The organization of the lamina muscularis mucosae in the human esophagus

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Summary. The structural organization of the lamina muscularis mucosae of the human esophagus was studied by light microscopy and scanning electron microscopy (SEM). The organization of the lamina muscularis mucosae varied considerably among the cervical, the thoracic, and the abdominal part of the esophagus. In the cervical part, the lamina muscularis mucosae was not well developed and only inlets of the smooth muscle bundles were scattered within the connective tissue. In the thoracic part, the lamina muscularis mucosae consisted of several layers of smooth muscle bundles, individual muscle cells of which ran in a longitudinal direction. In the abdominal esophagus near the cardia, the muscular bundles in the lamina muscularis mucosae ran in various directions forming a reticulated configuration. The differences in density and arrangement of the lamina muscularis mucosae are discussed in relation to the swallowing of food and submucosal invasion of esophageal cancer.

Introduction

The esophagus functions primarily to conduct food from the pharynx to the stomach, and the tunica muscularis and the lamina muscularis mucosae are apparently concerned with its movements. Anatomically, the human esophagus is divided into three parts: the cervical, thoracic and abdominal (Warwick et al., 1975). As for the tunica muscularis, this layer in the cervical esophagus, consists of striated muscle, which gradually replaces the smooth muscle in the lower esophagus. In contrast, no precise description has been made of the organization of the lamina muscularis mucosae; some histology textbooks state that the lamina muscularis mucosae consists of longitudinal smooth muscle cells (Fawcett, 1994), while some others note that the lamina muscularis mucosae consists of the inner circular and outer longitudinal layers (Warwick and Williams, 1975). A precise analysis of the organization of the lamina muscularis mucosae has been a waited, especially in relation to the physiological significance of this layer.

On the other hand, the importance of the lamina muscularis mucosae has been also reported from the viewpoint of an invasion of esophageal carcinoma; it has been established that the five-year survival rate of patients with intramucosal carcinoma is significantly superior to that of patients with submucosal invasion (Yoshinaka et al. 1991, Araki et al., 2002). Thus, the aim of this study is to clarify the structural organization of the lamina muscularis mucosae more clearly and precisely than before by the use of light microscopy and scanning electron microscopy (SEM). In the present study, we especially focused on the regional differences in structure of this layer for a better understanding its physiological and pathological significance.

Materials and Methods

Human esophagi were taken by autopsy from five adult subjects (aged from 20 to 77 years old) without any histories of esophageal disease. The tissues were divided into three parts: the cervical, thoracic and abdominal sections of each esophagus. Normal esophageal tissues were also obtained, with informed consent, from four adult patients (aged from 36 to 81 years old) who underwent surgery for esophageal carcinoma at Oita Medical University.
**Light microscopy**

Specimens were longitudinally cut into 5 mm thick slices with razor blades, fixed with 10% formalin, dehydrated in a series of ethanol, cleared by xylene, and embedded in paraffin. Sections (about 5 μm) were made and stained with hematoxylin and eosin.

**Scanning electron microscopy (SEM)**

The tissue blocks fixed in Karnovsky’s fixative were used for SEM. They were treated with 6N NaOH at 60°C for 10–15 min (Takahashi-Iwanaga et al. 1986) or with 8N HCl at 37°C for 3 h (Shimada et al., 1981) for the digestion of collagen fibers. After being rinsed 4 times in physiological saline, the blocks (about 2 cm × 2 cm) were placed in an elastase solution (1~5 mg/ml, Tohri and Company Ltd., Tokyo) at 37°C for 12–18 h. The remaining paraffin blocks for light microscopy were also used for conventional SEM.

They were deparaffined with xylene, hydrated, and fixed again in Karnovsky’s fixative and immersed in 2N NaOH at 37°C for 3 h to digest proteoglycan (Shimada et al., 1993). All specimens were then washed thoroughly in distilled water, and placed in cacodylate-buffered 1% osmium tetroxide, 1% tannic acid solution, and 1% osmium tetroxide for 1 h each. They were then dehydrated through a graded series of ethanol, dried by the t-butanol drying method, coated with gold and observed under a Hitachi S-800 SEM at an acceleration voltage of 15kV.

**Results**

**Light microscopy**

In the specimens stained with hematoxylin and eosin, the lamina muscularis mucosae in the human esophagus was clearly observed because of the presence of smooth muscle bundles in the mucosal connective tissues. The histological
Fig. 2. Light micrographs of the lamina muscularis mucosae (LMM) in the thoracic esophagus. a: Blood vessels cross perpendicularly in the gap of the lamina muscularis mucosae. b: A lymphatic vessel (L) is observed between two muscles bundles in the lamina muscularis mucosae. a, b: ×210

organization varied considerably among the cervical, thoracic and abdominal parts as follows.

The lamina muscularis mucosae in the cervical part was poorly developed, and only the islets of muscular bundles were scattered within the connective tissue. (Fig. 1a). In the region between two neighboring bundles the lamina propria mucosae was directly continuous with the tela submucosa. Arterioles, venules and lymphatic vessels freely entered the subepithelial connective tissue through the gap of the lamina propria mucosae. In the thoracic part, muscle bundles in the lamina muscularis mucosae became dense, and were piled up to form a continuous muscles layer about 75 μm to 550 μm in thickness. Each bundle of smooth muscle cells in this layer ran in a longitudinal direction along the axis of the esophagus. (Fig. 1b). Blood vessels and excretory ducts of the esophageal glands nearly vertically traversing the lamina muscularis mucosae were occasionally found in the region where the muscle layer was partially lacking. (Fig. 2a). Lymphatic vessels were sometimes sandwiched between the upper and lower muscle bundles, suggesting

that the vessels obliquely or longitudinally crossed the lamina muscularis mucosae. (Fig. 2b). In the abdominal part of the esophagus, the lamina muscularis mucosae ranged from 140 μm to 450 μm in thickness. Although the muscular bundles in this part ran roughly longitudinally, those near the cardia showed a more complicated cytoarchitecture (Fig. 1c).

