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MAIN TOPIC

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Megacystis-microcolon-intestinal hypoperistalsis syndrome: evidence of intestinal myopathy

Abstract We investigated small- and large-bowel specimens of three newborn infants presenting with the clinical and radiological symptoms of megacystis-microcolon-intestinal hypoperistalsis syndrome (MMIHS). Conventional histological staining revealed marked thinning of the longitudinal muscle layer. Electron-microscopic investigations showed typical "central core" vacuolic degeneration of smooth-muscle-cells combined with proliferation of col lagen fibres. The expression of α-smooth-muscle actin was absent or markedly reduced in the circular and longitudinal muscle layers and muscularis mucosae compared to the normal controls. These findings suggest that the intestinal obstruction in MMIHS is due to an abnormality of the smooth-muscle cells.

Keywords Megacystis-microcolon-intestinal hypoperistalsis syndrome · Newborn · Visceral myopathy · Chronic idiopathic intestinal pseudo-obstruction

Introduction

Megacystis-microcolon-intestinal hypoperistalsis syndrome (MMIHS), a rare cause of intestinal obstruction in the newborn, was first described in 1976 by Berdon et al [1]. The syndrome is characterised by abdominal distension caused by a distended, non-obstructed urinary bladder, microcolon, incomplete intestinal rotation, decreased or absent intestinal peristalsis, and failure to pass meconium. Although 89 cases of MMIHS have been reported to date, the aetiology of this syndrome is still not fully understood. Histological studies of the myenteric and submucous plexuses have revealed normal ganglion cells in the majority of patients, decreased in some, and hyperganglionosis and giant ganglia in others. In 1983, Puri et al. demonstrated vacuolar degenerative changes in the smooth-muscle cells with abundant connective tissue between muscle cells in the bowel and bladder in patients with MMIHS and suggested that a degenerative disease of smooth-muscle cells may be the cause of this syndrome [2].

The purpose of this study is to describe three infants with MMIHS in whom histological, immunohistochemical, and ultrastructural studies of the bowel revealed abnormalities of smooth-muscle.

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Materials and methods

Two female infants having clinical and radiological features of MMIHS died at the age of 2 and 21 months, respectively. An autopsy was performed in both infants soon after death, and full thickness biopsies were taken from the entire gastrointestinal (GI) tract. The third patient was a 6-month-old girl who presented with clinical and radiological features of MMIHS at birth and underwent a laparotomy. Full-thickness bowel-wall biopsies were taken from the distal ileum and caecum. Parts of the specimen were snap-frozen after fixation with Zamboni's solution for immunohistochemistry. Further parts were fixed in 4% buffered formaldehyde, processed, and paraffin-embedded. Normal control large-bowel specimens were collected during bladder augmentation operations from four patients and processed in the same manner. Serial 7-μm paraffin sections were stained with haematoxylin and eosin (HE) for normal bright-field microscopy.

Small pieces of the specimen were fixed in 2.5% phosphate buffered glutaraldehyde for 1 h at 4 °C, washed in phosphate buffer (PBS), and fixed in 1% buffered osmium tetroxide for 1 h at 4 °C. After rinsing in distilled water, the specimen was dehydrated in 2,2 dimethoxypropane twice for 15 min, embedded in resin TAAB, and polymerised at 60 °C for 24 h, 1-μm sections were stained with 1% toluidine blue in 1% borax and areas for thin sectioning were selected under light microscopy. Appropriate blocks were treated with uranyl acetate methanolic for 2 min, washed in distilled water,

and counterstained with Reynold's lead citrate for 15 min. Sections were viewed with an electron microscope.

Single-enzyme immunohistochemistry was performed using anti-α-smooth-muscle actin (SMA) antibody (Novocastra, SMA-NCL) in a 1:100 dilution on 8-μm frozen sections. For immunohistochemical investigation the streptavidin-alkaline-phosphatase (SAP) universal kit (Immunotech, MA, USA) was used. After incubation with the primary antibody for 24 h at 4°C and rinsing three times with PBS, the specimens were incubated with the biotinylated secondary antibody for 24 h at 4°C, rinsed three times, and incubated with (SAP) reagent for 24 h at 4°C. The last reaction with fresh prepared chromogen solution was observed microscopically and lasted about 30 min at room temperature. The staining results were evaluated with normal bright-field microscopy. For negative controls the primary antibody was omitted.

