Gastrointestinal Disorders Involving ICCs and the ENS

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The enteric nervous system (ENS) is organized into two plexuses—submucosal and myenteric—which regulate smooth muscle contraction, secretion, and blood flow along the gastrointestinal tract under the influence of the rest of the autonomic nervous system (ANS). Interstitial cells of Cajal (ICCs) are mainly located in the submucosa between the two muscle layers and at the intramuscular level. They communicate with neurons of the enteric nerve plexuses and smooth muscle fibers and generate slow waves that contribute to the control of gastrointestinal motility. They are also involved in enteric neurotransmission and exhibit mechanoreceptor activity. A close relationship appears to exist between oxidative stress and gastrointestinal diseases, in which ICCs can play a prominent role. Thus, gastrointestinal motility disorders in patients with neurological diseases may have a common ENS and central nervous system (CNS) nexus. In fact, the deleterious effects of free radicals could affect the fine interactions between ICCs and the ENS, as well as between the ENS and the CNS.

Keywords: enteric nervous system ; interstitial cells of Cajal ; oxidative stress

1. Hirschsprung's Disease

HD is a congenital disorder consisting of an absence of ganglion cells in the Auerbach's and Meissner's plexuses of the distal colon [1]; this disorder is either due to an abnormal migration of the primitive cells of the neural crest between the fourth and twelfth week of gestation [1] or a degenerative process of ganglion cells by necrosis, abnormal differentiation, or changes in the cellular microenvironment [1][2]. This results in an aganglionic colon segment with absent motility and symptoms of bowel obstruction [3]. Aganglionosis may influence the proper development of intestinal pacemaker cells [4]. The most common genetic cause of HD has been associated with *RET* proto-oncogene mutation [1].

Histopathological examination of the affected colon is of great diagnostic interest because the presence of ganglion cells under the microscope rules out HD, whereas the absence of such ganglion cells together with positive staining for acetylcholinesterase in mucosal and submucosal nerve fibers diagnoses HD [1].

Studies on ICCs in these aganglionic areas have yielded contradictory results. Some studies have shown a lower cell density in affected areas, especially at the innermost levels of the circular muscle layer and in ICC-SM [5]. There are also reductions in the numbers of ICCs at the myenteric level together with an abnormal decrease in their branching between nerve trunks [6]. Other studies have shown this reduction in both resected aganglionic and ganglionic sections, suggesting that the persistence of motility problems after resection of the affected area originates in the abnormal organization of the ICCs due to either a reduction in their number or defects in their connections with the myenteric ganglia [5][6]. In immuno-histochemistry of post-operative samples from patients diagnosed with HD, the expression of connexin 43, a component of the transmembrane channels in the gap junction that connect ICCs to other structures, was decreased in aganglionic segments, favoring the lack of communication between ICCs and smooth muscle cells as being responsible for motility dysfunction [7]. There is a more severe and rare variant of HD, total colonic aganglionosis, in which c-kit-dependent immuno-histochemical alterations have also been detected, with reductions in the density of myenteric ICC networks [6].

2. Achalasia

Achalasia is described as a motor disorder of the esophagus, encompassing an absence of peristalsis together with hypertonicity of the lower esophageal sphincter, making it unable to relax [5][8]. The main characteristic of its physio-pathogenesis consists of degeneration of the ganglion cells and nerve structures of the myenteric plexus over time [8], especially in the more distal areas and lower esophageal sphincter [5]. An inflammatory etiology based on an immune reaction, perhaps due to the herpes virus or an autoimmune phenomenon with antibodies directed to the Auerbach's plexus, has been suggested [5] as a T-lymphocyte infiltrate has been observed along the affected nerve plexuses [8]. A relationship of achalasia with paraneoplastic processes has also been proposed [9].

The damage to esophageal ICC-IM does not appear to be continuous, but patchy; this may be related to the intermembrane connection between these ICCs and the mast cells present in the muscle layer [8]. It seems that the key site of damage to these structures is located in the connections between the ICCs and the neurons, which could be responsible for the alterations in motility [10], possibly due to a defect in nitrergic neurotransmission [11]. Therefore, damage to ICCs translates ultra-structurally to a loss of connections between ICCs and neurons, a reduction in mitochondria [5], a clearer and more uncluttered cytoplasm, and sparser smooth endoplasmic reticulum [10].

Notably, in the autosomal recessive genetic syndrome Allgrove, which presents with achalasia, addisonianism, and alacrimia, there is evidence of a decrease in ICCs at the level of the gastric cardia [5][9][10].

