Original Article

The Electron Microscopic Findings in Colonic Tissue of Slow Transit Constipation

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Key Words
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Colectomy;
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Purpose. Slow transit constipation (STC) is a sub-type of chronic constipation. Data from pathophysiological studies support the hypothesis that an intrinsic neuromuscular abnormality may be associated with this disease entity. Under this hypothesis, we undertook an ultra-structural survey of colonic tissue in STC and compare to normal colonic tissue.
Methods. Tissue was obtained from 10 female patients with slow transit constipation who underwent therapeutic subtotal colectomy. Control tissue was obtained from 8 colectomy specimens that removed from neoplastic disease. Samples were taken from ascending, transverse, descending and sigmoid colon. All these samples examined under electronic microscope (EM).
Results. Under the EM examination, the smooth muscle cells of colon from STC were in disarray, tortuous, and condensed as compare to normal colonic smooth muscle. Other findings include cell fragmentation with disruption of myofilament. Nucleus of smooth muscle cell in STC was condensed and fragmented, too. In the cytoplasm, mitochondrion was swollen and loss of cristae. Multiple vacuoles formation in cytoplasm was noted in both smooth muscle cells and neural cells. Ballooning of axons, loss of neurotubules, and neurofilaments was found in colonic neuronal cells of STC patient.
Conclusion. Our findings would support the existence of an intrinsic neuromuscular abnormality in STC.
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Constipation is one of the most common symptoms of gastrointestinal track and involved 15~20% of general population. Most of them can manage effectively with diet habit control or laxative available on the corner pharmacy. About 1% of patientswho fair to response to diet control or laxative medication were transfer to specialists for further study and evaluation.[1] The normal transit constipation, defecatory disorder and slow transit constipation (STC) are the 3 most common types of constipation.[2] Among these subgroups, the STC is more refractory to treatment and usually need further surgical treatment.

The diagnosis of slow transit constipation dependson radiopaque transit study, scintigraphic or manometric study. There is an extreme type of STC that call colonic inertia which defined by (1) severe functional constipation (according to Rome Criteria); (2) absence of outlet obstruction; (3) delayed transit with markers

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distributed throughout the colon; (4) manometric and/or electromyographic documentation of absenct colonic motor activity; and (5) no response to pharmacologic stimulation during colonic motility recording.[3] However, the underlying pathophysiology of constipation is varied[4] and complex, the exact mechanism that induced STC still poor understood.

Unfortunately, under the light microscope examination of STC colonic tissue with Hematoxylin and eosin stain staining, there are only melanosis coli except normal colonic structure.[5] In recent decade, the advancement of molecular biology and development of animal model, there are several observational studies in this mysterious disease. One of the most important finding is the decrease number of interstitial cell of Cajal (ICC) in patient's colonic tissue.[6] The ICC play important role in intrinsic neuronal activity which act as a pacemaker in many autonomous muscular tissue. The decrease number of ICC in colonic tissue hints the possible defect of neuronal defect of these patients.

Other findings include abnormal mesenteric plexus, abnormal neurotransmitters like vasoactive intestinal polypeptide (VIP), Nitric oxide (NO), neuropeptide Y, 5-hydroxytryptamine (5-HT) had also been observed in several studies.[7]

From these findings, the urge to exam the ultrastructure changes between normal colonic tissue and STC can help us further elucidate the abnormal neuromuscular abnormalities. These may help us understand the underlying mechanism of this disorder.

Materials and Methods

Tissue was obtained from 10 female patients with slow transit constipation (age 26-42 years) who underwent therapeutic subtotal colectomy for this condition. All patients had a bowel frequency of 1 time/7-14 days (despite high fiber diet), and prolonged colonic transit studies (90-120 hours). Samples for electronic microscopy (EM) were taken from ascending, transverse, descending and sigmoid colon. Control tissues were obtained from 8 colectomy specimens removed for neoplastic disease (at least 10 cm away from the lesion site). There were 2 ascending colon, 1 transverse colon, 2 descending colon and 3 sigmoid colon samples which corresponding to the STC colonic sites.

