activities have not been well studied. In the present study, we applied electrophysiological techniques to urethane-anesthetized rats (10 – 12) to investigate the effects of intravenous injection of ghrelin on autonomic nerve activities, including the gastric and hepatic branches of the efferent vagus nerve and the sympathetic nerve that innervates brown adipose tissue, the adrenal gland, and the kidney. In addition, we studied the effect of neurectomy of the vagal gastric afferent nerve on the activation of the efferent vagal gastric nerve by ghrelin. As a result, we revealed for the first time that ghrelin stimulates the gastric efferent vagus nerve in an organ-specific manner without affecting the gastric afferent vagus nerve and that ghrelin does not acutely affect the efferent basal activity of the sympathetic nerve in rats.

Materials and Methods

Materials

Human ghrelin (> 95% purity) was synthesized via the chemical condensation of the N-terminal 7-amino-acid peptide and a recombinant 21-residue C-terminal fragment, as reported previously (13). Human desacyl-ghrelin (> 99% purity) was synthesized using a solid-phase synthesis method (Asubio Pharma Co, Ltd., Kobe). The following compounds were used: cholecystokinin-8 (CCK8; Peptide Institute, Osaka), hexamethonium bromide (hexamethonium; Tokyo Chemical Industry, Tokyo), D-(+)-glucose (glucose; Nacalai Tesque, Kyoto), saline (Otsuka Pharmaceutical Factory, Tokushima), and urethane (Sigma-Aldrich, Saint Louis, MO, USA).

Animals and surgical operation

All experiments were performed under the approval of the Ethics Committee of Asubio Pharma Co, Ltd. Male Wistar rats (8 – 9 weeks of age; Japan Charles River, Yokohama) were used in this experiment. Rats were housed under controlled temperature (25°C) and light cycle (lights on between 7:00 and 19:00) and were fasted for 16 h with free access to tap water before surgery. Under anesthesia of 1 g/kg of urethane, which was delivered by intraperitoneal injection, rats were placed in prone or supine positions. The left carotid artery was cannulated with a polyethylene catheter containing 50 U/mL of heparin in saline to monitor blood pressure using a pressure transducer (DX300; Nihon-Koden, Tokyo). The left femoral vein was also cannulated for drug administration. Heart rate was measured from the blood pressure waveform. The body temperature was kept at 36.5°C to 37.5°C using a heat controller (ATC-101B; Unique Medical, Tokyo), and a probe was placed into the rectum to monitor rectal temperature. A skin incision on the back or an abdominal horizontal incision was made, and under a stereomicroscope, the bundle of the ventral gastric or hepatic branch of the vagus nerve, or the sympathetic nerve branch that innervates brown adipose tissue, the adrenal gland, or the kidney, was carefully isolated from surrounding tissues using a sharp blade. The nerve filament was transected at the region close to the each organ; and to record efferent nerve activity, the upper bundle of the nerve filament was placed on a pair of platinum-wire electrodes (Unique Medical) and then covered with a paraffin-vaseline mixture (45:55). In the gastric afferent vagotomy group to study the gastric action of ghrelin, the dorsal vagal trunk was transected just under the diaphragm; the hepatic and celiac branches of the ventral vagus nerve were also transected. In addition, the ventral gastric nerve filament was transected at the region close to the stomach, and the upper bundle of the nerve filament was placed on the electrodes to record only the gastric efferent nerve activity (Fig. 1).

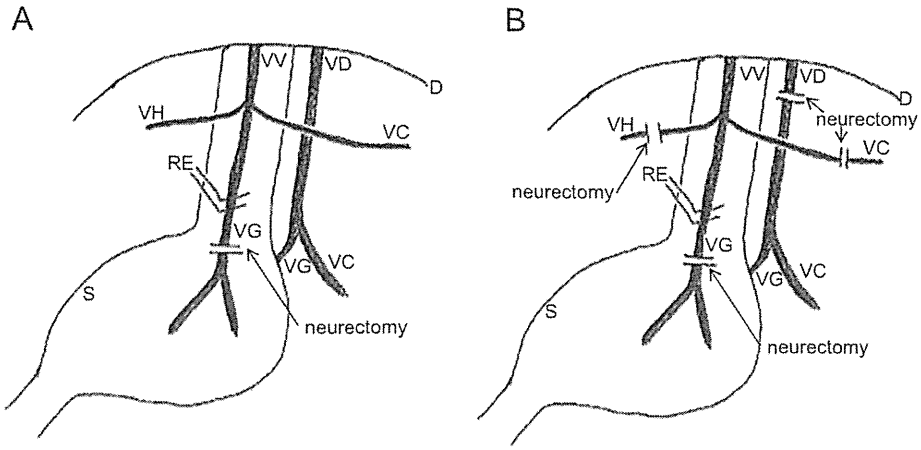

Fig. 1. Schema of the recording method for efferent activity of the gastric vagus nerve in rats with sham-operation (A) or gastric afferent vagotomy (B). In both groups, the ventral gastric nerve filament was transected at the region close to the stomach, and the upper bundle of the nerve filament was placed on the electrode to record only the gastric efferent nerve activity. Abbreviations are ventral vagal trunk (VV), dorsal vagal trunk (VD), hepatic branch of the vagus nerve (VH), gastric branch of the vagus nerve (VG), celiac branch of the vagus nerve (VC), recording electrode (RE), diaphragm (D), and stomach (S).

Electrophysiological study

The electrode was connected to a head stage (JB-101J, Nihon-Koden) and the signal was differentially amplified 5000 times before being filtered using a bandwidth of 150 Hz to 1 KHz (MEG-1200, Nihon-Koden). The neural signal output was acquired using an action potential measurement device (PowerLab, ADI Instruments) and was digitally sampled with a resolution power of 10 kHz. Nerve activity was separated from noise by setting the threshold so that the firing rate just before the administration of the drug was about 50 pluses/5 s using the rate meter of spike histogram extension (SHE; ADInstruments Japan, Nagoya). While nerve activity was monitored sequentially, the firing rate was calculated every 5 s, was averaged every 1 min, and was evaluated every 5 min in comparison with the basal firing rate measured just before the administration of the drug.

