An unusual case of intestinal leiomyositis in a Bernese mountain dog

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Summary

A 1-year-old, female Bernese mountain dog was presented to the Veterinary Teaching Hospital of Turin University with a 3-month history of weight loss, intermittent anorexia, vomiting, constipation, and abdominal distension. Full-thickness biopsies from the stomach, duodenum, jejunum and ileum were collected for histological and immunohistochemical examination. Microscopic lesions displayed severe diffuse degeneration and loss of leiomyocytes, with lymphocytic leiomyositis, fibroplasia, angiogenesis, severe diffuse neuronal atrophy, and ganglioneuritis in the myenteric (Auerbach’s) plexus. A diagnosis of chronic idiopathic intestinal pseudo-obstruction was made. Response to immunosuppressive therapy was poor and the dog was humanely euthanized. Unique findings were mononuclear infiltration composed predominantly of B-cell, angiogenesis and weak immunoreactivity for neuron-specific enolase.

Keywords: dog, pseudo-obstruction, intestine, leiomyositis, B-lymphocytes

Introduction

Chronic intestinal pseudo-obstruction (CIPO) is a rare gastrointestinal syndrome characterized by impaired intestinal motility that results in clinical signs of bowel obstruction without evidence of mechanical occlusion of the gut lumen. In human medicine, pseudo-obstruction syndrome may be either acute or chronic (Di Lorenzo, 1999). The chronic form has been described in different animal species including dogs (Dvir et al., 2001; Eastwood et al., 2005; Johnson et al., 2007; Vandenberge et al., 2009; Murtagh, 2013). The acute form has been associated with abdominal surgery, peritonitis, hypokalemia, spinal or pelvic trauma, viral enteritis, pancreatitis, intestinal infarction, anticholinergic and opioid therapy (De Giorgio et al., 2011). The chronic form, either congenital or acquired, is characterized by recurrent arrest of intestinal motility. The latter can be associated with neurologic, metabolic, endocrine, paraneoplastic, autoimmune and infectious diseases (De Giorgio et al., 2011). In humans, CIPO may involve interstitial cells of Cajal and components of the neuronal or muscular gastrointestinal motility control systems, either alone or in combination; more rarely, it may occur together with mesenchymopathies (De Giorgio et al., 2011). Fibrosis and infiltration of the smooth-muscle

Ein ungewöhnlicher Fall von Darmleiomyositis beim Berner Sennenhund


Schlüsselwörter: Hund, Pseudoobstruktion, Darm, Leiomyositis, B-Lymphozyten

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fibers of the tunica muscularis by mononuclear cells are the most frequently reported macroscopic lesions of CIPO in the dog (Dvir et al., 2001; Eastwood et al., 2005). Occasional involvement of gastric and bladder musculature was reported (Johnson et al., 2007). The suspicion of CIPO relies on the exclusion of a mechanical basis of subocclusion. A definitive diagnosis rests on full thickness biopsies that can provide clues to the nature of neuromuscular impairment (Knowles, 2009).

Clinical and laboratory examination

A 1-year-old, female Bernese mountain dog was referred to the Veterinary Teaching Hospital (VTH) of Turin University because of a 3-month history of weight loss, intermittent anorexia and vomiting, constipation and abdominal distension. Symptomatic treatment with antiemetics (maropitant, 60 mg, PO, q24h, for 5 days), antibiotics (metronidazole 10 mg/kg, PO, q12h for 2 weeks in addition to amoxicillin-clavulanate 20 mg/kg, PO, q12h), disaccharide laxatives (lactulose, 6 ml PO, q12h), warm water enemas and commercial diets (low-residue-producing diet and hydrolyzed diet) had brought no improvement. Abdominal radiographs performed by the referring veterinarian showed generalized, dilated small bowel loops suggestive of intestinal obstruction. Exploratory celiotomy ruled out mechanical obstruction. The dog was subsequently referred to the VTH. On clinical examination, the dog exhibited mild dehydration, tachycardia, intermittent polypnea and distended abdomen with fluid and gas-filled intestinal loops. Neurological examination was normal. Hematological analysis revealed mild normocytic normochromic nonregenerative anemia (4.43 × 10¹²/l, normal range 5.7-8.8 × 10¹²/l). Serum biochemical analysis and serum protein electrophoresis demonstrated a low total protein (46 g/l, normal range 54-75 g/l) with low albumin (21 g/l, normal range 29-35 g/l) and normal globulin (25 g/l, normal range 25-40 g/l). Arterial blood gas, urinalysis, pre- and post-prandial bile acids and adrenocorticotropic (ACTH) stimulation test were within normal limits. Thyroid stimulating hormone (TSH) was normal with decreased tT4 (11.71 nmol/l, normal range 12.87-45.04 nmol/l); a “euthyroid sick syndrome” was suspected. The antinuclear antibody test was negative and the serum Spec cPL® test (IDEXX laboratories) for pancreatic lipase was normal. Thoracic radiographs were unremarkable. Abdominal radiographs and ultrasonography confirmed a generalized, severe distension of the small intestine by gas and fluid. A second celiotomy was performed to collect full-thickness biopsies from the stomach, duodenum, jejunum and ileum.