Tissues were immediately transported to the EM laboratory in 0.5% Glutaraladhyde in Balanced salt solution (pH 7.4) at 4 °C. After dissecting into 1-2 mm blocks, the samples are fixed with 3% glutaraldehyde-2% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.4 at 4 °C for one hour. Then the samples are rinsed twice for 5 minutes each time in ice-cold 0.1 M cacodylate buffer (pH 7.4), and then post-fixed in ice-cold 1% osium tetroxide in 0.12 M cacodylate buffer for another one hour. After that the samples are again rinsed twice in ice-cold buffer and staining with 4% Tannic Acid and 0.5% Uranglacetate. The samples were sequentially dehydrated in ice-cold 30%, 50%, and 95% ethanol (5 minutes each). Then, the samples are allowed to come to room temperature and dehydration is completed with two changes in 100% ethanol. The samples are infiltrated and embedded in Epon embedding medium. After polymerization at 60 °C for 24 hours, semithin (1 um) sections are cut and stained with Mallory's Azure II-methylene blue for light microscopy. Silver ultrathin sections are cut and mounted on uncoated copper grid sections and attained with 4% uranyl acetate and Reynold's lead citrate. Samples were examined with Hitachi H-500 electron microscope operated at 75 KV.

Results

Control study

All along the colon, smooth muscle cells and neurons showed apparently normal features. The smooth muscle cells in each layer are arranged approximately parallel to one another, forming a sheet-like coat. There are thin strands of collagen within the muscle layers dividing them loosely into bundles. The collagen is in the form of septae and diffuse collagen deposition is not normally present. The smooth muscle cells of the outer circular muscle layers, of the longitudinal muscle layer and of the muscle sheaths enveloping the myenteric plexus nerve strands and ganglia are ap-

parently normal. (Fig. 1A) Well-maintained cell-to-cell junctions were present among these cells. Smooth muscle fibers are narrow and tapering. The nucleus is centrally located, and there are no transverse striations. (Fig. 1B) The cytoplasmic organelles, which include mitochondria, Golgi apparatus, scattered profiles of rough endoplasmic reticulum, and free ribosomes, are mostly confined to a conical region at each pole of the nucleus. The rest of the sarcoplasm is occupied largely by thin filaments. Characteristic dense bodies, into which the thin filaments appear to insert, are distributed throughout the sarcoplasm. (Fig. 1C) The neurons have large, round, centrally located nuclei containing large quantities of euchromatin and a prominent nucleolus. Mitochondria, endoplasmic reticulum, ribosomes and the Golgi apparatus are present within the cytoplasm.(Fig. 1C & Fig. 1D) Neurofibrils and neurotubules (microtubules) are well developed and occur through the perikaryon and cellular processes. (Fig. 1D) The fibers are enfolded by the cytoplasm of the Schwann cell.

Pathological study

The smooth muscle cells -Electron microscopy reveals several abnormalities of smooth muscle cells. Changes are characterized by a plasma membrane that is discontinuous and by myofilaments that are in disarray and no longer aligned with dense bodies. (Fig 2A & Fig. 2C) The mitochondria are vacuolated and decrease of crista. The cytoplasm is electron-lucent. Moreover, muscle cells appeared as fragmentation, (Fig. 2C) together with some disruption of normal myofilament architecture. (Fig. 2C) More advanced changes consist of frank degeneration of muscle cells. These degenerating cells are widely separated, (Fig. 2B) with abundant collagen in the spaces between them. (Fig. 2B & Fig. 2C) The nuclease of the smooth muscle cell was condensed and fragmented. (Fig. 3A) Mitochondria swelling with the loss of cristae and formation of vacuoles in the smooth muscle cell were noted. (Fig. 3B)