Experiments related with the gastric or hepatic branch of the vagus nerve were performed as follows: After confirmation of stable baseline signal recording for about 30 min, saline; 3 μg/kg of human ghrelin; or 3, 30, or 100 μg/kg of human desacyl-ghrelin, a main metabolite of human ghrelin (14, 15), was injected into the femoral vein, and efferent vagus nerve activity was recorded until 60 min after the injection. Intravenous injection of ghrelin at 0.8, 4, and 20 μg/kg dose-dependently increased gastric motility and gastric acid secretion in urethane-anesthetized rats (5). Therefore, 3 μg/kg of ghrelin was chosen as a dose that stimulates gastric functions. This dosage was also comparable to that used to stimulate growth hormone secretion and food intake by ghrelin (3). To test whether vagus nerve activity was appropriately measured, we confirmed the well-known change in nerve activity induced by intravenous injection of CCK8, as a positive control (10).

Experiments related with the sympathetic nerve that innervates brown adipose tissue, the adrenal gland, or the kidney was performed as follows: After confirmation of stable baseline signal recording for about 30 min, saline, or 5 μg/body (14 to 20 μg/kg in dose per the body weight of rats) of human ghrelin was injected into the femoral vein and efferent sympathetic nerve activity was recorded until 30 min after the injection. We set the dosage of ghrelin to be higher than that used in measurement from the efferent vagus nerve. To test whether sympathetic nerve activity was appropriately measured, we confirmed the well-known change in nerve activity induced by intravenous injection of positive-control substances, such as glucose and hexamethonium, as reported previously (11, 16, 17).

Blood sampling and plasma hormone measurements

Male Wistar rats (8 weeks of age) were used in this experiment. Under anesthesia of 1 g/kg of urethane, which was delivered via intraperitoneal injection, saline or 5 μg/body of human ghrelin was injected into the tail vein. Blood samples (0.5 mL each) were collected into a plastic tube containing EDTA through a catheter cannulated into the femoral artery just before and at 10, 30, and 60 min after saline or ghrelin injection and centrifuged at 8000 rpm for 5 min at 4°C. The plasma was frozen at −80°C until plasma hormone measurement (within 2 weeks).

The plasma concentrations of norepinephrine, corticosterone, and renin were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits specific to norepinephrine (Rat Norepinephrine ELISA kit; TSZ ELISA, Lexington, MA, USA), corticosterone (Rat Corticosterone ELISA kit; Uscn, Houston, TX, USA), and renin (Rat Renin ELISA kit, Uscn). The assays were carried out according to the instructions provided by the manufacturers.

Statistics

Data were expressed as the mean ± S.E.M. Statistical significance was analyzed using an unpaired *t*-test on EXSUS (CAC EXICARE, Tokyo), for comparison with the basal levels of the vehicle or sham-operation group, or with the levels observed just before test-drug injection. A probability of $P < 0.05$ was considered statistically significant.

Results

Effects of ghrelin on efferent vagus nerve activity

First, we confirmed that CCK8 inhibited the efferent activity of the gastric branch of the vagus nerve in rats, as reported previously (10) (Fig. 2A). Intravenous injection of 3 μg/kg of ghrelin significantly potentiated the firing rate of the gastric efferent vagus nerve (Fig. 2: B and D). The stimulatory effect peaked within 5 to 10 min after the injection of ghrelin and the neuronal firing rate returned to the basal level about 40 min later. Even when 100 μg/kg of ghrelin (used as an overdose) was injected intravenously into 2 animals, the maximal stimulatory effect was almost the same as the effect of the injection of 3 μg/kg. However, the stimulatory action of 100 μg/kg of ghrelin lasted longer (data not shown).

In rats that underwent the gastric afferent vagotomy, the basal activity of the gastric branch of the efferent vagus nerve fluctuated more than that in rats without vagotomy, and it was also stimulated by intravenous injection of 3 μg/kg of ghrelin similarly to the procedure used in rats without vagotomy. The maximal response was also reached within 5 to 10 min (Fig. 2: C and D). In contrast, when ghrelin was injected intravenously at

