Enteric neuropathology of congenital intestinal obstruction: A case report

Giovanni Di Nardo, Vincenzo Stanghellini, Salvatore Cucchiara, Giovanni Barbara, Gianandrea Pasquinelli, Donatella Santini, Cristina Felicani, Gianluca Grazi, Antonio D Pinna, Rosanna Cogliandro, Cesare Cremon, Alessandra Gori, Roberto Corinaldesi, Kenton M Sanders, Roberto De Giorgio

Abstract

Experimental evidence indicates that chronic mechanical sub-occlusion of the intestine may damage the enteric nervous system (ENS), although data in humans are lacking. We here describe the first case of enteric degenerative neuropathy related to a congenital obstruction of the gut. A 3-year and 9-mo old girl began to complain of vomiting, abdominal distension, obstruction of the gut. A 3-year and 9-mo old girl who presented with congenital intestinal obstruction underwent a Ladd’s band which was removed and the associated neuropathy along with reduced interstitial cells of Cajal network in dilated loops above the obstruction and a normal neuromuscular layer below the Ladd’s band.

One year after the latest surgery the patient tolerated oral feeding and did well, suggesting that congenital (partial) mechanical obstruction of the small bowel in humans can evoke progressive adaptive changes of the ENS which are similar to those found in animal models of intestinal mechanical occlusion. Such ENS changes mimic neuronal abnormalities observed in intestinal pseudo-obstruction.

Key words: Enteric neuropathy; Chronic intestinal pseudo-obstruction; Congenital intestinal obstruction; Ladd’s band; Enteric nervous system

INTRODUCTION

The enteric nervous system (ENS), the third component of the autonomic nervous system, plays a crucial role in the control of gastrointestinal functions, including motility, secretion, absorption, blood flow, mucosal growth and aspects of the local immune system[1]. Hence, any condition altering the integrity of the ENS is responsible for a wide spectrum of disorders affecting the gastrointestinal tract. Abnormalities of the ENS (also referred to as enteric neuropathies) may be secondary to a variety of inflammatory, infectious, metabolic and neurological diseases, or they can be labelled idiopathic when no causes can be found[2-6]. The most common clinical manifestation related to an underlying enteric neuropathy (either idiopathic or secondary) is a severe functional impairment of gastrointestinal motility as that identifiable in patients with chronic intestinal pseudo-obstruction (CIPO)[7,8]. The diagnosis of CIPO is based on the exclusion of any mechanical lesion occluding the...
induce ENS damage and treated obviously mechanical cause. The patient was discharged with laparoscopy showing duodeno-jejunal dilatation with no levels although a CT scan failed to reveal any mechanical distension of the proximal small bowel loops with air-blood tests. X-ray of the small bowel showed mild gaseous syndromes were excluded by physical examination and diseases, as well as infections and malabsorption and decreased bowel sounds. Systemic and neurologic prandial vomiting (often bilious) and constipation. Clinical and 9 mo of age when she began to complain of post-abdominal pain, early satiety and fullness. Because of the persistence of severe dyspeptic symptoms, at 5 years and 7 mo of age the patient was referred to a tertiary center in Bologna. Physical examination showed a highly distended (Figure 1A) and painful abdomen, with absence of peristaltic sounds. An X-ray examination showed multiple air-fluid levels in the upper abdomen with a striking elevation of the diaphragm. In order to achieve bowel decompression, she underwent surgery. At laparotomy a Ladd’s band was detected and removed with correction of the associated malrotation. Bowel loops with marked dilatation (Figures 1C and 1D) were resected and a protective ileostomy was created. Full-thickness biopsies were taken from the loops located proximally (dilated segments) and distally (macroscopically normal) to the Ladd’s band.

Following surgery, the clinical course was uneventful with immediate deflation of the abdomen. One year after the last laparotomy the patient was healthy and tolerated oral feeding, her height and weight were markedly increased, and her quality of life was good (Figure 1B).

**Immunohistochemical analysis**

Full-thickness tissue specimens were processed for immunohistochemistry according to standard protocols commonly applied in our laboratory. Compared to controls (jejunal specimens collected from patients operated on for intestinal bleeding due to angiodysplasia; \( n = 4, \) 2 females, age range: 6-16 years) (Figure 2A) and the non-dilated segment (Figure 2B), the immunohistochemical evaluation of biopsies taken from the dilated loop showed evidence of intrinsic neuropathy of the gastrointestinal tract characterized by severe myenteric and submucosal neuron depletion (Figure 2C), as identified by the reduced number of neural elements labeled by the general marker neuron specific enolase (NSE) (purchased from DakoCytomation, Milan, Italy). Furthermore, analysis of several transmitters/neuromodulators of the ENS demonstrated a marked decrease of substance P, vasoactive intestinal polypeptide, calcitonin gene-related peptide (all these antibodies were kindly donated by Dr. C. Sternini and H. E. Wong, Center for Ulcer Research and Education/Digestive Diseases Center, UCLA School of Medicine, Los Angeles, CA) and nitric oxide synthase (purchased from BD Biosciences, San Jose, CA) in the myenteric and submucosal neurons and related processes of the dilated segment compared to