The neuronal cells - Varying degrees of ultrastructural morphological changes were constantly demonstrated in the axon terminal. Degeneration is suggested by a spectrum of changes such that neurons appear swollen, distorted, vacuolated, or misshapen with clubbed or fragmented dendrites. The abnormalities consist of ballooning of axons, which have loss of neurotubules and neurofilaments. (Fig. 3D) Mitochondrial swelling with disruption of the cristae and

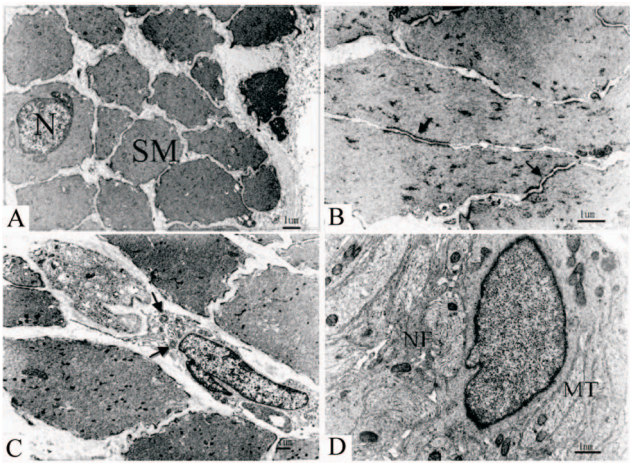

Fig. 1. (A) Cross section of smooth muscle cells (SM). Morphology of cell is normal. Nucleus (N) is centrally located. (6000 X); (B) Smooth muscle cell is arranged parallel with normal cell-cell junction (Arrow); (C) Schwann cell is normal. Axon terminal is normal with normal mitochondria (Arrow) and axolemma (4800 X); (D) Neurofilaments (NF) and microtubules (MT) are normal in the nerve ending (10,000 X).

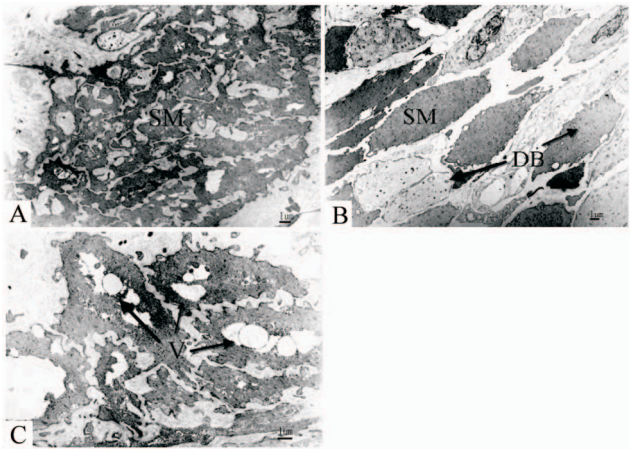

Fig. 2. (A) Smooth muscle (SM) cells were arranged in irregularity, and were tortuous and condensed (3600 X); (B) Dense bodies (DB) within smooth muscle cell (SM) were varied in size (3000 X); (C) Loss of normal myofilament architecture with formation of vacuole (V) was noted (4800 X).

formation of the vacuoles were also observed. (Fig. 3C & Fig. 3D)

Discussion

Severe idiopathic slow transit constipation is a troubling problem often afflicting young women.[8] The most disruptive symptoms are chronic tenesmus, straining with defecation, abdominal bloating and occasionally abdominal pain. The severity of symptoms and the lack of efficacy of medical therapeutic measures may eventually lead to colonic resection in these subjects.[9] The underlying pathophysiology of slow transit constipation is not clear. For these patients, the routine histological evaluation for these subtotal colectomy specimens usually appears normal. Some studies have characterized the major disturbances of motility in this condition,[10] but the enteric neural abnormality which produces these changes is unknown. Although the colon appears macroscopically normal, the abnormalities in argyrophilic neurons and axons in the myenteric plexus were observed in STC colonic tissue.[11]