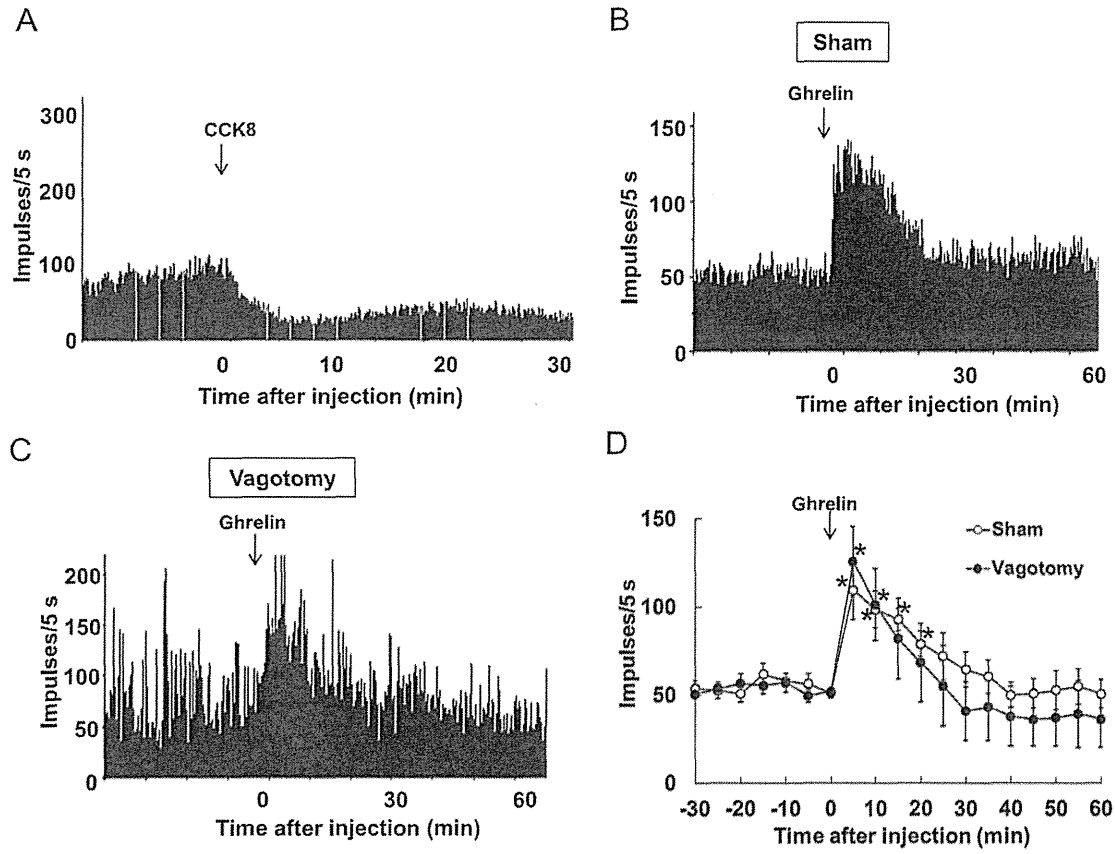

Fig. 2. Effects of CCK8 and ghrelin on the firing rate of the gastric efferent vagus nerve in urethane-anesthetized rats with or without vagotomy. A, B, and C show typical original traces of a sequential frequency histogram, as an index of gastric efferent nerve activity. At time 0, CCK8 (20 μg/body) was injected intravenously (A) and ghrelin (3 μg/kg) was injected intravenously into a rat with sham-operation (Sham) (B) or gastric afferent vagotomy (Vagotomy) (C). D shows the time course of the changes in the firing rate of the gastric efferent vagus nerve after injection of ghrelin into 5 rats with vagotomy or 4 rats without vagotomy. Each point and vertical bar indicate the mean ± S.E.M. (*$P < 0.05$ vs. 0 min). There were no significant differences between the sham and vagotomy groups (t-test).

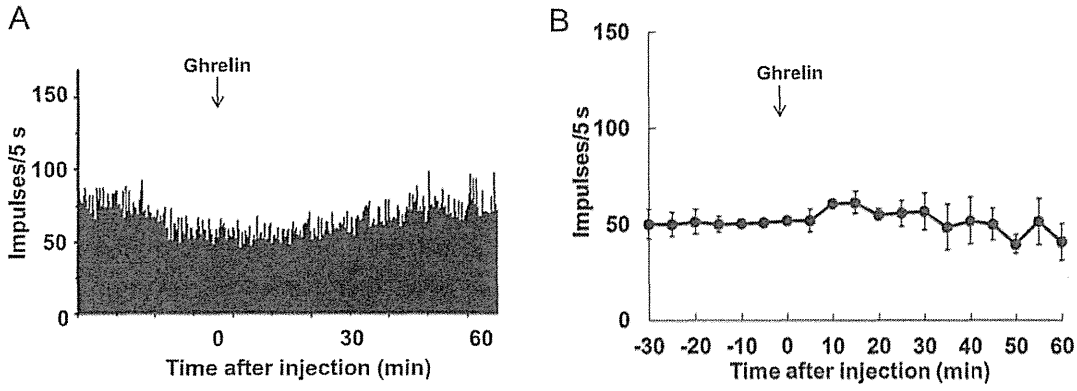

Fig. 3. Effect of ghrelin on the firing rate of the hepatic efferent vagus nerve in urethane-anesthetized rats. A) Typical original trace of a sequential frequency histogram used as an index of hepatic nerve activity. Ghrelin (3 μg/kg) was applied intravenously at time 0 min. B) Time course of the changes in the firing rate of the hepatic efferent vagus nerve by injection of ghrelin into 5 rats. Each point and vertical bar indicates the mean ± S.E.M. The data exhibited no significant differences (t-test) between the levels before and after the ghrelin injection.

