Modification of the Acetylcholinesterase (AchE) Staining Method in Hirschsprung's Disease

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Abstract

Demonstration of the increasing activity of acetylcholinesterase (AChE) in a segment of the colon has proved to be the most accurate diagnostic tool to diagnose Hirschsprung's disease. Two methods of histochemical assessment were tried to establish the most appropriate and effective method for this study within the limitation of available equipment. Lake's method was chosen and was modified as the standard histochemical examination.

Key word : Acetylcholiesterase Staining, Rectal Suction Biopsy, Hirschsprung's Disease

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Hirschsprung's disease is a functional disturbance characterized by obstruction mostly of the large intestine due to defective function of the aganglionic segment. The clues for diagnosis depend on the clinical history, physical examination and barium enema. If all the clues are suspicious, histological proof of the abscence of ganglion cells is essential to confirm the diagnosis. Until now, a full-thickness biopsy specimen that includes both muscular coats, submucosal and mucosal layers has been necessary for the diagnosis in all pediatric surgical centers in Thailand. However, there is the harzard of general anesthesia required to obtain the specimen and the more difficult subsequent operative procedure. The accuracy of the barium enema for diagnosis is also questionable.

Rectal suction biopsy with subsequent study of acetylcholinesterase (AChE) activity has been adopted since 1972[1] and has proved to be a simple, safe, effective and accurate method for

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diagnosis of such a disease[2] and is now used as an intermediate diagnostic procedure in most pediatric surgical centers in western countries[3-5].

In an attempt to introduce this procedure in Thailand and to assess its accuracy, the prospective study was launched in two parts. The first part of the project was the attempt to find the most appropriate method for AChE activity staining. The experiment was performed in 1989. The method and result are reported here. The second part was the assessment of such a procedure in the clinical field for diagnosis of Hirschsprung's disease and will be published separately.

MATERIAL AND METHOD

The authors surveyed all the staining methods for demonstrating AChE activity that could be employed with the equipment available. We decided to try two possible methods. One was the direct coloring thiocholine method for cholinesterase proposed by Karnovsky and Roots in 1964[6]. The second was Lake's method[2]. We used rectal suction biopsy specimens from known cases of Hirschsprung's disease for testing the methods. We could not demonstrate acetylcholinesterase by Karnovsky and Roots' method, but we succeeded in Lake's method with some modifications.

Ten rectal suction biopsy specimens from known cases of Hirschsprung's Disease (HD) and two specimens of non HD cases were obtained by using Noblett instruments. The specimens were put into the freezing microtome and embedded in the OCT compound as the cryoprotective agent. The temperature in the freezing microtome was approximately -30°C. Cryostat sections were cut at 8-20 μ in a plane perpendicular to the mucosal surface. Some sections were checked with hematoxylin and eosin staining to ensure that the specimen was covered with columnar epithelium and that both mucosa and submucosa were present. The sections were fixed in 4 per cent formaldehyde solution in 0.1 M calcium acetate for 3 seconds, rinsed in distilled water for 10 seconds and incubated for one hour in a mixture containing acetylthiocholine iodide as the substrate for acetylcholinesterase. The mixture contained 5.0 mg acetylthioholine iodide, 6.5 ml of 0.1 M acetate buffer pH 6.0, 0.5 ml of 0.1 M sodium citrate, 1.0 ml of 30 mM copper sulfate, 1 ml distilled water, 1 ml of 5 mM potassium ferricyanide and 0.2 ml of tetraisopropyl pyrophosphoramide. The section was washed briefly with distilled water (10 seconds) then treated with 0.05 per cent diaminobenzidine tetrahydrochloride (DAB) in distilled water for 45 minutes at room temperature. The sections were washed with distilled water (10 seconds) then treated with 1 per cent aqueous osmium tetroxide for 5 minutes and washed well with distilled water. The sections were dehydrated in graded alcohol, cleared in xylene and mounted with permount. The sections were examined under a light microscope.

The other sections were treated identically but omitting the substrate (acetylthiocholine iodide).

RESULT

The AChE stained slides were examined under a light microscope. Abscence of dark stained nerve fibers or presence of occasional small thin nerve fibers in the lamina propria, muscularis mucosae and submucosa constituted normal AChE activity or a negative pattern (Fig. 1A). Presence of a network of coarse nerve fibers in the lamina propria, muscularis mucosae and/or a thick nerve trunk in the submucosa was designated a positive pattern or indicated increased AChE activity (Fig. 1B).

All known cases of HD showed the increased AChE activity but exhibiting different patterns which will be further discussed. No AChE activity was seen in the sections incubated in the solution without substrate. In the non- HD cases, only occasional small thin nerve fibers were seen in the lamina propria. (Fig. 1A)

DISCUSSION

We have modified the acetylcholinesterase staining method proposed by Lake BD in 1978. In our procedure the main equipment is the freezing microtome available in every anatomical and pathological laboratory. Instead of mounting the specimens on blocks of animal liver, we embedded them in the OCT compound precooled in the freezing microtome. We did not freeze the specimen in hexane maintained at –80°C and distilled water was used instead of tap water. The duration for treating the specimen in osmium tetroxide was reduced from 10 to 5 minutes in order to minimize precipitation. The authors also did not counterstain with Carazzi's hematoxylin in the last step. Our