Slow transit constipation is a disorder of colonic motility characterized by a reduction in the frequency, amplitude, and duration of propulsive contraction in the large bowel.[12] The enteric neural alterations producing these abnormalities are not completely understood. Although histological and functional studies have shown morphological changes of the myenteric plexus, abnormalities of interstitial cell of Cajal and unusual distribution of some neurotransmitters.[13] This has especially been true regarding the concentration of vasoactive intestinal peptide (VIP). Immunohistochemical studies in colonic circular muscle of slow transit constipation patients showed non-consistent findings. Some authors demonstrated an increase of neural tissue in the myenteric plexus,[14] however other authors showed a reduced number of myenteric plexus neurons with a decreased concentration of VIP-positive neurons and increased nitric oxide synthase positive nerves.[13,15,16]

Many authors have suggested that an abnormality in the myenteric plexus can affect the functional activity ofcholingeric nerve fibers.[17,18] Bureleigh[18] demonstrated a reduced activity of cholinergic nerves in the longitudinal muscle of bowel wall of the colon removed from patients with slow transit constipation. Abnormal cholinergic innervation in severely constipated subjects was expected, since the role of acetycholine as a neurotransmitter within the enteric nervous system is well established.[11] Benson et al. performed an immunohistochemical study for neurofilament included S-100 protein, and neuro-specific enolase in patients with slow transit constipation. They observed an increase in small nerve fibers of the muscularispropria, but no other neural or myocyte abnormalities were detected by light microscopy. Studies carried out by Cortesini et al.[15] and Schouten et al.[19] on colon specimens from STC found morphological alterations in the myenteric nervous plexus and a reduction of the total density of intrinsic inhibitory neurons. The apparently normal axon bundles in the myenteric plexus showed a reduced or absent neurofilaments.

Autonomic dysfunction is an established cause of constipation in conditions such as diabetes, and multiple epidemiological studies have confirmed the associations between constipation and neurological dis-

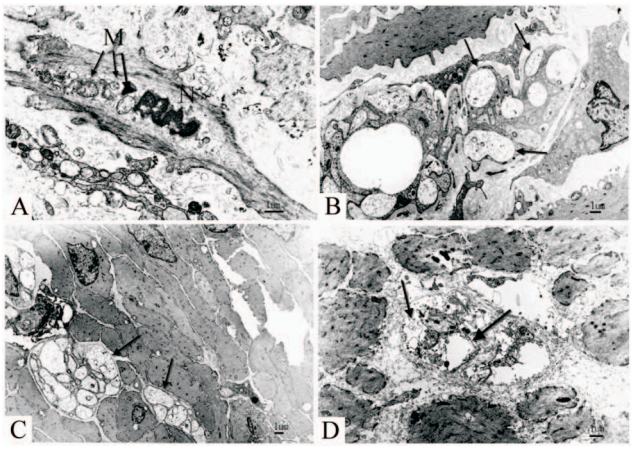

Fig. 3. (A) Nucleus (N) of the smooth muscle cell was condensed and fragmented (7000 X). Mitochondria (M) were swollen and disrupted; (B) Mitochondria (Arrow) are swollen with disruption of the cristae and formation of the vacuoles (3600 X); (C) Mitochondria swelling and disruption of the cristae (Arrow) were seen in neural cell (3000 X); (D) Vacuole formation with loss of neurofilament (Arrow) was observed (4800 X).

orders.[20] It has been hypothesized that slow transit constipation may be related to defective enteric innervation[17] and there is some evidence of neural dysfunction in patients with slow transit constipation.[21,22]

Up to now, there was less direct evidence or observation of their ultrastructural change in these resected specimens. More recently, Wedel et al.[23] use new smooth muscle specific antibodies for immunohistochemistry studies and revealed the abnormalities of smooth muscle myosin heavy chain, smoothelinand Histone deacetylase 8 in longitudinal and circular muscles of colon form patient with STCand all these proteins play important roles in smooth muscles contractility. These findings combined with our results demonstrate not only smooth muscle cells phenotypic abnormalities but also structural degeneration in STC smooth muscle cells. Our studies reveal that the smooth muscle cells were arranged in irregularity and were rather tortuous and condensed. Moreover, some smooth muscle cell nucleus appeared as fragmentation. Loss of normal myofilament architecture is also noted in the STC colonic muscles. Mitochondria swelling with the loss of cristae, and formation of vacuoles were noted in both STC smooth muscle cell and STC neural cells.