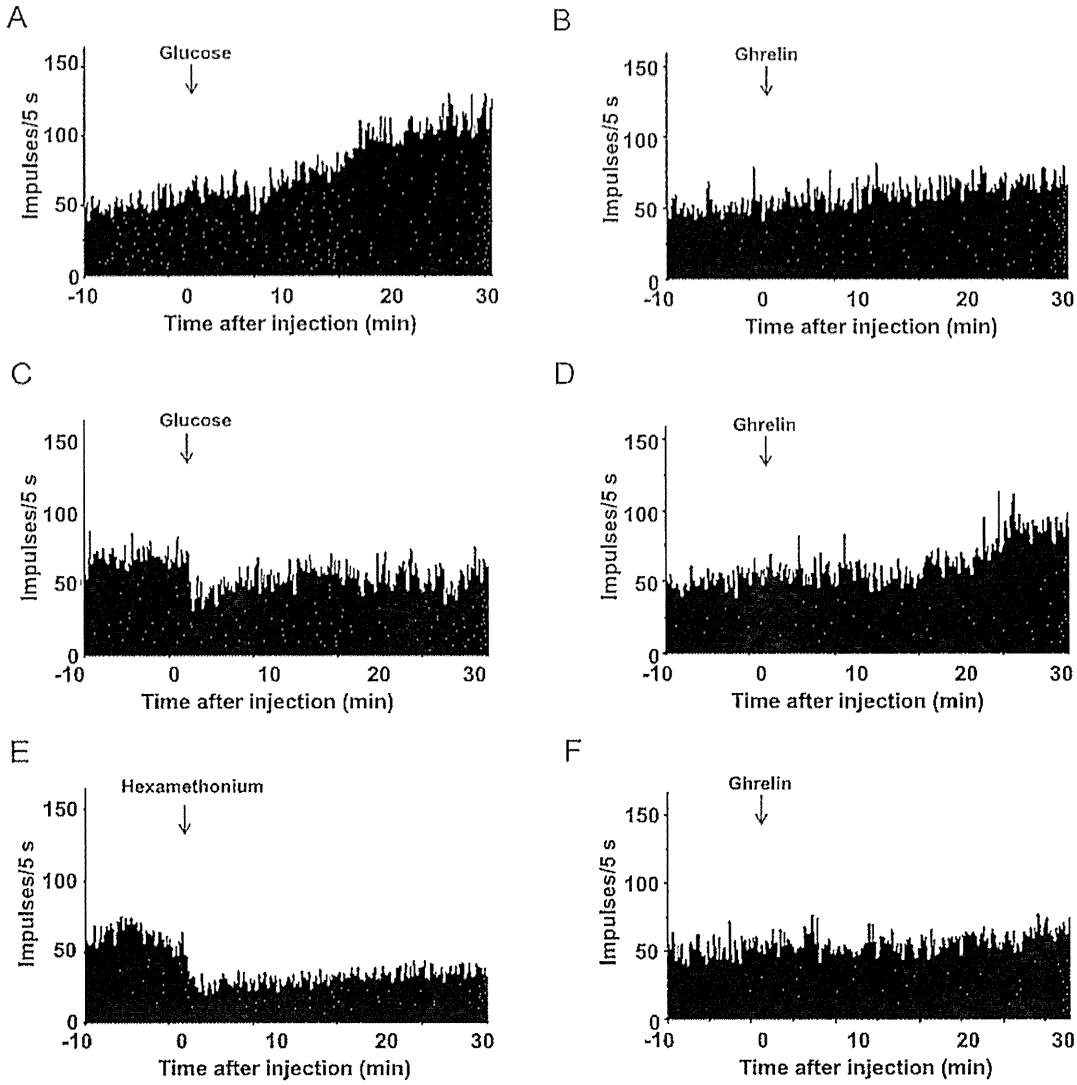

Fig. 4. Effects of glucose, hexamethonium, and ghrelin on the firing rate of the various efferent sympathetic nerves in urethane-anesthetized rats. Typical original traces of a sequential frequency histogram of brown adipose tissue sympathetic nerve activity (BATSNA) (A and B), adrenal sympathetic nerve activity (AdSNA) (C and D), and renal sympathetic nerve activity (RSNA) (E and F) in 4 or 5 rats in each group are shown. Glucose (50 mg/body) (A and C), hexamethonium (3 mg/body) (E), or ghrelin (5 μg/body) (B, D, and F) was applied intravenously at time 0 min.

3 μg/kg, the firing rate of the hepatic efferent vagus nerve was not changed (Fig. 3).

Desacyl-ghrelin, a main metabolite of ghrelin, did not affect the firing rate of the gastric efferent vagus nerve at 3, 30, or 300 μg/kg. The vital signs, including blood pressure and heart rate, of rats were not altered by any of the doses of ghrelin and desacyl-ghrelin tested in the present study (data not shown).

Effects of ghrelin on efferent sympathetic nerve activity

We investigated the effect of ghrelin on the efferent sympathetic activity of the nerve that innervates brown adipose tissue. The inhibitory effect of intravenous injection of 50 mg/body (140 to 200 mg/kg) of glucose on the firing rate was confirmed in rats, as reported previously (17) (Fig. 4A). Ghrelin injected intravenously at 5 μg/body (14 to 20 μg/kg) did not yield any significant changes in the firing rate of the efferent sympathetic nerve that innervates brown adipose tissue in rats (Fig. 4B and 5).

Next, we investigated the effect of ghrelin on the efferent sympathetic activity of the nerve that innervates the adrenal gland and the kidney. We confirmed that glucose at 50 mg/body (140 to 200 mg/kg) or hexamethonium at 3 mg/body (8 to 12 mg/kg) inhibited the neuronal firing rate innervating the adrenal gland or

kidney, respectively, as reported previously (11, 16) (Fig. 4: C and E). Ghrelin injected intravenously at 5 μg/body (14 to 20 μg/kg) did not yield any significant changes in the firing rate of the efferent adrenal and renal sympathetic nerve (Fig. 4: D, F and Fig. 5).

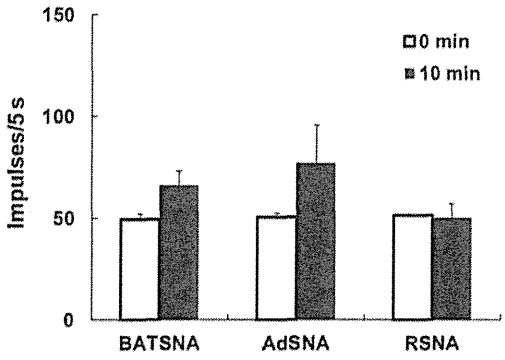

Fig. 5. Effects of ghrelin on brown adipose tissue sympathetic nerve activity (BATSNA), adrenal sympathetic nerve activity (AdSNA), and renal sympathetic nerve activity (RSNA) in urethane-anesthetized rats. Sympathetic nerve activities recorded just before and 10 min after the intravenous injection of ghrelin (5 μg/body) were compared. Each column shows the mean ± S.E.M. of 4 or 5 rats in each group. The data exhibited no significant differences (*t*-test).

Effects of ghrelin on body temperature and plasma hormone levels

Finally, we investigated the effects of ghrelin on body temperature and plasma norepinephrine, corticosterone, and renin levels because body temperature and these hormones are known to be regulated by the sympathetic nerve systems (18 – 20). We found that rectal temperature and the plasma levels of norepinephrine, corticosterone, and renin were not changed over 30 or 60 min after the intravenous injection of 5 μg/body of ghrelin (Fig. 6).

Discussion

Ghrelin is known to influence the autonomic nervous system. However, the direct effect of peripheral injection of ghrelin on neuronal electrical activity has hardly been reported, with the exception of the suppression of the afferent activity of the gastric branch of the vagus nerve by intravenous injection of ghrelin in rats (2, 3).