Results

In all MMIHS cases thinning of the longitudinal muscle layer of the small and large bowel was found using HE and SMA immunohistochemistry (Fig. 1). Additionally, marked connective-tissue proliferation was evident within the intestinal smooth-muscle layers. Electron microscopy showed vacuolar degeneration in the centre of the intestinal smooth-muscle cells. Some vacuoles were composed of granular material in which remnants of cytoplasmatic organelles were visible in a background of fibrillar material, possible representing actin filaments. The majority of the vacuoles appears empty and were surrounded by a thin rim of smooth-muscle cytoplasm. Furthermore, disorganization of the smooth-muscle-fibre contractile proteins was demonstrable. Severe reduction of contractile fibres and central storage of granular material appearing as "central core"-like lesions was detectable. Peripheralisation of contractile fibres around the central, developed vacuoles was evident (Fig. 2). This disorganisation was more noticeable in the longitudinal-muscle layer, but was also present in the circular-muscle layer. Additionally, marked proliferation of collagen fibres was evident around the smooth-muscle cells. These pathological findings were expressed similarly in small- and large-bowel specimens of all investigated infants.

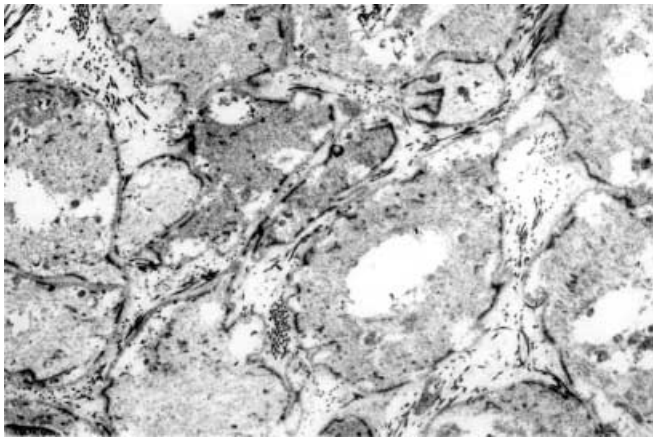

Fig. 2 Electron microscopic view of vacuoled degenerated smooth-muscle cells and proliferation of collagen fibres (×6,800)

Smooth-muscle actin was strongly expressed in normal human bowel in the circular and longitudinal muscle layers as well as in the muscularis mucosae. Additionally, SMA-immunoreactive filaments were expressed between the crypts of the large bowel in the lamina propria mucosae. The expression of SMA was strikingly different in the affected bowel compared to normal controls: SMA-immunoreactivity was markedly reduced in the circular smooth-muscle layer and absent in the longitudinal smooth-muscle layer of MMIHS small and large bowel (Fig. 3a and b). SMA expression in the muscularis mucosae was markedly reduced in MMIHS small bowel and absent in the large bowel compared to normal controls (Fig. 4a and b). No SMA-immunoreactive structures were evident in the lamina propria of the affected bowel. SMA-immunoreactive filaments were strongly expressed around submucosal vessels in MMIHS bowel and normal controls.