Histopathologic and immunohistochemical findings

Tissue samples were fixed in 10% neutral buffered formalin and routinely processed. Tissue sections 5μm in thickness were stained for histology with haematoxylin and eosin. Tissue sections were also stained with the following antibodies (Table 1). The antibody panel used in the study included smooth muscle actin (Dako, Glostrup, Denmark), desmin (Novocastra, Newcastle, UK), neuron specific enolase (NSE) (Dako, Glostrup, Denmark), CD79a (Dako, Glostrup, Denmark), CD3ε (Serotec, Oxford, UK), and CD20 (Neomarkers, CA, U.S.A.).

Table 1: Antibody panel used on gastrointestinal tissue samples.

<table>
<thead>
<tr>
<th>Target antigen</th>
<th>Clone</th>
<th>Dilution</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth muscle actin</td>
<td>14A</td>
<td>1:2000</td>
<td>Dako, Glostrup, Denmark</td>
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<tr>
<td>Desmin</td>
<td>DE-R1I</td>
<td>1:400</td>
<td>Novocastra, Newcastle, UK</td>
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<tr>
<td>Neuron Specific Enolase (NSE)</td>
<td>BBS/NC/VI-H14</td>
<td>1:25</td>
<td>Dako, Glostrup, Denmark</td>
</tr>
<tr>
<td>CD79a</td>
<td>HM-57</td>
<td>1:20</td>
<td>Dako, Glostrup, Denmark</td>
</tr>
<tr>
<td>CD3 ε</td>
<td>CD3-12</td>
<td>1:10</td>
<td>Serotec, Oxford, UK</td>
</tr>
<tr>
<td>CD20</td>
<td>Rabbit Polyclonal</td>
<td>1:40</td>
<td>Neomarkers, CA, U.S.A.</td>
</tr>
</tbody>
</table>

Figure 1: Longitudinal and circular muscular layers of the duodenum are completely replaced by collagen and angiogenesis. Multifocal mild lymphoplasmacytic inflammation is also present. Hematoxylin and Eosin stain, 25×. Bar: 25 μm.
An unusual case of intestinal leiomyositis in a Bernese mountain dog

P. Gianella et al.

and eosin, Masson’s trichrome, periodic acid-Schiff (PAS) for histology, and by a standard avidin–biotin peroxidase method for immunohistochemistry. Table 1 lists the primary antibodies utilized. Negative controls consisted of substitution of specific antibodies with an isotype-matched, irrelevant monoclonal antibody or omission of the primary antibody. Histological findings in the stomach included mild gastric submucosal fibrosis with occasional lamina propria infiltration by small mature lymphocytes. Gastric muscularis mucosa, muscular layers and the plexuses were normal. Histological findings in duodenum, jejunum and ileum were similar, with maximal severity in jejunum. The intestinal epithelium of all tracts contained increased numbers of mucous cells. The lamina propria was severely infiltrated by small mature lymphocytes and plasma cells. The lymphatic vessels in the ileum were dilated. Ileal muscularis mucosa was characterized by a moderate infiltrate of mature lymphocytes, mild fibrosis and angiogenesis. The most severe changes were observed in the circular and, to a lesser extent, in the longitudinal muscular layers of the small intestine (Fig. 1). Severe atrophy of the circular muscular layer was associated with replacement of the smooth-muscle fibres with angiogenesis, fibroplasia and infiltration by small numbers of mature lymphocytes and occasional plasma cells. The longitudinal muscular layer was replaced by fibrosis, with greatest loss of myocytes observed in the duodenum, the jejunum and, to a lesser degree, the ileum. An abrupt transition between the circular and longitudinal muscular layers was evident in the duodenum and jejunum, often together with haemorrhages. In addition, occasional non-degenerated neutrophils and rare eosinophils were observed. The submucosal (Meissner’s) plexus was not visible, while the ganglia of the myenteric (Auerbach’s) plexus were characterized by fibrosis and small mature lymphocyte infiltration with neuronal atrophy.

Masson’s trichrome evidenced a normal gastric muscular structure. In the duodenum and ileum, the muscularis mucosa was normal in thickness, however, weaker staining intensity and thinning was detected in the jejunum. Overall, there was complete loss of circular muscularis mucosa, while some areas of the longitudinal layer in the ileum stained in red (Fig. 2). Severe fibrosis extended from or under the muscularis mucosa to the serosal lining of the duodenum and the jejunum and to a lesser extent in the ileum. These findings were confirmed by smooth-muscle actin and desmin stains that demonstrated the almost complete absence of leiomyocytes in the circular and longitudinal layers of the duodenum (Fig. 3).