In the present study, intravenous injection of 3 μg/kg

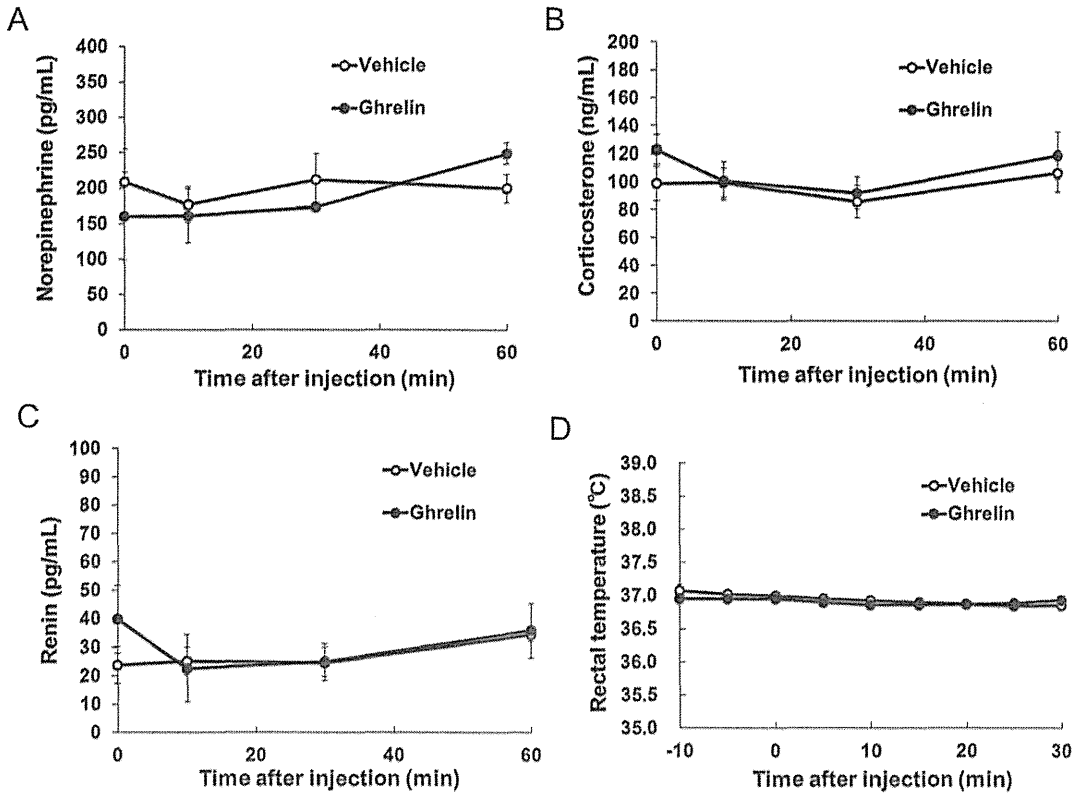

Fig. 6. Effects of intravenous injection of ghrelin (5 μg/body) on plasma levels of norepinephrine (A), corticosterone (B), and renin (C), and on rectal temperature (D) in urethane-anesthetized rats. Each point and vertical bar indicate the mean ± S.E.M. (A and B, n = 4 in each group; C, n = 3 or 4 in each group; D, n = 4 to 6 in each group). There were no significant differences between the vehicle and ghrelin groups (*t*-test).

of ghrelin significantly potentiated the firing rate of the gastric efferent vagus nerve in rats with or without the gastric afferent vagotomy. Because desacyl-ghrelin, a main metabolite of ghrelin, did not yield any effect on the efferent activity of the gastric vagus nerve, it was suggested that the action of ghrelin was induced by ghrelin itself. We reported previously that ghrelin stimulates gastric motility and gastric acid secretion via the vagus nerve (5). Based on these results, it was suggested that ghrelin stimulates gastric motility and gastric acid secretion by the activation of the gastric efferent vagus nerve. Signal transduction via the gastric afferent vagus nerve has been recognized to play an essential role in the ghrelin-induced stimulation of growth hormone secretion from the pituitary gland and food intake (2 – 4). It has also been reported that ghrelin induces gastric motility and gastric acid secretion via the vagal gastric afferent nerve (21 – 23). However, our study, which measured neuronal electrical activity directly, suggests that the gastric stimulating actions of ghrelin are not induced via the gastric afferent signal and are evoked via a mechanism different from stimulation of growth hormone secretion and food intake. In addition, we revealed that ghrelin stimulated the gastric branch of the efferent vagus nerve in an organ-specific manner, as ghrelin had no effect on the efferent activity of the hepatic branch of the vagus nerve.

Several physiologically active substances have different actions on various vagus nerves. For example, intravenous glucose injection activates the hepatic efferent vagus nerve in rats; in contrast, it inhibits the gastric efferent vagus nerve (24, 25). The precise signal transduction pathway that is stimulated by glucose remains unknown, but it is thought that those signals are transmitted via different neurons and result in different physiological responses. Growth hormone secretion, stimulation of gastric motility and gastric acid secretion, stimulation of food intake, and energy accumulation are well-known physiological and pharmacological actions of ghrelin (2). The results of the present study suggest that exogenously applied ghrelin has no effect on the acute phase of glycogen synthesis that is associated with hepatic vagus nerve activity; however, it is expected to contribute to energy accumulation via the stimulation of gastric motility and gastric acid secretion.

It is recognized that the nucleus of the solitary tract (NTS) and the dorsal motor nucleus of the vagus (DMNV) play important roles as regions that control the central nervous system of the gastric nerve (26 – 28), and the growth hormone secretagogue receptor is expressed in these regions (29). Furthermore, it has been reported that the intracerebroventricular injection of ghrelin stimulated gastric acid secretion and enhanced c-*fos* expression in the NTS and DMNV (30). These observations and the present results suggest that the stimulatory effects of ghrelin on gastric motility and gastric acid secretion are caused by its direct action or indirect action via the peripheral nerves other than the gastric afferent vagus nerve on the NTS or/and DMNV.

Here, we also found that intravenous injection of 5 μg/body (14 to 20 μg/kg) of ghrelin into rats did not yield significant changes in the firing rate of the efferent sympathetic nerve that innervates brown adipose tissue. In addition, none of the parameters studied, including plasma norepinephrine concentration, blood pressure and heart rate, and rectal temperature, were significantly changed over the 60-min period after ghrelin injection. Thus, it was concluded that peripheral application of ghrelin has no physiologically meaningful action on the efferent sympathetic nerve that innervates brown adipose tissue and its related physiological parameters. It has been reported that the intracerebroventricular injection of ghrelin transiently decreased the body temperature in conscious rats (31); however this inconsistency might be caused by differences in the experimental conditions such as the application route and the consciousness of animals.