3. Hypertrophic Pyloric Stenosis

Hypertrophic pyloric stenosis (HPS) is a pediatric disease that presents with a clinical picture of post-prandial, non-bilious projectile vomiting together with a palpable “olive” at the level of the pyloric sphincter due to pyloric sphincter hypertrophy and an inability to coordinate with gastric contractions [5]. At this level, decreases in NO-producing neurons and ICCs at the level of the hypertrophic circular muscle layer have been described in both immuno-histochemical and electron microscopy morphological studies [5]. Lack of ICCs is associated with reduced anthropyloric co-ordination as slow wave generation is compromised [10].

Pylorus samples from 18 children with HPS were found to have a reduced number of ICCs at the level of the muscular layer of the hypertrophic pylorus relative to healthy controls and a decrease in anti-HO-2 immunoreactivity, which was interpreted as a decrease in CO [12]. After surgical treatment of HPS by pyloromyotomy, the set of ICCs return to their normal pattern [5].

4. Gastroesophageal Reflux

Gastroesophageal reflux (GER) is caused by transient relaxation of the lower esophageal sphincter [10]. Sphincter control in response to increased pressure in the stomach depends on vagal reflexes [13]. The presence of gastric contents in the esophagus produces esophagitis, which can lead to loss and injury of the ICCs, especially in the more advanced stages of reflux [10]. There seems to be a direct relationship between the severity of esophagitis and ICC destruction, which causes motility problems and reflux to be maintained, exacerbating GER symptoms [9][10].

5. Inflammatory Bowel Disease

IBD includes ulcerative colitis (UC) and Crohn’s disease (CD). At the microscopic level, structural abnormalities in the ICCs have been evidenced in both UC and CD, with accumulation of lysosomes and lipid vacuoles, which could represent damage secondary to inflammatory changes [5].

The relationship between ENS and IBD remains controversial. In some cases of IBD, an abundance of enteric neurons has been seen in the inflamed tracts, raising the question of whether this enteric hyperinnervation pre-exists in genetically pre-disposed patients or whether it is a consequence of cytokine-mediated inflammatory damage [14]. Rodent studies have found an increase in macrophages in the submucosal plexus [8], suggesting that the cytokines they produce are involved in structural and functional alterations in ICCs, nerves and myocytes [5]. Nevertheless, despite such structural changes, samples from IBD patients have stable c-kit labeling, possibly due to the presence of mast cells, which label positive for c-kit and form intermembrane interactions with the altered ICCs [8]. In an experimental rodent model in which an IBD-like state was induced by administration of trinitrobenzene sulfonic acid (TNBS) and dextran sulfate sodium (DSS), enteric neuronal hyperplasia was found to be a marker of severity in IBD [14].

In conclusion, reductions in the ICCs have been seen in CD in the affected areas of the small intestine, especially the circular and longitudinal muscle layer, with some changes occurring at the level of Auerbach’s plexus and ICC-DMP [5]. The involvement of the myenteric plexus is still under discussion because KIT immunoreactivity is reduced in ICC-IM and ICC-MP from small intestine samples [8]. In UC, there are increases in KIT+ immunoreactivity at the level of the ICC-IM, with mast cells found near ICC-SMP and nerve endings [8].

6. Chagas Disease

Chagas disease is caused by the protozoan *Trypanosoma cruzi* and chronic infection can cause problems visceromegaly at both the cardiac and digestive level [15]. In the digestive tract, infection can lead to degeneration of enteric neurons and

cause colorectal motility disorders, including megacolon [13]. Reductions in colon ICC density have been reported [5].

There seems to be a relationship between the chagasic megacolon and serotonin levels [16]. When comparing intestinal samples from 24 Chagas disease patients to 14 controls, patients exhibiting megacolon presented a higher concentration of mast cells and lower serotonergic expression compared to those without megacolon, who had a marked increase in serotonin [17]. Another study, which involved esophageal biopsies from 10 Chagas disease patients and 5 controls and compared the number of ICCs in the myenteric plexus, found that patients had a reduced number of ICCs in the muscle layers, which was thought to contribute to the pathogenesis of megaesophagus [15].

