Chronic Intestinal Pseudo-Obstruction due to Buserelin-Induced Formation of Anti-GnRH Antibodies

BODIL OHLSSON,* BÉLA VERESS,‡ SABINA JANCIAUSKIENE,* AGNETA MONTGOMERY,§ MONICA HAGLUND,‡ and ANDERS WALLMARK*

*Department of Clinical Sciences, Gastroenterology Division, ‡Department of Laboratory Medicine, Pathology Division, and §Department of Surgery, Malmö University Hospital, Lund University, Malmö, Sweden

Background & Aims: **A 30-year-old woman, treated with buserelin, an analogue of gonadotropin-releasing hormone (GnRH) (also called luteinizing hormone-releasing hormone, LH-RH), developed chronic intestinal pseudo-obstruction (CIPO). The sudden onset of this disease in a previously healthy woman perplexed us. CIPO refers to a gastrointestinal disorder that can have a variety of causes, such as drugs, among others. Thus, we wanted to examine whether in this patient the development of CIPO is related to the treatment with buserelin.** ***Methods:*** **The patient was examined using esophagogastroduodenoscopy, esophageal, and antroduodenojejunal manometry, gastric emptying tests, and histologic analyses and immunohistochemistry on full-thickness biopsies including staining with anti-GnRH antibody. Plasma samples were examined by the standard serologic analyses and specifically for the occurrence of anti-GnRH antibodies by enzyme-linked immunosorbent assay methods.** ***Results:*** **CIPO was diagnosed based on symptoms (abdominal pain, vomiting, and constipation), and the results of the clinical examinations, such as signs of esophageal aperistalsis, delayed gastric emptying, and small intestinal bursts. Histologic examination revealed a decreased number of myenteric neurons as well as increased neuronal degeneration and an abnormal immune profile. There was a loss of GnRH-containing neurons. The patient had high plasma titers of anti-GnRH antibodies, which occurred on the occasions of the treatment with buserelin.** ***Conclusions:*** **Our findings suggest that the patient has developed CIPO due to buserelin-induced formation of anti-GnRH antibodies destroying GnRH-producing neurons of the myenteric plexus.**

Gastrointestinal motility requires coordination between the intrinsic and extrinsic nervous system, the interstitial Cells of Cajal (ICCs) and smooth muscle cells.[1,2] Disturbances in digestive motility can occur as a result of a variety of abnormalities affecting each of these elements (alone or in combination) involved in the physiology of gut motor function.[3]

Chronic intestinal pseudo-obstruction (CIPO) is a particularly difficult clinical challenge, characterized by the presence of chronic dysmotility and intestinal dilatation in the absence of mechanical obstruction.[4] There are 2 main forms of CIPO, that is, myopathic, involving the intestinal musculature, and neurogenic, involving the extrinsic innervation of the gut or intrinsic (enteric) nerves and pacemaker cells (ICCs).[5,6]

Here we report on a patient who participated in an in vitro fertilization program (IVF), and who, following repeated pretreatment with buserelin, a synthetic gonadotropin-releasing hormone (GnRH) agonist,[7] developed a degenerative neuropathic type of CIPO. The patient developed antibodies against buserelin. Our hypothesis is that these antibodies cross-reacted with endogenous GnRH, destroying GnRH-immunoreactive neurons of the myenteric plexus. To our knowledge, this is the first report demonstrating the presence of GnRH-positive neurons in the human gastrointestinal tract.

Materials and Methods

Case Report

A 30-year-old woman had been bilaterally salpingectomized due to ectopic pregnancies in 1991–1992, complicated by the rupture of the fallopian tubes, and had therefore developed secondary infertility. Her first IVF attempt took place in December 1992, and she was treated with intranasal buserelin (total dose 16 mg). The attempt was unsuccessful, wherefore a second IVF procedure with intranasal buserelin (total dose 24 mg) was initiated in March 1993, but was discontinued after 3 weeks because of excessive uterine bleeding. A third IVF attempt in August 1993 using subcutaneous buserelin (total dose 37.5 mg) was successful, and she delivered a child in May 1994.

Abbreviations used in this paper: CIPO, chronic intestinal pseudo-obstruction; ELISA, enzyme-linked immunosorbent assay; GnRH, gonadotropin releasing hormone; ICCs, interstitial cells of Cajal; IVF, in vitro fertilization; LH-RH, luteinization hormone-releasing hormone.

0016-5085/07/$32.00
doi:10.1053/j.gastro.2006.10.036

Six years later the patient desired another child, and she was accepted for a fourth IVF program using subcutaneous buserelin (total dose 17 mg). This was initiated in April 2000. However, the treatment was discontinued after 17 days because of nausea. Two weeks later, the patient experienced acute onset of chest, and abdominal pain combined with dysphagia. These symptoms progressed, together with vomiting, constipation, bloating, and extensive belching. To avoid symptoms she reduced her food intake, resulting in weight loss. Despite discontinuing the treatment, her symptoms remained and she had lost 14 kg of body weight. She was then referred to us in December 2000 due to nausea and dysphagia.