Several previous reports have demonstrated the effects of ghrelin on brown adipose tissue: daily subcutaneous injections of ghrelin for 8 weeks improved the down-regulation of uncoupling protein 1 in the brown adipose tissue of gastrectomized mice (32); the genetic suppression of the growth hormone secretagogue receptor increased uncoupling protein 1 expression (33); and the intracerebroventricular injection of ghrelin decreased the firing rate of the efferent sympathetic nerve that innervates brown adipose tissue in rats (34). These effects seem to be inconsistent with the results presented here. However, this discrepancy might be caused by differences in the experimental conditions such as the application route and the dosing period. Mano-Otagiri et al. (35) reported that intravenous injection of 30 nmol/body (ca 100 μg/body) of ghrelin decreased norepinephrine secretion from brown adipose tissue in conscious rats and that 6 nmol/body (ca 20 μg/body) of ghrelin did not yield any effects. Therefore, to induce an effect in brown adipose tissues, it might be necessary to apply a higher dose than that used in the present study.

Intravenous injection of 5 μg/body (14 to 20 μg/kg) of ghrelin did not yield any significant changes in the firing rate of the efferent sympathetic nerve that innervated the adrenal gland and did not affect plasma corticosterone concentration in rats. It was reported that ghrelin activates corticosterone secretion via the stimulation of adrenocorticotropic hormone secretion in humans (36, 37) or via direct action on rat adrenocortical cells (38).

In the latter study, the stimulation of corticosterone secretion was induced by exposure to ghrelin for 24 h, suggesting that the effect did not occur via an acute action of ghrelin. However, in the former study, the stimulatory effect on adrenocorticotropic hormone secretion was observed during the initial 60 min after the injection of ghrelin; this was different from the results of the present study. This discrepancy is estimated to be caused by study conditions (i.e., presence or absence of urethane anesthesia). Sympathetic nerve activity is accelerated during urethane-based anesthesia, and Ishida et al. (39) reported that corticosterone secretion is controlled by the sympathetic nerve. Under urethane anesthesia, during which the activity of the sympathetic nerve and corticosterone secretion were stimulated, ghrelin may not affect these parameters. Nevertheless, our results indicate that the action of ghrelin on the efferent sympathetic nerve that innervates the adrenal gland was too small to affect the related physiological parameters.

We also found that intravenous injection of 5 μg/body of ghrelin did not induce any changes in the firing rate of the efferent renal sympathetic nerve or in plasma renin concentration. These results suggest that the peripheral application of ghrelin does not affect the renal sympathetic nerve. Matsumura et al. (7) also reported that intravenous injection of ghrelin did not affect the firing rate of the efferent renal sympathetic nerve in conscious rabbits.

From these results, it is suggested that peripheral injection of ghrelin at the dose that stimulates growth hormone section or food intake does not acutely affect the activities of the sympathetic nerve that innervates brown adipose tissue, adrenal gland, and kidney at least in urethane-anesthetized rats. In contrast, Schwenke et al. (8) reported that peripheral injection of ghrelin reduced an increased cardiac sympathetic nerve activity in the acute phase after myocardial infarction in rats. This discrepancy might be caused by differences in not only the basal activity of the cardiac sympathetic nerve but also in the innervated tissues.

In conclusion, we demonstrated for the first time that ghrelin stimulates the gastric branch of the efferent vagus nerve in an organ-specific manner without affecting the gastric afferent vagus nerve, which may lead to the stimulation of gastric motility and gastric acid secretion. It was also suggested that ghrelin does not acutely affect basal activities of the sympathetic nerve that innervates brown adipose tissue, adrenal gland, and kidney in urethane-anesthetized rats. Additional investigation of the roles and actions of ghrelin on various neurons may provide a better understanding of the multiple physiological and pharmacological functions of ghrelin.

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CASE REPORT

Boerhaave's syndrome in a tracheoesophageal speaker: report of a case

Ryu Kanzaki · Masahiko Yano · Masaaki Motoori · Kentaro Kishi · Isao Miyashiro · Kunitoshi Yoshino · Yasuhiko Tomita · Osamu Ishikawa

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Abstract Boerhaave's syndrome is still associated with a high mortality rate and remains a therapeutic challenge. Pharyngo-laryngo-esophagectomy is performed as the standard treatment for advanced hypopharyngeal cancer and tracheoesophageal speech is an option for esophageal speech rehabilitation. We report what, to our knowledge, is the first case of Boerhaave's syndrome developing in a tracheoesophageal speaker.

Keywords Larynx · Esophageal perforation · Tracheoesophageal speak

R. Kanzaki · M. Yano (✉) · M. Motoori · K. Kishi · I. Miyashiro · O. Ishikawa
Department of Surgery, Osaka Medical Center for Cancer and Cardiovascular Diseases, 1-3-3 Nakamichi, Higashinari-ku, Osaka 537-8511, Japan
e-mail: yano-ma@mc.pref.osaka.jp

R. Kanzaki
e-mail: rkanzaki@tj8.so-net.ne.jp

R. Kanzaki
Department of General Thoracic Surgery, Osaka University Graduate School of Medicine, Osaka, Japan

K. Yoshino
Department of Otolaryngology Head and Neck Surgery, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan

Y. Tomita
Department of Pathology, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan

Introduction

An abrupt increase in intraesophageal pressure is thought to be the most common cause of Boerhaave's syndrome. We report a rare case of Boerhaave's syndrome that occurred in a tracheoesophageal speaker 20 years after he underwent pharyngo-laryngo-esophagectomy for advanced hypopharyngeal cancer.

Case report

A 54-year-old man presented to our hospital with a 2-day history of progressive chest pain and hematemesis, which had started after he drank a glass of cola and then burped. About 20 years earlier, at the age of 34 years, he had undergone a pharyngo-laryngo-esophagectomy with bilateral neck dissection, left thyroid lobectomy, tracheostomy, and reconstruction of the alimentary tract using a skin tube made of a local skin flap for advanced hypopharyngeal cancer. He had also undergone metastectomy for the lymph node metastasis on the right side of the neck and voice restoration by tracheoesophageal puncture at 38 years of age. He used a Blom–Singer voice prosthesis to speak and suffered dysphagia as a result of stenosis of the skin tube tract (Fig. 1). His medical history apart from the hypopharyngeal cancer was unremarkable. On arrival to our hospital, he was in moderate distress, with a heart rate of 115 beats/min, a blood pressure of 84/44 mmHg, a respiratory rate of 24 breaths/min, and a body temperature of 37.5 C. Laboratory findings revealed a white blood cell count of 9400/mm^3 and C-reactive protein of 0.3 mg/dL. Chest computed tomography with oral water-soluble contrast material showed the extravasation of contrast into the mediastinum and bilateral pleural effusions (Fig. 2).