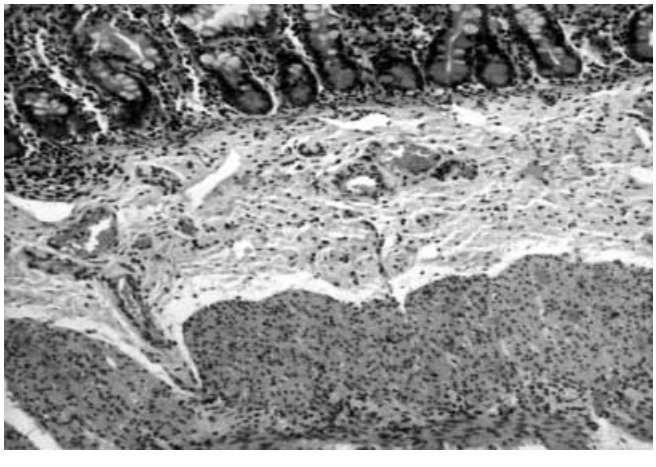

Fig. 1 Thinning of longitudinal muscle layer (H&E ×50)

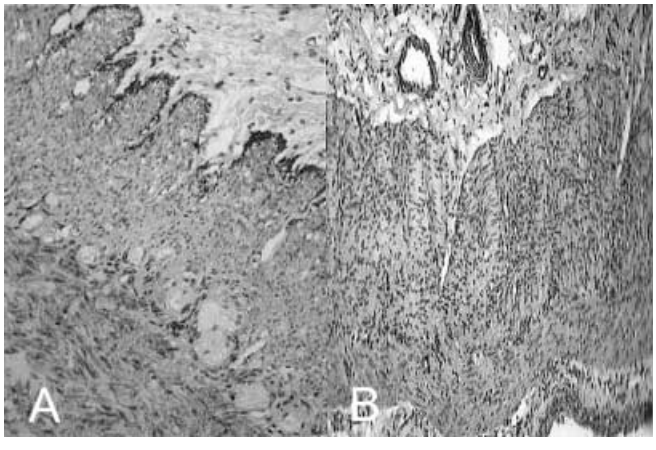

Fig. 3 **A** Absent SMA-immunoreactive filaments in longitudinal- and circular-muscle layer compared to **B** normal bowel specimen (×100)

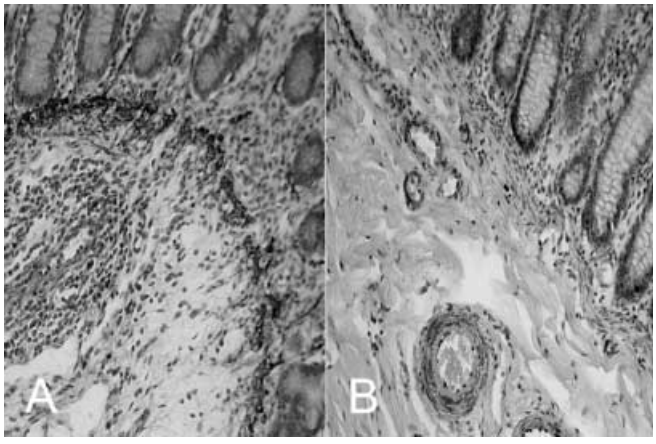

Fig. 4 **A** Markedly reduced SMA-immunoreactive filaments in muscularis mucosae of MMIHS compared to **B** normal bowel specimen. Note similar SMA-immunoreactivity in submucosal vessels in both specimens (×100)

Discussion

The aetiology of MMIHS is unknown. Several reports have mentioned defective autonomic innervation and described a variety of innervation abnormalities in single cases including hypoganglionosis, aganglionic zones, hyperganglionosis, and immature ganglia [3–14]. Srikath et al. [15] speculated that the initial event in the pathogenesis is an intramural inflammatory process that effects the GI and urinary tract, leading to extensive fibrosis that destroys the intestinal neural network and produces hypoperistalsis. In contrast, the majority of reported MMIHS cases reveal normal intrinsic GI tract innervation.

Hypoperistalsis as the leading symptom in MMIHS is variously attributed to an imbalance in gut peptides [16]; defective autonomic inhibitory neuroeffector activity [17]; neuroaxonal dystrophy [18] and visceral myopathy [2]. Characteristic clinical features of a visceral myopathy include abdominal distension and pseudo-obstruction [19] which were evident in all our patients.