Scanning electron microscopy

The treatment of the human esophagus with 6N NaOH or 8N HCl followed by elastase treatment resulted in the removal of collagenous elements, thus exposing the stromal surfaece of smooth muscle cells in the lamina muscularis mucosae (Fig. 5, 6). On the other hand, treatment of the tissues with 2N NaOH removed only an extracellular ground substance, which was useful for observation of the smooth muscle cells in relation to the arrangement of reticular, elastic and nerve fibers by SEM (Fig. 3, 4).

In the cervical part of the esophagus, the lamina muscu-
Fig. 4. SEM image of the lamina muscularis mucosae (LMM) in the thoracic esophagus treated with 2N NaOH. The lamina muscularis mucosae is very thick, showing a plate like profile because of the presence of smooth muscle cells piling up densely in this layer. $\times 90$

Fig. 3. SEM images of the lamina muscularis mucosae (LMM) in the cervical esophagus treated with 2N NaOH (a) or 8N HCl (b) followed by elastase treatment. a: The esophagus wall is cut longitudinally along the axis. A small muscular bundles of the lamina muscularis mucosae is seen within the connective tissue. b: The inner surface of the lamina muscularis mucosae. Note muscular bundles running longitudinally. a: $\times 180$, b: $\times 680$
Fig. 5. The outer surface of the lamina muscularis mucosae (LMM) in the thoracic esophagus. Smooth muscle cells are arranged in a sheet in the lamina muscularis mucosae. Note an esophageal gland (G) and blood vessels (BV) producing gaps of the muscular sheet. ×80. The inset shows a higher magnification of smooth muscle cells. ×680
laris mucosae was composed of small longitudinal bundles of smooth muscle cells (Fig. 3a), which were arranged very sparsely beneath the lamina propria mucosae. Individual muscular bundles were 15 μm thick consisting of 5 layers of smooth muscles cells. Thus, the border between the lamina muscularis mucosae and the tela submucosa was not distinct. In the thoracic part of the esophagus, the lamina muscularis mucosae was observed as a rather continuous sheet consisting of two to five layers of smooth muscle bundles. These muscular bundles were composed of longitudinally running smooth muscle cells (Fig. 4). Even though individual variations were present in thickness of the lamina muscularis mucosae, this part was thickest among the three parts of the esophagus.

Viewed from the side of the tela submucosa, the lamina muscularis mucosae appeared as a sheet of longitudinally arranged bundles of smooth muscle cells, where the blood vessels and the esophageal glands closely adhered (Fig. 5).
Gaps in this muscular layer were apparently produced by the presence of the esophageal glands and large vessels. At a higher magnification, smooth muscle cells in each bundle were observed like a long slender cord, which appeared to contact each other with adjacent smooth muscle cells (Fig. 5).

The lamina muscularis mucosae in the abdominal part near the cardia differed from the other two parts in both the cytoarchitecture and density of smooth muscle cells. The bundles of smooth muscles cells ran in various direction to form a complicated network (Fig. 6).

Discussion

The present study demonstrated the three-dimensional organization of the lamina muscularis mucosae of the human esophagus, and clarified the regional differences in the structure of this layer by both light microscopy and SEM. In this study, the lamina muscularis mucosae of the cervical esophagus was poorly developed. This may provide enough room for the wide expansion of the esophageal lumen during the swallowing of foods. Because the tunica muscularis consisted of the striated muscle (Warwick et al., 1975), the movement of the cervical part is probably regulated exclusively by the voluntary striated muscle.

On the other hand, the present study showed that the lamina muscularis mucosae of the thoracic and abdominal esophagus is well developed. The thick layer of the lamina muscularis mucosae in these portions may be useful for maintaining pressure against the pressure from the internal cavity similar to these smooth muscles in blood vessels (Farquharson et al., 1989). Dilatation of the esophageal lumen may be limited when food passes down through the esophagus to the stomach.

Another noteworthy finding is that the lamina muscularis mucosae near the cardia consists of complicated networks of smooth muscles cells. It is known that the lower esophageal portion near the cardia is a high pressure zone; intraluminal pressure in this zone is 15 to 40 mmHg, which is higher than the intragastric pressure (Bell et al., 1980). Thus, the reticular arrangement of smooth muscle cells in the lamina muscularis mucosae near the cardia may be related to the intramural pressure, and probably, together with the tunica muscularis, play a role in preserving the reverse flow from the stomach to the esophagus.

Clinically, intraepithelial or mucosal carcinoma in the superficial esophageal cancer has almost no risk of lymph node metastasis and recurrence, while the submucosal carcinoma had a high frequency of lymph node metastasis and recurrence (Yoshinaka et al., 1991, Araki et al., 2002). It is likely that the lamina muscularis mucosae serves as a limiting barrier to block submucosal invasion of carcinoma. In the present study, regional differences in structural organization of the lamina muscularis mucosae were clarified by both light microscopy and SEM. These findings throw new light upon the interpretation of the submucosal invasion of carcinoma cells. The presence of developed lymphatic vessels in and around the lamina propria mucosae, as demonstrated by Hashimoto et al. (2002), is also noteworthy in connection with the traffic of the immune cells between the lamina propria and submucosa, although a precise morphological analysis is still awaited for this point.

References