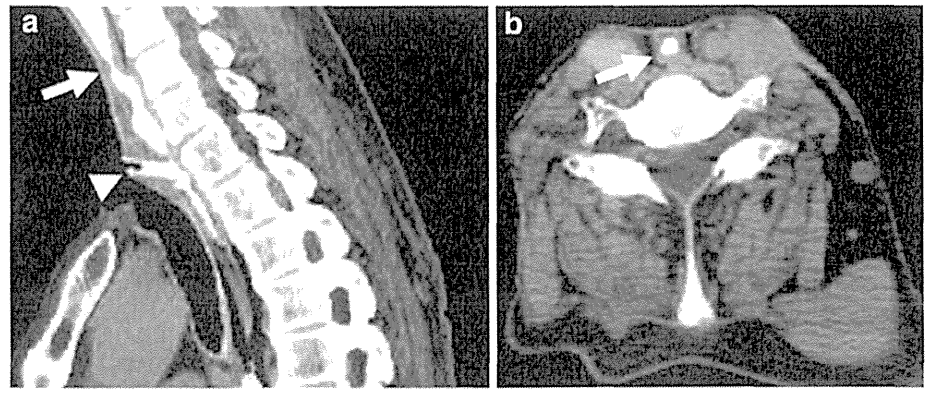

Fig. 1 **a** Sagittal section and **b** transverse section of the neck computed tomography with oral water-soluble contrast material showing stenosis of the cervical esophagus made by the skin tube (*arrow*) and the tracheoesophageal fistula with the Blom–Singer voice prosthesis (*arrowhead*)

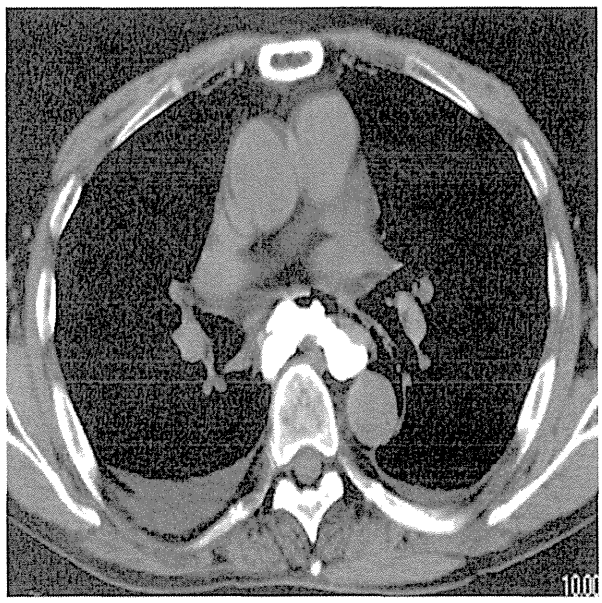

Fig. 2 Chest computed tomography with oral water-soluble contrast material revealed the extravasation of contrast agent into the mediastinum and bilateral pleural effusions. These findings are consistent with a ruptured esophagus

These findings were consistent with a ruptured esophagus. Broad spectrum antibiotics were initiated and an emergency thoracotomy was performed.

Under general anesthesia with single lung ventilation, the patient was placed in the left lateral decubitus position and a lateral thoracotomy via the right fifth intercostal space revealed a full-thickness, 4-cm longitudinal tear in the right side of the mid-thoracic esophagus (Fig. 3). Purulent material was found in both the mediastinum and bilateral pleural space; however, considering the weakened esophageal wall caused by the inflammation, we decided against a primary repair. We instead performed subtotal esophagectomy and placed drainage tubes in the mediastinum and right pleural cavity. Histological examination revealed diffuse atrophy of the internal circular muscle and chronic inflammation of the lamina propria mucosa and submucosa of the mid-thoracic

Fig. 3 Lateral thoracotomy via the right fifth intercostal space revealed a full-thickness 4-cm longitudinal tear in the right side of the mid-thoracic esophagus

esophagus (Fig. 4). A decompression tube was also placed in the cervical esophagus through the nostril for salivary diversion. The patient had a good postoperative recovery with total parenteral nutritional support.

We completed the esophagectomy with reconstruction of the alimentary tract 5 weeks after this operation. The skin tube was resected, a gastric conduit was made via an antethoracic route, and a free jejunal graft was transplanted for reconstruction between the pharynx and the gastric conduit. The patient recovered uneventfully and was discharged 3 months after the first operation. He used an artificial larynx to speak and did not suffer dysphagia or regurgitation postoperatively.

Discussion

The precise etiology of Boerhaave's syndrome is unclear; however, it is most commonly attributed to an abrupt increase in intraesophageal pressure, possibly caused by

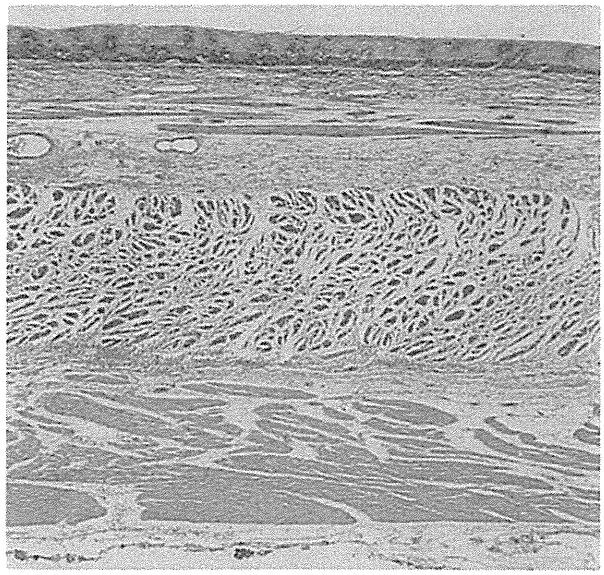

Fig. 4 Hematoxilin-eosin staining of the esophageal wall (×20) showing diffuse atrophy of the internal circular muscle and chronic inflammation of the lamina propria mucosa and the submucosa of the mid-thoracic esophagus

vomiting or the Valsalva maneuver. Predisposing factors that have been proposed include gastroesophageal reflux, regular consumption of irritating substances, focal defects in the muscularis mucosa, and other functional disorders [1]. To the best of our knowledge, there has never before been a report of Boerhaave's syndrome occurring in a tracheoesophageal speaker.