Standard histopathological techniques are often inadequate for the diagnosis of visceral myopathies. Specific histologic features are vacuolic degeneration of smooth-muscle cells with extracellular oedema and increased fibrosis of the intestinal smooth-muscle layers. The muscularis propria is predominantly affected, with loss of the internal structures. The smooth-muscle cells show loss of alignment of contractile elements. Electron microscopic findings in visceral myopathy are degenerative intestinal smooth-muscle cells with intervening proliferation of collagen fibres [19]. These histological and electromicroscopical abnormalities have been found in all investigated patients in the present study. Similar findings on light and electron microscopy have been reported in adults with chronic idiopathic intestinal pseudo-obstruction (CIIP) [20].

The major component of the smooth-muscle filaments is actin. Among different isoforms, smooth-muscle α-isoactin is an important component of the smooth-muscle cells and plays a role in the interaction of the filaments in smooth-muscle contraction. The immunohistochemical investigations in the present study revealed absent or clearly reduced α-SMA immunoreactive filaments in the intestinal muscle layers of MMIHS. Similar findings were reported in adult patients suffering from (CIIP) [20, 21].

The combination of reduced smooth-muscle filaments and vacuolic degeneration of smooth-muscle cells contributes to the hypoperistaltic obstruction of the bowel in MMIHS. The finding of reduced SMA expression in our study supports the hypothesis of the inability of MMIHS patients to synthesise adequate amounts of appropriately structured and arranged contractile fibres in smooth muscle. Disorganization or lack of smooth-muscle contractility is assumed to be the primary pathological dysfunction in CIIP in children [22]. A recent report demonstrated evidence of a primary myocellular defect of contractile fibre synthesis in MMIHS [23].

MMIHS is known to occur in families. The occurrence of this condition in siblings, together with a history of consanguity of the parents, suggests an autosomonal pattern of inheritance [13, 24–26]. Penman and Lilford have proposed that MMIHS is the result of an autosomal recessive end–organ receptor defect confined to the smooth muscle of the urinary and GI tract [27].

MMIHS is the most severe form of functional intestinal obstruction. It presents at birth and is usually fatal. Our study clearly shows that the smooth-muscle cells of the GI tract in MMIHS are structurally abnormal. The motility dysfunction in this condition is therefore due to an intestinal myopathy.

References

1. Berdon WE, Baker DH, Blane WA, Gay B, Santulli TV, Donovan C (1976) Megacystis-microcolon-intestinal hypoperistalsis syndrome. A new cause of intestinal obstruction in the newborn. Report of radiological findings in five newborn girls. Am J Roentgenol 126: 957–964
2. Puri P, Lake BD, Gorman F, O'Donnell B, Nixon HH (1983) Megacystis microcolon intestinal hypoganglionosis syndrome: a visceral myopathy. J Pediatr Surg 18: 64–69
3. Garber A, Shohat M, Sarti D (1990) Megacystis-microcolon-intestinal hypoperistalsis syndrome in two male siblings. Prenat Diagn 10: 377–387
4. Bindl L, Emons D, Haverkamp F, Fahnenstich H, Kowalewski S, Meier-Ruge W (1989) Das Megazystis-mikrokolon-intestinale hypoperistaltik-syndrom: eine Neuropathie? Z Kinderchir 44: 249–252
5. Jona JZ, Werlin SL (1981) The megacystis-microcolon-intestinal hypoperistalsis syndrome: report of a case. J Pediatr Surg 16: 749–751
6. Kirtane J, Talwalker V, Dastur DK (1984) Megacystis, microcolon, intestinal hypoperistalsis syndrome: possible pathogenesis. J Pediatr Surg 19: 206–208
7. Krook PM (1980) Megacystis-microcolon-intestinal hypoperistalsis syndrome in a male infant. Radiology 136: 649–650

