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SHORT ORIGINAL COMMUNICATION

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A systematic analysis of polyglucosan bodies in the human gastrointestinal tract in health and disease

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Abstract Polyglucosan inclusion bodies have been described in smooth muscle of the gastrointestinal tract of aged dogs, and rarely in association with enteric dysmotility in humans. We have systematically examined the human small and large bowel for the presence of such inclusions in health and motility disorders. Systematic, blinded, dual observer analysis of colonic and ileal tissue from patients (n=80, age 20–92 years) undergoing large bowel resections for non-dysmotile conditions, principally neoplasia was performed, as well as retrospective review of all intestinal tissues referred for specialist histochemistry from patients undergoing surgery for motility disorders. All sections were stained with haematoxylin and eosin and periodic acid-Schiff stains. No polyglucosan bodies were identified in any specimen without dysmotility, regardless of age, but were a feature of 4/104 patients with diverse severe gastrointestinal motility disorders. In contrast to dogs, polyglucosan bodies are not a feature of normal ageing in human gastrointestinal smooth muscle but, in accord with previous suggestions, are seen in rare cases of human gut dysmotility. The significance of this difference is unclear.

Keywords Ageing · Inclusion bodies · Myopathy · Polyglucosan bodies

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Introduction

Kamiya et al. [8, 9] reported the finding of polyglucosan inclusion bodies in the digestive tract of aged dogs, without associated muscle dysfunction [9]. These were clearly evident on light microscopy with periodic acid-Schiff staining (PAS), were 5–20 μm in diameter, were variably stained with other methods and shown to be immunoreactive with polyglucosan antibody [8]. Ultrastructural study revealed a filamentous composition, which was identical to previously reported Lafora bodies found in the central nervous system [6]. The number of inclusions increased with age, and were found throughout the intestinal tract, although predominantly in the ileum and large intestine, as well as in the brain and spinal cord [9].

Polyglucosan bodies may be seen in smooth muscle in some traditional hereditary muscle glycogenoses and in disorders such as Lafora's disease [6] and adult polyglucosan disease [5, 15]. The latter disorder, while systemic, includes symptoms of bladder and bowel dysfunction [15]. Specific to visceral smooth muscle, PAS-positive material has been discovered in detrusor muscle in association with bladder dysfunction [1], and in the gastrointestinal tract, where we have described a familial myopathy affecting the smooth muscle of the internal anal sphincter in a number of women who presented with proctalgia fugax and hypertrophy of the internal sphincter [10, 13] (Fig. 1a). The characteristic pathological features of this disorder were highlighted by PAS staining, with ovoid inclusion bodies, 2–30 μm in length, demonstrated. These inclusion bodies resisted diastase predigestion, and had a similar staining profile [2] and ultrastructural appearance [19] to the polyglucosan structures of corpora amylacea, seen as a usual feature in brains from the older population [7]. Similar or perhaps identical inclusion bodies have been reported in three patients with intestinal pseudo-obstruction and visceral myopathy, which was either idiopathic (two patients: colonic inclusions) [4] or found (a single case) in association with a probable diagnosis of scleroderma (ileal inclusions) [20, 21].