In summary, our results demonstrate the presence of an intrinsic neuromuscular abnormality in slow transit constipation (Table 1). It is still not possible to say whether the neuronal changes are secondary to smooth muscle celldegeneration or vice-versa, or whether either/both of these changes represent a primary defect or are a consequent response to this functional abnormality.

Table 1. The difference between normal smooth muscle/neuron cells and slow transit constipation smooth muscle/neuron cells

	Normal smooth muscle cell	STC smooth muscle cell
Nucleus	Round	Fragmented
Myofilament	aligned and parallel	Disarray
Mitochondria	Oval shape with crista	Swollen with loss of crista
ER	Peri-nucleus distribution	
	Normal Neuron	STC neuron
Nucleus	Round and large	small size
Neurotubules	Present	Absence
Neufofibrils	Present	Absence
Mitochondria	Oval shape with crista	swollen with loss of crista

STC: slow transit constipation; ER: endoplasmic reticulum.

Conclusion

STC is a type of bowel motility disorder which induced intractable constipation and involved mostly in young women. The pathophysiology of this disease is still not well known. Here we examined the ultrastructure of the diseased colonic tissue by electron microscopy and reveal the abnormality of smooth muscle and neuron cell. These findings may help us more closely to elucidate the actual mechanism of this disorder.

References

1. Camilleri M, Thompson Wg Fau - Fleshman JW, Fleshman Jw Fau - Pemberton JH, Pemberton JH. Clinical management of intractable constipation. *Annals Intern Med* 1994;121 (0003-4819 (Print)):520-8.
2. Lembo A, Camilleri M. Chronic constipation. *N Engl J Med* 2003(1533-4406 (Electronic)):1360-8.
3. Bassotti G, Roberto GD, Sediari L, Morelli A. Toward a definition of colonic inertia. *W J Gastroenterol: WJG* 2004;10: 2465-7.
4. Alame AM, Bahna H. Evaluation of constipation. *Clini Colon Rectal Surg* 2012;25:5-11.
5. Knowles CH, Farrugia G. Gastrointestinal neuromuscular pathology in chronic constipation. *Best Practice Res Clini Gastroenterol* 2011;25:43-57.
6. He CL, Burgart L, Wang L, Pemberton J, Young-Fadok T, Szurszewski J, Farrugia G. Decreased interstitial cell of cajal volume in patients with slow-transit constipation. *Gastroenterology* 2000;118:14-21.
7. Dinning PG, Smith TK, Scott SM. Pathophysiology of colonic causes of chronic constipation. *Neurogastroenterol Motility: Official J Eur Gastrointestinal Motility Soc* 2009;21 Suppl 2:20-30.
8. PRESTON DM, LENNARD-JONES JE; Severe chronic constipation of young women: 'idiopathic slow transit constipation'. *Gut* 1986;27:41-8.
9. Lubowski D, Chen F, Kennedy M, King D. Results of colectomy for severe slow transit constipation. *Dis Colon Rec* 1996;39:23-9.

