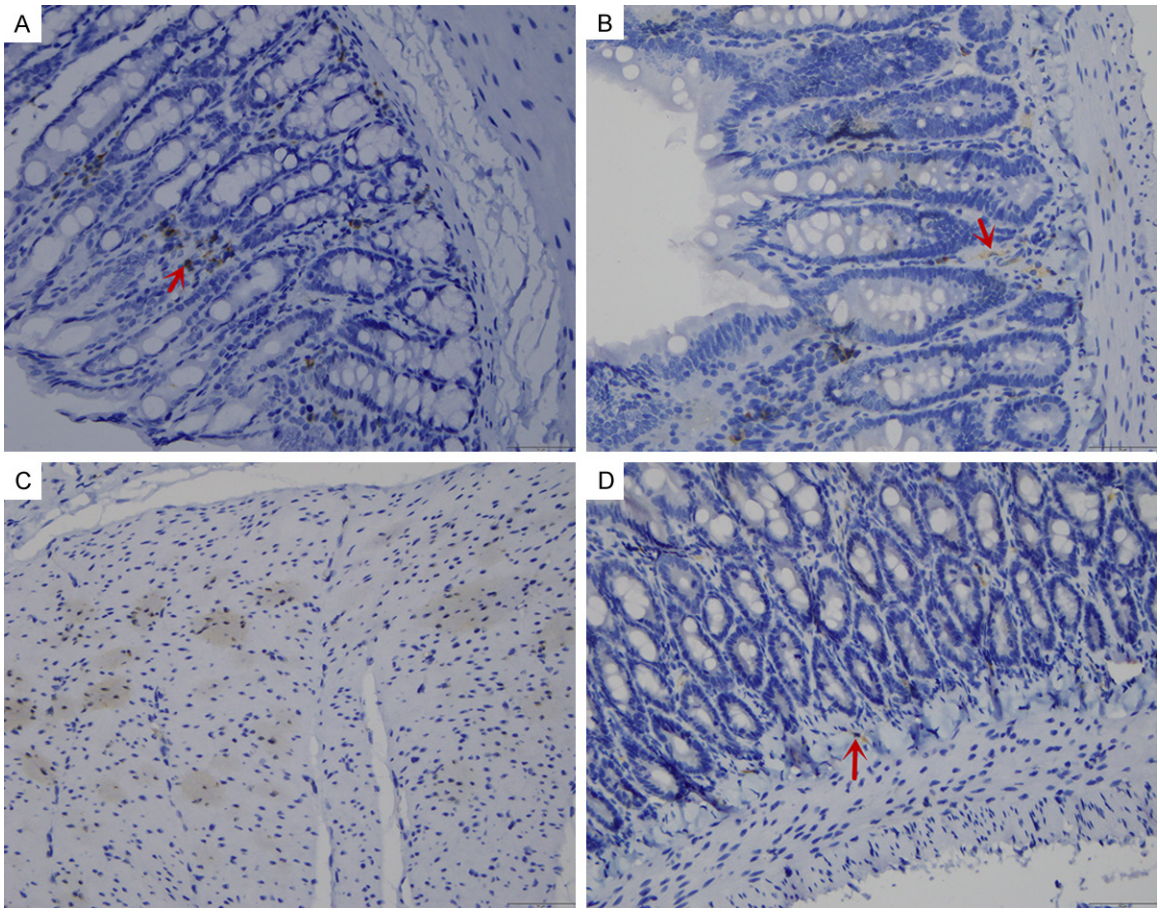

Figure 2. Immunohistochemistry for c-kit. A: Mucosal mast cells in rats of the control group show c-kit positivity (internal control); B: c-kit-positive interstitial cells of Cajal (ICC) in the submucosal layer in the control group; C: c-kit-positive interstitial cells of Cajal (ICC) in the muscularis propria in the control group; D: Decreased c-kit-positive ICC in the severe acute pancreatitis group. Magnification × 400.

between nerves and smooth muscle cells, network with them (**Figure 3A-D**).

In contrast with control tissues, confluent vacuoles were frequently present in ICC in tissue from the SAP group. Mitochondria appeared damaged in some vacuolated processes (**Figure 3E**). Ultrastructural preservation of other cellular elements and organelles was mostly unaffected. ICC processes were weakened. Depletion of ICC network was also seen (**Figure 3F**).