8. Manco LG, Osterdahl P (1984) The antenatal sonographic features of megacystis microcolon-intestinal hypoperistalsis syndrome. J Clin Ultrasound 12: 595–598
9. Shalev J, Itzchak Y, Avigad I, Hertz M, Straus S, Serr DM (1983) Antenatal ultrasound of megacystis-microcolon-intestinal hypoperistalsis syndrome. Isr J Med Sci 19: 76–78
10. Vezina WC, Morin FR, Winsberg F (1979) Megacystis-microcolon-intestinal hypoperistalsis syndrome. Antenatal ultrasound appearance. Am J Roentgenol 133: 749–750
11. Young LW, Yunis EJ, Girdany BR, Seiber WK (1981) Megacystis-microcolon intestinal hypoperistalsis syndrome. Am J Roentgenol 137: 749–755
12. Puri P, Tsuji M (1992) Megacystis-microcolon-intestinal hypoperistalsis syndrome (neonatal hollow visceral myopathy). Pediatr Surg Int 19: 206–208
13. Granata C, Puri P (1997) Megacystis-microcolon-intestinal hypoperistalsis syndrome. J Pediatr Gastroenterol Nutr 25: 12–19
14. Puri P (2000) Megacystis-microcolon-intestinal hypoperistalsis syndrome. In: Hirschsprung's disease and allied disorders, 2nd edn. Holschneider W, Puri P (eds) Harwood, Amsterdam, 185–197
15. Skirath MS, Ford EG, Isaacs H Jr, Mahour GH (1993) Megacystis microcolon intestinal hypoperistalsis syndrome: late sequelae and possible pathogenesis. J Pediatr Surg 28: 57–59
16. Taguchi T, Ikeda K, Shono T, Goto S, Kubota M, Kawana T, Hirose R, Toyohara T (1989) Autonomic innervation of the intestine from a baby with megacystis microcolon intestinal hypoperistalsis syndrome: I. Immunohistochemical study. J Pediatr Surg 24: 1264–1266
17. Kubota M, Ikeda K, Ito Y (1989) Autonomic innervation of the intestine from a baby with megacystis microcolon intestinal hypoperistalsis syndrome: II. Electrophysiological study. J Pediatr Surg 24: 1267–1270
18. Al Rayess M, Ambler MW (1992) Axonal dystrophy presenting as the megacystis microcolon-intestinal hypoperistalsis syndrome. Pediatr Pathol 12: 743–750
19. Moore SW, Schneider JW, Kaschlua ROC (2001) Unusual variations of gastrointensinal smooth-muscle abnormalities associated with chronic intestinal pseudo-obstruction. Pediatr Surg Int: (in press)
20. Smith VV, Lake BD, Kamm MA, Nicholls RJ (1992) Intestinal pseudo-obstruction with deficient smooth-muscle α-actin. Histopathology 21: 535–542
21. Gattuso JM, Smith VV, Kamm MA (1998) Altered contractile proteins and neural innervation in idiopathic megarectum and megacolon. Histopathology 33: 34–38
22. Glassmann M, Spivak W, Mininberg D, Madara J (1989) Chronic idiopathic intestinal pseudo-obstruction: a commonly misdiagnosed disease in infants and children. Pediatrics 83: 603–609
23. Ciftci Ao, Cook RCM, van Velzen D (1985) Megacystis microcolon intstinal hypoperistaltis syndrom: evodence of a primary myocellular defect of contractile fibre synthesis. J Pediatr Surg 31: 1706–1711
24. Anneren G, Meurling S, Olsen L (1991) Megacystis-microcolon-intestinal hypoperistalsis syndrome (MMIHS), an autosomal recessive disorder: Clinical reports and review of the literature. Am J Med Genet 41: 251–254
25. Winter RM, Knowles SAS (1986) Megacystis-microcolon-intestinal hypoperistalsis syndrome: Confirmation of autosomal recessive inheritance. J Med Genet 25: 360–362
26. McNamara HM, Onwude JL, Thornton JG (1994) Megacystis-microcolon-intestinal hypoperistalsis syndrome: a case report supporting autosomal recessive inheritance. Prenat Diagn 14: 153–154
27. Penman D, Lilford R (1989) The megacystis-microcolon-intestinal hypoperistalsis syndrome: a fatal autosomal recessive condition. J Med Genet 26: 66–67