Figure 2: Longitudinal and circular muscular layers of the duodenum are partially and totally replaced by collagen, respectively. Masson’s Trichrome stain, 25×. Bar: 25 μm

Figure 3: Variable severe loss of desmin-positive myocytes in longitudinal and circular muscular layers of the duodenum. Anti-Desmin immunohistochemical stain, Carbazole chromogen, Mayer’s hematoxylin contrast stain, 25×. Bar: 25 μm
An unusual case of intestinal leiomyositis in a Bernese mountain dog

P. Gianella et al.

Neuron Specific Enolase (NSE) identified neurons of the myenteric plexus of Auerbach, while no neurons were found in the myenteric plexus of Meissner. In the duodenum and jejunum, CD20+, CD3- B cell predominated, while T cell represented 15% or less of the inflammatory lymphoid component. In the ileum, B cells were prevalent in nodular aggregates. In contrast, CD3+, CD20- T cells predominated in the jejunum. PAS stain did not demonstrate fungal hyphae but confirmed a severe increased number of mucous cells.

Diagnosis, treatment and outcome

The dog was initially treated with intravenous fluids, metoclopramide (2 mg/kg/24 hours constant rate infusion), amoxicillin-clavulanate (20 mg/kg, IV, q12h), metronidazole (10 mg/kg, IV, q12h), buprenorphine (0.01 mg/kg, IV, q8h), ranitidine (2 mg/kg, IV, q12h) and erythromycin (2 mg/kg, PO, q8h). A poor response to treatment was seen. A histopathological diagnosis of severe, diffuse chronic degeneration and loss of leiomyoctes with lymphocytic leiomyositis, fibroplasia, angiogenesis, severe diffuse atrophy and chronic lymphoplasmacytic ganglioneuritis in the myenteric (Auerbach’s) plexus causing CIPO was made. Following the histopathological diagnosis of intestinal leiomyositis an immunosuppressive therapy with prednisone (1 mg/kg, PO, q12h) was started. However, two weeks later, due to the poor response to treatment and, upon owner request, the dog was humanely euthanized. Necropsy was not permitted.

Discussion

As compared to previous reports of CIPO in the dog (Dvir et al., 2001; Vandenberghe et al., 2009), the distinctive pathological findings in the current case were the extensive angiogenesis, particularly in the circular muscle layer, the low number of inflammatory cells, predominantly of B-cells, and the reduced immunoreactivity for NSE. Moreover, the ganglia of the submucosal plexus were not microscopically visible perhaps because of the severe changes or because of a true aplasia. The inflammation of the myenteric plexus was interpreted as being secondary to the interstitial leiomyositis. Several cases of canine CIPO have been associated with secondary inflammation of the myenteric plexuses (Dvir et al., 2001), however, no cases of CIPO due to primary visceral neuropathy have been reported. Furthermore, human neuropathic CIPO is generally not associated with severe muscular lesions like those observed in the current case. The muscular angiogenesis and fibrosis observed here shared common features with human “hollow primary visceral myopathy” characterized by fibrosis and leiomyocte degeneration (Muñoz-Yagüe et al., 2006). However, the stomach was normal and no information about oesophagus, colon or bladder involvement could be gleaned from the clinical history.

An immune-mediated pathogenesis involving “effector” lymphoid cells has been suggested in both humans and dogs (Vandenberge, 2009), but the low number of inflammatory cells and the abundance of B-lymphocytes reported here seem to contrast partially with this hypothesis. The reason for these unique features is not clear. A dense T-cell infiltrate without fibrosis has been associated with early-stage of disease (Murtagh, 2013), while B-cell infiltrates may represent either a later inflammatory response or a consequence of superficial inflammation. The latter, also observed in this dog, is frequent in secondary CIPO and has been associated with bacterial overgrowth (Connor and Di Lorenzo, 2006). Bacteria or their toxins may also be responsible for a reduction in intestinal NSE immuno-reactivity, as has been demonstrated in equine grass sickness (Griffiths et al., 1993).

There is no cure for CIPO and management strategies include a wide array of nutritional and pharmacologic options which are mainly directed to minimize malnutrition, promote gut motility, reduce inflammation and complications of stasis (De Giorgio et al., 2011). Particularly, a variety of prokinetics drugs, eg, erythromycin, metoclopramide, domperidone, neostigmine, serotoninergic agents, prostaglandins and somatostatins analogs have been used with variable results (Di Lorenzo, 2006). Bacteria or their toxins may also be responsible for a reduction in intestinal NSE immuno-reactivity, as has been demonstrated in equine grass sickness (Griffiths et al., 1996).

This is the first report to describe leiomyositis with mononuclear cell infiltration predominantly composed of B-lymphocytes, angiogenesis and reduced immunoreactivity for NSE in a dog with CIPO. Since lesions are frequently confined to the muscular layers, full-thickness biopsy is essential for making a definitive diagnosis, while superficial biopsies may delay correct diagnosis.

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References


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An unusual case of intestinal leiomyositis in a Bernese mountain dog

P. Gianella et al.