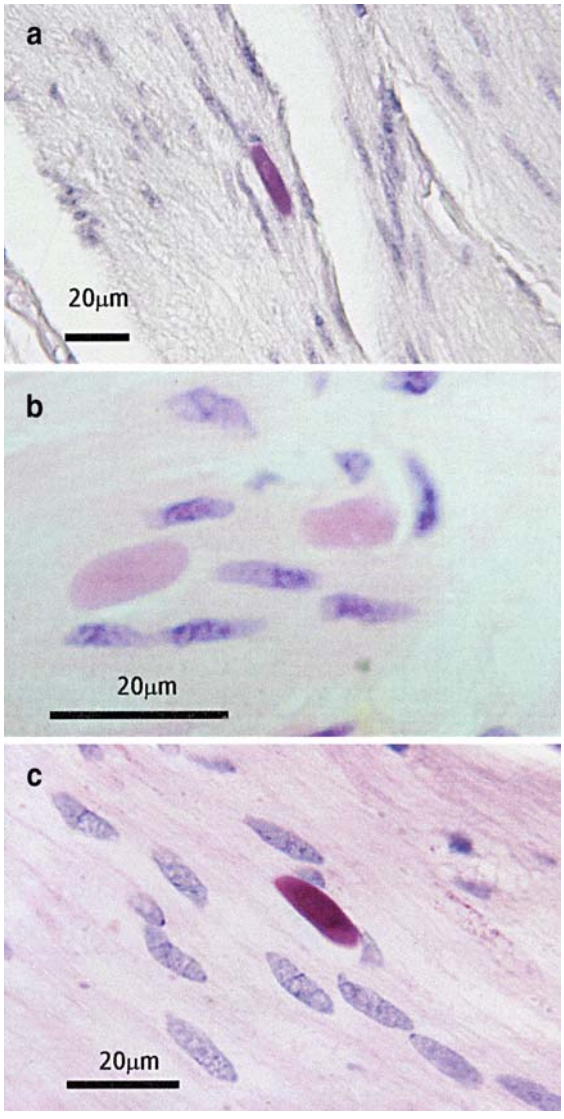

Fig. 1 **a** PAS preparation after diastase predigestion showing an ovoid inclusion body in the muscle of the internal anal sphincter of a female patient with hereditary internal anal sphincter myopathy causing proctalgia fugax and constipation. **b** H-E staining of (non-polyglucosan) amphophilic inclusion bodies in the ascending colon of a control subject, aged 90 years. **c** PAS preparation after diastase predigestion showing an ovoid inclusion body in the circular muscle of the jejunum of a female patient with intestinal pseudo-obstruction (*PAS* periodic acid-Schiff, *H-E* haematoxylin and eosin). *Bar* 20 μm

We tested the hypothesis that (1) polyglucosan bodies are a feature of ageing in normal intestinal smooth muscle in humans, and (2) such inclusions may be a finding in the intestine of patients with severe gastrointestinal motility disorders.

Materials and methods

Study of ageing

Tissues included normal ileal and colonic tissue removed for the treatment of colorectal cancer (n=75), familial adenomatous polyposis (FAP) (n=2), and Crohn's disease (n=3). The latter groups were included to provide a comprehensive age range of control tissue, and were only selected if the region of the colon was macroscopically normal, with muscularis propria microscopically unaffected by the disease process (age range 20–92 years, mixed for sex). A total of 110 tissues were studied (70 colon, 40 ileum). Specimens were removed from the anti-mesenteric aspect of the colonic and/or ileal wall from inter-taenial portions.

Table 1 Presence of polyglucosan bodies by motility disorder

Condition	n	Polyglucosan inclusions
Oesophageal motility disorder	1	0
Gastric motility disorder	3	1
Intestinal pseudo-obstruction	19	2
Intractable constipation (unspecified)	11	0
Slow transit constipation	51	1
Idiopathic megarectum	6	0
Rectal evacuation disorder	10	0
Chronic idiopathic abdominal pain	3	0
Total	104	4 (4%)

Patients with enteric dysmotility

Tissue from patients (n=104) who had undergone surgery (either resection or biopsy: details omitted for brevity) over an 11-year period for diagnosis or treatment of a variety of severe motility disorders were examined (Table 1). The tissue(s) of study was dependent on the presumed underlying disorder, i.e. idiopathic vomiting: stomach; intestinal pseudo-obstruction: jejunum or ileum; slow transit constipation: colon. In all cases, full-thickness tissue had been obtained.

Methods

Specimens were fixed overnight in 10% formalin solution, and mounted in paraffin blocks by standard methodology. Routine 5-μm sections were stained with haematoxylin and eosin (H-E), and PAS preparations made with and without diastase predigestion (D-PAS). Whole H-E and PAS sections were systematically analysed blind to age and diagnosis at a magnification of ×125 or ×250 by two observers using a double-headed Olympus BH2 microscope. The presence, distribution and number of inclusion bodies were noted.