**CASE REPORT**

G. S. was a full term baby girl weighing 2040 g at delivery whose clinical history was unremarkable until 3 years and 9 mo of age when she began to complain of post-prandial vomiting (often bilious) and constipation. Clinical examination revealed abdominal distension, dehydration and decreased bowel sounds. Systemic and neurologic diseases, as well as infections and malabsorption syndromes were excluded by physical examination and blood tests. X-ray of the small bowel showed mild gaseous distension of the proximal small bowel loops with air-fluid levels although a CT scan failed to reveal any mechanical obstruction. Due to the severity of the clinical picture and the marked small bowel distension, the patient underwent laparoscopy showing duodeno-jejunal dilatation with no obvious mechanical cause. The patient was discharged with a diagnosis of intestinal pseudo-obstruction and treated with prokinetics and a low-fiber diet.

Subsequently, she had recurrent episodes of vomiting, abdominal pain, early satiety and fullness. Because of the persistence of severe dyspeptic symptoms, at 5 years and 7 mo of age the patient was referred to a tertiary center for pediatric gastroenterology where, based on the clinical history and previous examinations, a decision to undertake an explorative laparotomy was reached. The intra-operative evaluation showed a marked degree of duodeno-jejunal dilatation but no mechanical obstruction.

Analysis of full-thickness biopsies taken from the dilated jejunal loop revealed enlarged myenteric and submucosal neurons whose number was increased as compared to sex-age-matched normal controls. A further important feature was the increased density of the nerve fibers in the lamina propria and submucosa. Based on the histopathology suggesting a case of hyperganglionosis, the diagnosis of CIPO was confirmed and the patient received several courses of prokinetics, metronidazole and high-caloric liquid diet supplementation.

**Figure 1** Representative pictures showing clinical (A and B) and laparotomic views (C and D) observed in this case. Note (A) the hugely distended abdomen and (C and D) abnormally dilated loops located proximally to the removed Ladd’s band (not visible in these examples) at laparotomy. B illustrates the considerable deflation of the abdomen following surgery.

During a subsequent recovery the patient underwent an upper gastro-intestinal manometry which showed a pattern indicative of neurogenic-type CIPO. Due to the progressive worsening of her clinical status, at 14 years and 2 mo of age, the patient was referred to our center in Bologna. The correct pathology remained undiagnosed for a long time, this case can be considered a human model of mechanical partial obstruction of the gut. Specifically the case indicates that a longstanding (“chronic”) intestinal sub-obclusion can evoke progressive changes of the ENS similar to those found in animal models, and that the observed ENS changes mimic abnormalities often found in CIPO related to idiopathic neuropathies.
the non-dilated segment and controls. In order to explore the possible abnormalities of neuronal cell survival, we used a specific antibody against the product of B-cell lymphoma-2 (BCL-2) (DakoCytomation, Milan, Italy), a gene encoding a protein involved in cellular pathways of neuronal apoptosis.[10,11] Compared to the controls (Figure 2D) and the non-dilated segment (Figure 2E), BCL-2 immunoreactivity of the dilated segment was markedly reduced in myenteric (Figure 2F) and submucosal neurons and nerve processes. The interstitial cells of Cajal (ICC), visualized by an antibody against c-Kit (DakoCytomation, Milan, Italy), were markedly decreased in the dilated segment compared to the non-dilated segment and controls. Compared to the controls, the anti-α-smooth muscle actin antibody (DakoCytomation, Milan, Italy) showed an apparently normal muscular layer both in dilated and in non-dilated segments.

**Transmission electron microscopy study**

Full-thickness tissue specimens were also processed for electron microscopy according to standard protocols commonly applied in our laboratory.

Compared to the controls (Figure 2G) and the non-dilated segment (Figure 2H), electron microscopy analysis of myenteric (Figure 2I) and submucosal neurons of the dilated loop showed degenerative features characterized by nuclear chromatin clumping, shrinkage of the cell body and cytoplasmic vacuoles mostly deriving from the enlargement and matrix clearing of mitochondria.

**DISCUSSION**

The present case illustrates the occurrence of gut failure as a result of longstanding mechanical small bowel obstruction due to a Ladd’s band, which has been unrecognized for more than 10 years despite extensive radiological investigation and 3 explorative laparotomies. A Ladd’s band, which arises from the posterior abdominal peritoneum and extends from the liver to the colon (passing anteriorly to the duodenum), is responsible for gut malrotation and extends from the liver to the colon (passing anteriorly to the duodenum), which arises from the posterior abdominal peritoneum and extends from the liver to the colon (passing anteriorly to the duodenum), which has been unrecognized for more than 10 years despite extensive radiological investigation and 3 explorative laparotomies. A Ladd’s band, which arises from the posterior abdominal peritoneum and extends from the liver to the colon (passing anteriorly to the duodenum), is responsible for gut malrotation which has been unrecognized for more than 10 years despite extensive radiological investigation and 3 explorative laparotomies.