Clinical Investigation

Esophagogastroduodenoscopy 1 month before referral gave normal results. Simultaneous videoradiography of the pharynx (barium swallow) indicated a specific swallowing defect with an initial retention of the bolus, which, after a certain time, could pass down gradually. The following esophageal videomanometry demonstrated an impaired motility in which 80% of the swallowing process lacked propagating peristalsis. Furthermore, the lower esophageal sphincter was short and had a reduced resting pressure of 8 mm Hg. The opening of the lower esophageal sphincter was normal. The result of computer tomography of the thorax and upper abdomen was normal, as were the results of extensive analyses of blood, urine, and cerebrospinal fluid. Tick-borne encephalitis, Borrelia, amyloidosis, and myasthenia gravis could be excluded. A consultant rheumatologist excluded collagen-vascular disease by several serologic analyses. Autonomic nerve function tests showed an abnormal vasoconstriction [z-score = 2.97 compared with the normal value <1.64[8]], indicating sympathetic neuropathy.

Ambulatory, 24-hour pH monitoring of the esophagus sphincter indicated mild reflux. As the symptoms intensified, she was reexamined by esophagogastroduodenoscopy, which showed a mild *Helicobacter pylori*-induced gastritis, for which eradication treatment was given. Further examination with enterography demonstrated extremely slow passage through both the stomach and small intestine, and intestinal manometry displayed abnormal fasting bursts, which raised the suspicion of CIPO. Gastric emptying scintigraphy performed after ingestion of a meal consisting of egg and bread showed a pronounced gastroparesis. After 70 minutes, 75% of the content remained in the ventricle (T50 for healthy controls; 40 ± 28 minutes).[9] A measurement of the gastric emptying by using a transit method showed that after ingestion of porridge, milk, and bread, only 30% of the particles were emptied after 8 hours (reference value for complete emptying: 1.3–5.6 hours).[10] A succeeding diagnostic laparoscopy showed no macroscopic pathology of the small intestine, but a markedly reduced peristalsis was noted. There were no signs of endometriosis, adhesions, Meckel's diverticulum or tumors. A diamond-shaped, full-thickness wall biopsy was taken 1 meter proximal to the ileocecal valve and was immediately transported in wet compress for pathologic examination.

The follow-up period is now 5 years. The dysphagia is unaltered and the patient is incapable of full-time work due to abdominal pain, vomiting, and nutritional problems. The patient is currently being considered for an implantation of an electric gastric stimulator.

Histopathology

The biopsy was a piece of full-thickness small intestinal wall, 1.5 × 1.0 cm in size. Following fixation overnight in 4% formalin at room temperature, 2 transverse, full-thickness slices perpendicular to each other were cut and embedded in paraffin for conventional transversal sections. The remaining, larger part of the biopsy was embedded in toto for tangential sectioning, which makes it possible to examine the whole myenteric plexus.

H&E-stained and transversally cut sections were used to determine the number of neurons/mm myenteric plexus. All nerve cells containing nuclei and large, unequivocal neuron bodies without nuclei were counted. Small, suspected neuron–cytoplasmic structures were disregarded. The damaged myenteric neurons were counted in both transversal and tangential sections, and were presented as a percentage of the total number of neurons.

Serial sections from all of the three blocks were stained according to a protocol for CIPO analysis, which includes different antibodies for neural tissue and smooth muscle cell staining.[11] The specific immunohistochemical stainings were applied for the analysis of neurons [protein gene product 9.5 (PGP 9.5), neurofilaments, synaptophysin, bcl-2, substance P, neuron-specific endolase and α-internexin], glial cells, and Schwann cells (S100), T-lymphocytes (CD3), intermediate/microfilaments of the smooth muscle cells (α-actin, desmin, vimentin), and the ICCs (CD117 and CD34). For the negative controls, the specific antibody was replaced by nonimmunized serum.

The amount of abnormally stained/negative neurons for PGP9.5 and bcl-2 was determined on tangential sections and is given as the percentage of the total neuron population in these sections.

In addition, the sections were stained with anti-GnRH antibody [anti-luteinization hormone-releasing hormone (LH-RH); PROGEN Biotechnik GmbH, Heidelberg, Germany] at 1:100 dilution, following microwave pretreatment. The transverse sections were used to determine the amount of GnRH-positive neurons because tangentially cut sections were no longer available. The sections of normal small intestinal tissue from four cases of bowel resection due to intestinal carcinomas were used as controls, and were stained as described previously.