Discussion

SAP is a very common and serious clinical disease and its mortality rate ranges from 10% in the case of sterile necrosis to 25% in the case of infected pancreatic necrosis [14, 15]. Many studies have indicated that gastrointestinal dysmotility in rats with SAP could lead to the translocation of bacteria from the gut, thus resulting in pancreatic infections which have been suggested to be a major cause of death in SAP [16, 17]. So it is very important to investigate the possible mechanisms of gastrointestinal dysmotility in SAP in order to reduce the mortality rate of SAP.

We used retrograde injection of 5% sodium taurocholate from the duodenal papilla to establish an SAP rat model. Pancreatic pathological changes, such as pancreatic hemorrhage, necrosis and infiltration of inflammatory cells, could be observed at 24 h after modeling. All these changes were consistent with patients with SAP. This demonstrated that the animal model of SAP was successfully established. Our results confirmed that experimental SAP induced intestinal motility disturbances as previously shown [18].

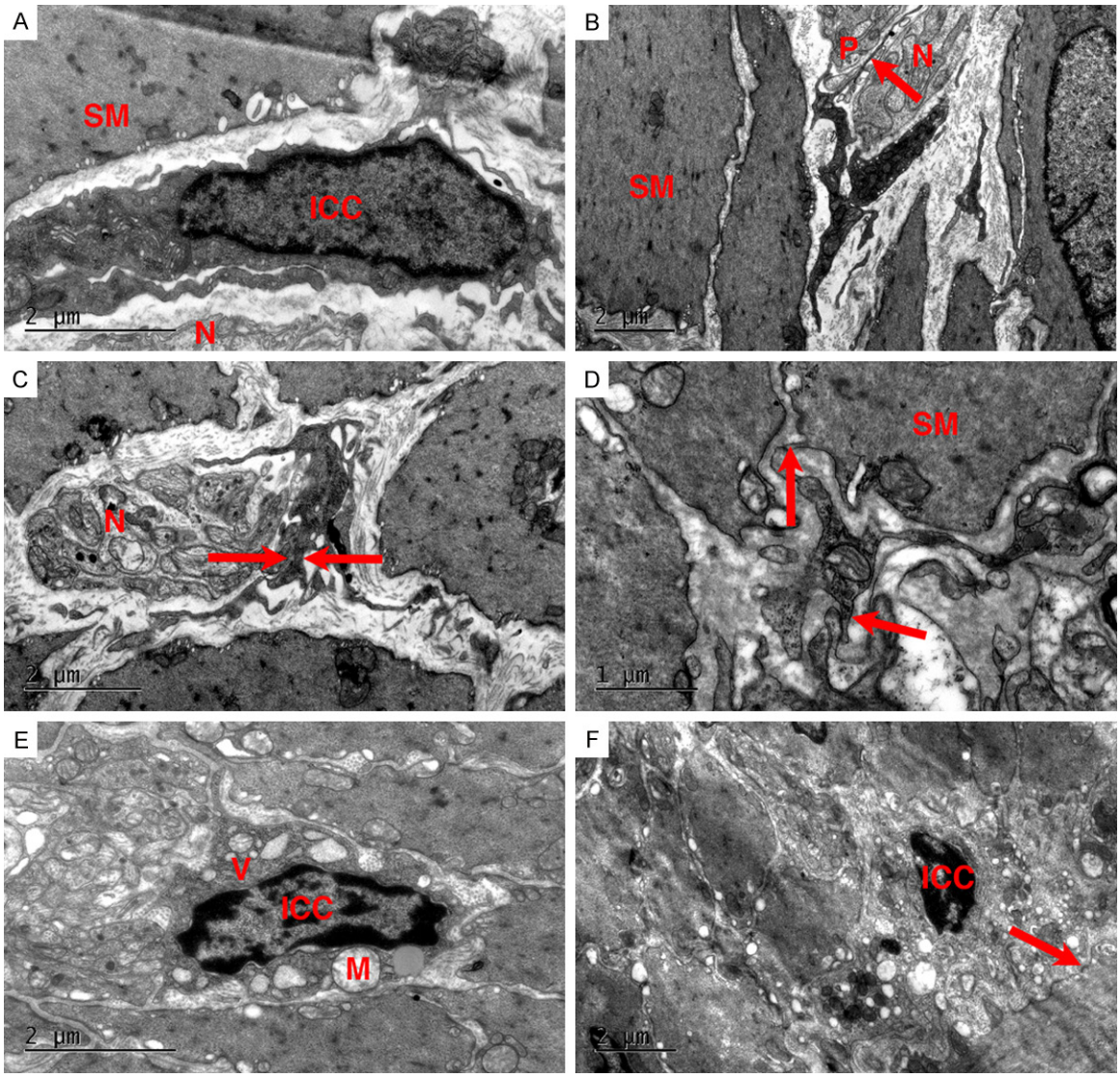

Figure 3. Ultrastructure of interstitial cells of Cajal. A-D: Control. A: Interstitial cells of Cajal (ICC) with fusiform nuclear morphology show an elongated nucleus with scarce perinuclear cytoplasm, and are situated between the smooth muscle (SM) and the enteric nerve (N), network with them; B: The cytoplasmic processes (P) extended among enteric nerves(N); C: Closed associations and synaptic-like specializations between ICC and enteric neurons (arrows); D: Gap junctions between ICC and smooth muscle (arrows); E, F: Severe acute pancreatitis. E: Vacuolated mitochondria (M) and Vacuoles (V) are present in ICC; F: The density of desmosome-like junctions between ICC and smooth muscle is lower (arrows).

So far, the pathogenic mechanisms of pancreatitis-induced intestinal motility disturbances have not been clearly elucidated. It is well documented that ICC are implicated in the control of gastrointestinal motility. For example, decreased numbers or disrupted networks of ICC are associated with a number of human gastrointestinal motility disorders [19], including slow transit constipation, pseudo-obstruction and diabetic enteropathy. The potential role of ICC in the pathogenesis of gastrointestinal dysmotility in SAP has attracted attention. ICC can be classified into several subtypes according to their location in the gut wall; ICC-MY are multipolar and possess multiple primary processes which branch further contact and connect electrically with neighboring cells to produce a complex 3-dimensional network [4]. The ICC-MY network has been implicated as the primary pacemaker region in the stomach and small intestine and to be involved in generating higher frequency activity in the colon.