Symptomatic patients usually present either acutely with bowel obstruction and intestinal ischemia, or chronically with vague abdominal pain. Chronic symptoms can often make diagnosis difficult, as was the case in our patient. Although Ladd’s band and associated gut malrotation are more common in the first 2 wk of life,[12,13] our case provides evidence that patients having this developmental abnormality may be found in childhood.

**JA**: In the current case, early tissue analysis of dilated bowel segments (i.e., from the region which was subsequently identified as proximal to the Ladd’s band) showed hyperganglionosis of the ENS. These neuronal changes, which were reported when the patient was 5 years old, may be considered the result of adaptive phenomena to a long-standing mechanical stimulus, such as obstruction limiting bowel propulsion. Several experimental models have been instrumental in supporting the concept that adaptive changes to the ENS occur proximal to a partially obstructed gut.[15,16-21]. These studies showed increased density and size in both myenteric[16,18,19,21] and submucosal neurons[19-21], along with neurochemical[18,20] and cytoskeletal abnormalities of myenteric neurons[21]. Recently, Galvez et al[22] induced surgical stenosis (about 20% of the lumen) of rat sigmoid colon and reported changes in ENS structure after 6-12 wk. The molecular mechanisms involved in enteric neuroplasticity secondary to a partially obstructed bowel remain to be elucidated. Further, enteric neuronal changes, ICC (the pace-maker cells of the gut, which act in concert with neurons in governing gut motility) have been found to be reduced in experimental mechanical subocclusion of the small bowel as indicated by a decrease in c-Kit immunoreactivity and loss of functions attributed to ICC[17,18]. In contrast, other data do not support the existence of ICC abnormalities (according to c-Kit immunostaining) in dilated gut segments of patients with Crohn’s disease, a well-known cause of intestinal mechanical subocclusion[23]. The possible explanations for the discrepancy between this and other studies remain to be defined.

About nine years after the initial observation of hyperganglionosis, the evaluation of new full-thickness biopsies from the dilated bowel above the stenosis showed evidence of a significant loss of both enteric neurons and ICC, which may explain the marked deterioration of the digestive function observed in our patient. Enteric neuron depletion is supported by the evidence of neurodegenerative changes detected by electron microscopy along with the reduced expression of the protein encoded by BCL-2,
a gene related to one of the intracellular pathways involved in the inhibition of programmed cell death[2,3,10,11]. These findings indicate that neuronal cell loss may be due to apoptosis triggered by the persistent mechanical sub-occlusion of the gut. The neurodegenerative abnormalities observed in this phase of the clinical history of the patient may resemble ENS changes described both in humans[22] and in an experimental model of small bowel atresia[24]. In human intestinal atresia, Masumoto et al.[22] have shown hypoplasia of myenteric ganglia and marked reduction of both intramuscular nerve fibers and ICC in the dilated bowel segments above atresia. Similarly, in a chick embryo model of intestinal atresia, Schoenberg and Kluth[24] have found an almost complete loss of both ganglionated plexuses in proximal dilated loops.

Taken together, enteric hyperganglionosis followed by degenerative changes can be considered the result of a bi-phasic adaptive process of the ENS in response to a persistent mechanical obstruction of the gut.

A further consideration which can be drawn from the present case concerns the accuracy of ENS pathology in patients with intestinal sub-occlusion. This case report clearly indicates that the enteric neuropathologic changes observed in different stages of a mechanical obstruction could not be distinguished from the neurodegenerative findings often detected in cases of CIPO. Neuropathology of CIPO includes a wide spectrum of ENS changes ranging from hyperganglionosis up to marked reduction of intramural (especially myenteric) neurons associated with swollen cell bodies, variable neurochemical abnormalities and fragmentation and loss of axons sometimes accompanied with proliferation of glial cells[23,24]. If the mechanical obstruction remains unrecognized, as it did in our case, the ENS abnormalities mimic those identifiable in CIPO associated with an underlying neuropathy. Therefore, our case should be considered as a reminder that ENS changes observed in patients with suspected CIPO may be secondary in nature and should not necessarily be interpreted as definitive evidence for a primary neuropathy.

In conclusion, congenital (partial) mechanical obstruction of the upper small bowel leads to progressive adaptive/neuroplastic changes of the ENS similar to those described in experimental models of intestinal occlusion. Interestingly, such ENS abnormalities mimic those often observed in cases of CIPO.

ACKNOWLEDGMENTS

The authors thank Dr. C. Sternini and Mrs. HE Wong (Center for Ulcer Research and Education/Digestive Diseases Center, UCLA School of Medicine, Los Angeles, CA) for the generous gift of the following antibodies: rabbit polyclonal anti-substance P/tachykinin (SP/TK+)

REFERENCES


12. Ladd WE. Congenital obstruction of the small intestine. JAMA 1933; 101: 1453-1458