10. Bassotti G, Gaburri M, Imbimbo B, Rossi L, Farroni F, Pelli M, et al. Colonic mass movements in idiopathic chronic constipation. *Gut* 1988;29:1173-9.
11. Krishnamurthy S, Schuffler MD, Rohrmann CA, Pope II CE. Severe idiopathic constipation is associated with a distinctive abnormality of the colonic myenteric plexus. *Gastroenterology* 1985;88:26-34.
12. Bassotti G, Chiarioni G, Vantini I, Betti C, Fusaro C, Pelli MA, et al. Anorectal manometric abnormalities and colonic propulsive impairment in patients with severe chronic idiopathic constipation. *Digest Dis Sci* 1994;39:1558-64.
13. Milner P, Crowe R, Kamm MA, Lennard-Jones J, Burnstock G. Vasoactive intestinal polypeptide levels in sigmoid colon in idiopathic constipation and diverticular disease. *Gastroenterology* 1990;99:666-75.
14. Park H, Kamm M, Abbasi A, Talbot I. Immunohistochemical study of the colonic muscle and innervation in idiopathic chronic constipation. *Dis Colon Rec* 1995;38:509-13.
15. Cortesini C, Cianchi F, Infantino A, Lise M. Nitric oxide synthase and VIP distribution in enteric nervous system in idiopathic chronic constipation. *Digest Dis Sci* 1995;40:2450-5.
16. Koch TR, Carney JA, Go L, Go V. Idiopathic chronic constipation is associated with decreased colonic vasoactive intestinal peptide. *Gastroenterology* 1988;94:300-10.
17. Bassotti G, Chiarioni G, Imbimbo BP, Betti C, Bonfante F, Vantini I, Morelli A, et al. Impaired colonic motor response to cholinergic stimulation in patients with severe chronic idiopathic (slow transit type) constipation. *Digest Dis Sci* 1993; 38:1040-5.
18. Burleigh D. Evidence for a functional cholinergic deficit in human colonic tissue resected for constipation. *J Pharmacy Pharmacol* 1988;40:55-7.
19. Schouten WR, ten Kate FJ, de Graaf EJ, Gilberts EC, Simons JL, Klück P. Visceral neuropathy in slow transit constipation: an immunohistochemical investigation with monoclonal antibodies against neurofilament. *Dis Colon Rec* 1993;36:1112-7.
20. Johanson JF, Sonnenberg A, Koch TR, Mccarty DJ. Association of constipation with neurologic diseases. *Digest Dis Sci* 1992;37:179-86.
21. Raethjen J, Pilot M-A, Knowles C, Warner G, Anand P, Williams N. Selective autonomic and sensory deficits in slow transit constipation. *J Autonomic Nervous System* 1997;66: 46-52.
22. Altomare D, Pilot M, Scott M, Williams N, Rubino M, Ilincic L, et al. Detection of subclinical autonomic neuropathy in constipated patients using a sweat test. *Gut* 1992;33:1539-43.
23. Wedel T, Van Eys GJ, Waltregny D, Glenisson W, Castronovo V, Vanderwinden JM. Novel smooth muscle arkers reveal abnormalities of the intestinal musculature in severe colorectal motility disorders. *Neurogastroenterol Motility: Official J Eur Gastrointest Motility Soc* 2006;18:526-38.

原　著

電子顯微鏡下大腸無力症腸道細胞的表現

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目的　大腸無力症的真正致病機轉尚不清楚，一般顯微鏡下的組織結構並無異常，本文利用電子顯微鏡下觀察其超微構造有無變化。

方法　共收集 10 位女性患有大腸無力症並接受大腸切除的病人，有 8 位患有大腸癌接受手術時，距腫瘤 10 公分以上處當作正常組。在適當的製備下以電子顯微鏡觀察其不同及變化。

結果　電子顯微鏡下觀察到平滑肌細胞的排列紊亂，並失去整體性。肌細胞看起來成破碎分裂狀。正常的肌纖維結構已遭破壞。肌細胞的細胞核也成碎狀。粒腺體腫大成空泡狀。神經細胞也明顯退化。神經管狀構造及神經纖維也遭破壞。

結論　本文的結果也證實大腸無力症的大腸神經細胞及肌肉細胞在電子顯微鏡下有異常的變化。

關鍵詞　大腸無力症、大腸切除、超微構造、電子顯微鏡。