Compelling evidences have suggested that ICC-MY are the primary pacemakers of the GI tract [4]. ICC-MY generates slow waves, and studies have confirmed that damage in the network of ICC-MY resulted in change of spontaneous mechanical contractions of the gut in a variety of human disease processes. All these studies were focused on ICC-MY and spontaneous mechanical contractions. In addition to generating slow waves, other subsets of ICC are engaged in mediating enteric neural signals to the smooth muscles and acting as mechanosensors. In the present study, total ICC were observed by immunohistochemical staining. Within the submucosal layer and in the intramuscular bundle, we have demonstrated a decrease of c-kit-positive cells in these regions in the SAP group.

Investigators have examined the ultrastructure of ICC by transmission electron microscopy in their intestinal obstruction model [20] and surgical resection model [21]. These findings all suggested that an actual change in ICC phenotype occurred from the ultrastructural appearance. Moreover, functionally mature ICC redifferentiated toward a smooth muscle cell phenotype when kit receptors were blocked [22]. Similarly, in our study, morphological changes such as vacuolation of mitochondria, irregular vacuoles and loosened gap junctions were present in ICC in the SAP group, while the ultrastructure of ICC is normal in the control group. Although we did not investigate the amplitudes and frequencies of slow waves of the jejunum generated by ICC, it could be speculated that loss of ICC and altered network integrity influenced the function of ICC and eventually resulted in gastrointestinal dysmotility.

In the gastrointestinal tract, development and maintenance of the ICC phenotype have been linked to intracellular signaling *via* c-kit. Beckett et al [23] have shown that blocking c-kit signaling during late gestation results in failure of ICC networks and pacemaker function to develop in the murine small intestine.

In conclusion, this study has disclosed that decreased c-kit-positive cells and degenerative ICC network were present in the jejunum of rats with SAP, and that all these changes resulted from blockade of the c-kit signaling pathway. This study may provide new insights into pathological mechanisms of gastrointestinal motility disturbances in SAP. Since loss and proliferation of c-kit-positive cells lead to a variety of human gastrointestinal motility disorders [21] and gastrointestinal stromal tumors, thus developing the means to manipulate the ICC phenotype may have profound therapeutic benefits for these patients.

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Disclosure of conflict of interest

None.

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References

[1] Aranishi H, Kunisawa Y and Komuro T. Characterization of interstitial cells of Cajal in the subserosal layer of the guinea-pig colon. Cell Tissue Res 2009; 335: 323-329.

[2] Blair PJ, Bayguinov Y, Sanders KM and Ward SM. Interstitial cells in the primate gastrointestinal tract. Cell Tissue Res 2012; 350: 199-213.

[3] Komuro T. Structure and organization of interstitial cells of Cajal in the gastrointestinal tract. J Physiol 2006; 576: 653-658.

[4] Blair PJ, Rhee PL, Sanders KM and Ward SM. The significance of interstitial cells in neurogastroenterology. J Neurogastroenterol Motil 2014; 20: 294-317.