Results

Study of ageing

No inclusions were demonstrated in any tissue on PAS staining with or without diastase predigestion. With advancing age, amphophilic, round or ovoid inclusion bodies, were evident on H-E staining, as previously described [11] (Fig. 1b).

Patients with enteric dysmotility

Of the 104 patients, 4 (4%) with diverse gastrointestinal motility disorders had PAS-positive, diastase-resistant, round or ovoid inclusions varying in size from approxi-

mately 5–40 μm (Table 1, Fig. 1c). These inclusions were present in both longitudinal and circular layers of the muscularis propria, but were not seen in the muscularis mucosa or in neural tissue. The frequency of inclusions varied from 0 to 3 per high-power field on microscopic examination. Inclusions were weakly, but non-specifically stained with a polyclonal antibody to ubiquitin (Dako UK, using a standard protocol). Two such patients (both female, aged 46 and 49 years) had intestinal pseudo-obstruction diagnosed on clinical findings and prolonged ambulatory jejunal manometry (both laparoscopic full-thickness jejunal biopsies). Further patients included a female, aged 56 with physiologically proven slow transit constipation, and a male with a predominantly gastric motility disorder (idiopathic vomiting and gastroparesis) who has been described previously [14]. None of these patients had evidence of scleroderma. A significant proportion of patients with slow transit constipation had colonic amphophilic inclusions (as above) [11], but these were not seen in the 4 patients with polyglucosan bodies.

Discussion

Kamiya et al. [9] elegantly and clearly demonstrated inclusion bodies of polyglucosan composition in ageing dogs. In contrast, in humans, we failed to demonstrate polyglucosan bodies in a large number of small and large bowel resection specimens from patients with non-myopathic conditions, regardless of age. However, a small proportion of patients with a diverse spectrum of severe gastrointestinal motility disorders had evidence of polyglucosan bodies in the colon (a previously described site [4]), jejunum and stomach (not previously described).

We have previously reported inclusion bodies found with increasing age in the human ileum and colon [11]. These were evident on H-E staining, and considered to have similar morphology and staining characteristics to previously reported ‘M’ bodies in the human gastrointestinal tract in diabetics with severe autonomic neuropathy affecting the gut [3, 16]. These bodies were thought to represent individual “transformed” smooth muscle cells, as a result of necrobiosis and atrophy. While they are of similar size and shape, they do not resemble polyglucosan bodies in staining characteristics, failing to stain with PAS.

The disparity in type of inclusions found with ageing in dogs and man is not easily explained. The genetic background of dogs can be less diverse than that of humans due to selective breeding. If present, there is a relatively high incidence of genetic disorders in inbred strains [12, 22]. In their study, Kamiya et al. [8], however, used dogs selected at random for autopsy, and the differences in our findings in humans are thus unlikely to relate to an inherited predisposition in dogs. It is conceivable that dietary differences may have a role.

In adult polyglucosan disease, there is involvement of the central nervous system, presenting with progressive sensorimotor impairment. Polyglucosan inclusions have been demonstrated in this condition in skeletal muscle and also in the smooth muscle of the bowel wall [5]. There was no clinical evidence of involvement of the central or peripheral nervous system, or of skeletal muscle myopathy in the cases we describe.

The pathological significance of such polyglucosan inclusions is not clear. On the basis that PAS-positive inclusions were only found in cases of gastrointestinal dysmotility in this study, and that such inclusions have only previously been reported by others [4, 20, 21] in similar conditions, it seems likely that they would be of some significance. However, in view of the wide spectrum of disorders concerned, it is probable that they represent a marker or possibly a bystander effect rather than a cause of abnormal contractile activity per-se. In neurodegenerative disorders there have been recent reports [17, 18] suggesting that intraneuronal inclusion bodies, specifically neurofilament accumulations, may act as a form of neuroprotection in disease states. It is conceivable that in the gastrointestinal tract, polyglucosan bodies may perform a similar function acting as a ‘sink’ for toxic phosphorylated elements that might otherwise result in more direct cell damage. In the absence of information regarding the underlying pathogenetic processes in affected patients, the biochemical nature of the precise cellular insult can only serve as a subject for speculation.

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