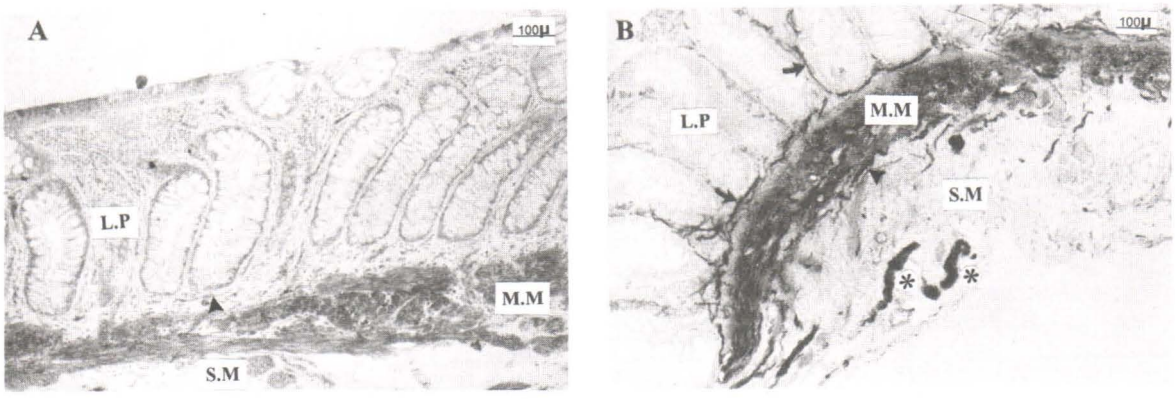

Fig. 1. Rectal suction biopsy stained for AChE activity.
(A) From a normal control, occasional small thin nerve fibers (arrow) were seen in the lamina propria (LP).
(B) From known case of HD, coarse nerve fibers in the lamina propria (LP), muscularis mucosae (MN) and thick nerve trunk in the submucosa (SM) were seen.

modified technique was successful in demonstrating acetylcholinesterase activity in the nerve as shown in Fig. 1B. The prominent nerve fibers staining for AChE were seen in the lamina propria and muscularis mucosae. The finding was in agreement with several previous studies(1,2,7-11).

SUMMARY

The histochemical study for acetylcholinesterase activity was first introduced in Thailand to diagnose Hirschsprung's disease in 1989. The staining method was modified from the standard technique.

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REFERENCES

1. Mier-Runge W, Luttervuck PM, Herzog B, Morger R. Acetylcholinesterase activity in suction biopsies of the rectum in the diagnosis of Hirschsprung's disease. J Pediatr Surg 1972; 7: 11-7.
2. Lake BD, Puri P, Nixon HH, Chaireaux AE. Hirschsprung's disease. An appraisal of Histochemically demonstrated Acetylcholinesterase Activity in Suction Rectal Biopsy Specimens as an Aid to Diagnosis. Arch Pathol Lab Med 1978;102: 244-7.
3. Monforte-Munoz H, Gonzalez-Gomez I, Rowland JM, Landing BH. Increased submucosal nerve trunk caliber in aganglionosis : a "positive" and objective finding in suction biopsies and segmental resections in Hirschsprung' disease. Arch Pathol Lab Med 1998;122:721-5.
4. Alizai NK, Batcup G, Dixon MF, Stringer MD. Rectal biopsy for Hirschspurung's disease : what is the optimum method? Pediatr Surg Int 1998; 13:121-4.
5. Qualman SJ, Jaffe R, Bove KE, Monforte-Munoz H. Diagnosis of Hirschsprung disease using the

rectal biopsy: multi-institutional survey. Pediatr Dev Pathol 1999;2:588-96.
6. Karnovsky MJ, Roots L. A "direct-coloring" thiocholine method for cholinesterase. J Histochem Cytochem 1964;12:219-21.
7. Chow CW, Chan WC, Yue PC. Histochemical criteria for the diagnosis of Hirschsprung's disease in rectal suction biopsies by acetylcholinesterase activity. J Pediatr Surg 1977;12:675-80.
8. Dale G, Bonham JR, Riley KW. An improved method for the determination of acetylcholinesterase activity in rectal biopsy tissue from patients with Hirschsprung's disease. Clin Chem Acta 1977; 77:407-13.
9. Huntley CC, Shaffner LD, Challa VR, Lyerly AP. Histochemical diagnosis of Hirschsprung disease. Pediatrics 1982; 69:755-61.
10. Hamoudi AB, Reiner CB, Boles ET Jr, Meclung HJ, Kerzner B. Acetylthiocholinesterase staining activity of rectal mucosa. Its use in the diagnosis of Hirschsprung's disease. Arch Pathol Lab Med 1982; 106: 670- 2.
11. Wakely PE Jr, Mc Adam AJ. Acetylcholinesterase histochemistry and the diagnosis of Hirschsprung's disease : a 3 1/2 year experience. Pediatr Pathol 1984; 2:35-46.

การดัดแปลงวิธีการย้อมอะซิติลโคลินเอสเทอเรสในโรคเฮอร์สปรุง

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วิธีการที่แม่นย่าที่สุดในการวินิจฉัยโรคเฮอร์สปรุง คือ การที่สามารถแสดงให้เห็นว่ามีการเพิ่มขึ้นของอะซิติลโคลินเอสเทอเรสในชั้นเนื้อของลำไส้ใหญ่ คณะผู้วิจัยได้ทำการทดลองวิธีการย้อมเอนซัยม์ชนิดนี้โดยใช้ 2 วิธี ภายใต้เงื่อนไขของความจำกัดในเรื่องของเครื่องมือและอุปกรณ์ที่มีอยู่ ซึ่งพบว่าวิธีการของ Lake และการดัดแปลงเพื่อให้เหมาะกับเครื่องมือที่มีอยู่สามารถใช้เป็นวิธีมาตรฐานของการตรวจทางฮีสโตเคมีได้

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