[5] Sanders KM. A case for interstitial cells of Cajal as pacemakers and mediators of neurotransmission in the gastrointestinal tract. Gastroenterology 1996; 111: 492-515.

[6] Sanders KM, Ordog T, Koh SD, Torihashi S and Ward SM. Development and plasticity of interstitial cells of Cajal. Neurogastroenterol Motil 1999; 11: 311-338.

[7] Takayama I, Horiguchi K, Daigo Y, Mine T, Fujino MA and Ohno S. The interstitial cells of Cajal and a gastroenteric pacemaker system. Arch Histol Cytol 2002; 65: 1-26.

[8] Sanders KM, Ordog T and Ward SM. Physiology and pathophysiology of the interstitial cells of Cajal: from bench to bedside. IV. Genetic and animal models of GI motility disorders caused by loss of interstitial cells of Cajal. Am J Physiol

Gastrointest Liver Physiol 2002; 282: G747-756.
[9] Hirst GD and Ward SM. Interstitial cells: involvement in rhythmicity and neural control of gut smooth muscle. J Physiol 2003; 550: 337-346.
[10] Zhou H, Liu L, Bai Y, Wu W, Li G, Li J, Zou D, Gao J and Li Z. Damage of the interstitial cells of Cajal and myenteric neurons causing ileus in acute necrotizing pancreatitis rats. Surgery 2011; 149: 262-275.
[11] Wang X, Gong Z, Wu K, Wang B and Yuang Y. Gastrointestinal dysmotility in patients with acute pancreatitis. J Gastroenterol Hepatol 2003; 18: 57-62.
[12] Seerden TC, De Man JG, Holzer P, Van den Bossche RM, Herman AG, Pelckmans PA and De Winter BY. Experimental pancreatitis disturbs gastrointestinal and colonic motility in mice: effect of the prokinetic agent tegaserod. Neurogastroenterol Motil 2007; 19: 856-864.
[13] Leveau P, Wang X, Soltesz V, Ihse I and Andersson R. Alterations in intestinal motility and microflora in experimental acute pancreatitis. Int J Pancreatol 1996; 20: 119-125.
[14] Swaroop VS, Chari ST and Clain JE. Severe acute pancreatitis. JAMA 2004; 291: 2865-2868.
[15] Dervenis C, Johnson CD, Bassi C, Bradley E, Imrie CW, McMahon MJ and Modlin I. Diagnosis, objective assessment of severity, and management of acute pancreatitis. Santorini consensus conference. Int J Pancreatol 1999; 25: 195-210.
[16] Van Felius ID, Akkermans LM, Bosscha K, Verheem A, Harmsen W, Visser MR and Gooszen HG. Interdigestive small bowel motility and duodenal bacterial overgrowth in experimental acute pancreatitis. Neurogastroenterol Motil 2003; 15: 267-276.
[17] Beger HG, Bittner R, Block S and Buchler M. Bacterial contamination of pancreatic necrosis. A prospective clinical study. Gastroenterology 1986; 91: 433-438.
[18] Chen X, Valente JF and Alexander JW. The effect of sennosides on bacterial translocation and survival in a model of acute hemorrhagic pancreatitis. Pancreas 1999; 18: 39-46.
[19] Streutker CJ, Huizinga JD, Driman DK and Riddell RH. Interstitial cells of Cajal in health and disease. Part I: normal ICC structure and function with associated motility disorders. Histopathology 2007; 50: 176-189.
[20] Chang IY, Glasgow NJ, Takayama I, Horiguchi K, Sanders KM and Ward SM. Loss of interstitial cells of Cajal and development of electrical dysfunction in murine small bowel obstruction. J Physiol 2001; 536: 555-568.
[21] Rolle U, Piaseczna-Piotrowska A and Puri P. Interstitial cells of Cajal in the normal gut and in intestinal motility disorders of childhood. Pediatr Surg Int 2007; 23: 1139-1152.
[22] Torihashi S, Nishi K, Tokutomi Y, Nishi T, Ward S and Sanders KM. Blockade of kit signaling induces transdifferentiation of interstitial cells of cajal to a smooth muscle phenotype. Gastroenterology 1999; 117: 140-148.
[23] Beckett EA, Ro S, Bayguinov Y, Sanders KM and Ward SM. Kit signaling is essential for development and maintenance of interstitial cells of Cajal and electrical rhythmicity in the embryonic gastrointestinal tract. Dev Dyn 2007; 236: 60-72.