7. Gastrointestinal Stromal Tumors

GISTs are the most common GI tract tumors of mesenchymal origin. The majority originate in the stomach (60–70%), though 20–30% originate in the small intestine and ~10% in the esophagus, colon, and rectum [10]. They exhibit KIT and CD34 immunoreactivity, common markers of ICCs, suggesting that GISTs derive from ICCs [10]. The treatment for inoperable and metastatic GISTs is imatinib mesylate, a tyrosine kinase inhibitor targeting mutant KIT and PDGFRα isoforms [10][18].

An immuno-histochemical study found that the marker CD34 appears to be associated with GIST malignancy [19]. The fact that benign GISTs do not express CD34 could indicate that they are composed of more mature ICCs, whereas malignant GISTs may be derived from less differentiated ICCs [19]. However, another study on intestinal samples from 10 patients with GISTs to compare mutations in both the c-kit and PDGFRα genes in tumor cells with respect to ICCs adjacent to the cells found no mutations in these genes [18]. This is contrary to the theory that GISTs have their origin in ICCs or could indicate that these mutations are not germline, but acquired locally by GIST precursor cells, which contribute to tumor development along with other genetic factors [18].

8. Gastroparesia

Slow wave genesis is altered in gastroparesis, as well as in other types of functional dyspepsia [5]. In the examination of stomach samples affected by severe gastroparesis, a reduction has been demonstrated in the number of ICCs in the myenteric and intramuscular plexuses, together with hypoganglionosis and neuronal dysplasia [5]. Similar ICC findings were reported in jejunal biopsies and the *muscularis propria* of the colon from diabetic gastroenteropathy patients [5].

In gastric samples from patients with type 1 diabetes, a loss of ICC density was reported in 60 to 100% of cases [10]. Diabetic neuropathy damage extends to the vagus nerve, NO-producing neurons, ICCs, and smooth muscle [10]. The complex outcome involving ICC dysfunction as a result of alterations in the expression of their chloride–calcium channels, or even their loss, may underlie the observed delayed gastric emptying and aberrant peristalsis [20][21]. A relationship between gastric viral infections and some cases of gastroparesis has also been proposed on account of local harmful inflammation [9].

9. Post-Operative Paralytic Ileus

Post-operative ileus is not a disease in itself but a temporary reaction of the gastrointestinal system to an external aggression as direct as surgery. The motility defect has been related to an overproduction of NO at the enteric level [22]. Immediately after surgery, inhibitory neuronal mechanisms are activated, followed by another phase of infiltration of inflammatory cells, such as monocytes, neutrophils, and mast cells, into the *muscularis mucosae* [8]. However, the involvement of mast cells in pathogenesis is under discussion because no motility problems have been observed in samples from mice without mast cells but with a normal ICC network, though problems did occur in groups of ICC-deficient mice [23]. Another curious relationship between the ENS and post-operative ileus was evidenced by pharmacological stimulation of the vagus nerve at the central level, improving paralytic ileus in both the recovery of GI motility and its anti-inflammatory actions [24].

On the other hand, surgical trauma and the inflammatory cascade it triggers may influence the pacemaker activity of ICCs, temporarily decreasing it [5]. A study investigating this relationship found that inflammation appears to be mediated by NO which, in turn, causes alterations in ICCs at both the electrophysiological level, with a decrease in pacemaker activity and reduced generation and coordination of slow waves, and structural level, with increased cytoplasmic vacuolization [23].

10. Constipation

As the structure and function of organs change over the years, these changes are reflected in an increased prevalence of certain diseases associated with aging; gastrointestinal tract conditions, such as GER, irritable bowel syndrome, constipation, and fecal incontinence, are more prevalent with age [25]. There appears to be a decrease in gastric emptying and colon motility; however, the magnitude of this change remains unclear as most patients maintain relatively good function [26], though the results are conflicting [27]. Gastrointestinal motility requires normal ICC distribution and function, which is profoundly changed by ICC depletion [28] or abnormal secretion of gut neurotransmitters. Among these, NO has turned out to be the most important, though other neurotransmitters are involved as co-transmitters, such as 5-HT or vasointestinal peptide (VIP) [29]. In 2011, Gomez-Pinilla et al. [27] carried out a study aiming to clarify the effect that age has on the number of ICCs and the volume of the networks they form in both the human stomach and colon. Identifying the initial cause of ICC-related motility disorders is difficult because early damage can decrease the volume of the networks, but not by enough to cause dysfunction. Therefore, in older patients, gastrointestinal motility problems could be more common not only because the lesions on the ICC networks have become evident with age, but also because they do not possess the functional reserve that can compensate for increased aggression [27].

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