Esophageal rupture usually occurs in the left side of the lower third of the esophagus, whereas a rupture in the middle third of the esophagus, as in the present case, is rare [1, 2]. This tendency of the rupture location is thought to be attributable to the anatomical features of the esophagus; namely, decreased thickness of the musculature in the lower esophagus, and the anterior angulation of the esophagus at the left diaphragmatic crus [1, 2].

Pharyngo-laryngo-esophagectomy has been performed as the standard treatment for advanced hypopharyngeal cancer for several decades. In 1980, Singer and Blom [3] introduced the concept of tracheoesphageal puncture and the placement of a voice prosthesis for postlaryngectomy esophageal speech rehabilitation. This procedure involves the creation of a fistula between the esophagus and the trachea and the placement of a valve that allows air to flow into the esophagus for esophageal speech, and keeps food matter out of the trachea. The vibrating source of tracheoesophageal speech is called a "pseudoglottis," which represents the mucosal fold arising from the posterior wall of the reconstructed pharyngoesophagus. Because of higher pseudoglottal impedance and a higher respiratory effort, the intraesophageal pressure in tracheoesophageal speakers has been reported to be higher [4]. It is thought that the esophagus becomes tense below the position of the prosthesis to prevent the airflow from entering the distal portion of the esophagus or the stomach. Two studies using different designs support this hypothesis. Using manometry, Aguiar-Ricz and co-workers [5] demonstrated that the intraluminal pressure of the middle and distal portions of the esophagus of tracheoesophageal speakers in the presence of voice and speech emission is significantly higher than that of non-speaking laryngectomees. Using fluoroscopy, Mohri and co-workers [6] demonstrated dilatation of the esophageal lumen below the point of vibration and the concentric closure of the esophagus in its distal part.

We suspect that multiple factors were involved in the mechanism of esophageal rupture in the present case. First, the increased intraluminal pressure of the esophagus in tracheoesophageal speakers, as described above, might have caused the mechanical fragility of the mid-thoracic esophagus. Second, the intraluminal pressure of the esophagus might also have increased as a result of stenosis of the skin tube tract. Third, repeated esophagitis, as suggested by the resected specimen, might have caused the mechanical fragility of the mid-thoracic esophagus. Further case collection with precise pathological and anatomical description is needed to clarify the mechanism of esophageal rupture in tracheoesophageal speakers.

The treatment strategy for Boerhaave's syndrome depends on the extent of delay in diagnosis and treatment [7]. The prolonged leakage of saliva, gastric juice, bile, and bacteria into the mediastinum causes local inflammation and worsens the general condition. When diagnosed within 24 h, a primary repair is favored, but when diagnosed late, the optimal treatment remains controversial [8]. A variety of approaches have been recommended for late-diagnosed Boerhaave's syndrome, including primary repair [1], esophagectomy, and the use of a T-tube to create a controlled fistula [9].

As Boerhaave's syndrome was diagnosed more than 24 h after onset in our patient, the progression of local inflammation resulted in weakness of the esophageal tissues, making them unable to hold sutures. As a result, primary repair could not be performed at that time, so the patient underwent esophagectomy as the first step of treatment.

Conflict of interest Ryu Kanzaki and his co-authors have no conflict of interest.

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Tissue factor predicts response to chemotherapy in esophageal cancer

Masaaki Motoori, MD,[a,] Masahiko Yano, MD,[a] Yasuhiko Tomita, MD,[b] Hidenori Takahashi, MD,[a] Koji Tanaka, MD,[a] Keijiro Sugimura, MD,[a] Kentaro Kishi, MD,[a] Yoshiyuki Fujiwara, MD,[a] Norikatsu Miyoshi, MD,[a] Hirofumi Akita, MD,[a] Kunihito Goto, MD,[a] Shigeru Marubashi, MD,[a] Shingo Noura, MD,[a] Masayuki Ohue, MD,[a] Osamu Ishikawa, MD,[a] and Masato Sakon, MD[a]*

[a] *Department of Surgery, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan*
[b] *Department of Cytology and Pathology, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan*

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ABSTRACT

Background: Neoadjuvant chemotherapy (NACT) improves the prognosis of patients with esophageal cancer who respond, but it is not effective in nonresponders. Therefore, it is crucial to establish a reliable method of predicting response before initiation of chemotherapy. Hypercoagulability, which is thought to be because of upregulation of tissue factor (TF) in cancer cells, was reported to be associated with chemoresistance. The aim of this study was to investigate the association between TF expression and response to NACT in esophageal cancer.

Methods: In 67 patients with advanced esophageal cancer, TF expression in pretreatment biopsy samples was evaluated immunohistochemically and correlated with clinicopathologic factors and response to chemotherapy.

Results: TF was expressed by 43.3% of the tumors, but there were no correlations observed with any clinicopathologic parameters examined. Clinical and histologic responses to chemotherapy were significantly worse in TF-positive patients compared with TF-negative patients. Multivariate analysis revealed that TF expression was significantly associated with a poor clinical response ($P = 0.0431$). TF expression was also independently associated with poor progression-free survival ($P = 0.0353$).

Conclusions: TF expression levels in pretreatment biopsy samples are useful for predicting response to NACT in advanced esophageal cancer. Further studies of mechanisms underlying the relationship between TF expression and chemosensitivity are needed.

* *Corresponding author.* Department of Surgery, Osaka Medical Center for Cancer and Cardiovascular Diseases, 1-3-3 Nakamichi, Higashinari-ku, Osaka, 537-8511, Japan. Tel.: +81 6 6972 1181; fax: +81 6 6981 8055.
E-mail address: motoori-ma@mc.pref.osaka.jp (M. Motoori).
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1. Introduction

Despite recent advances in surgical technique and perioperative management, surgery alone does not satisfactorily improve the prognosis of advanced esophageal cancer. Even after curative esophagectomy with extended three-field lymphadenectomy, cancer recurs in approximately 50% of patients [1]. Thus, it is highly likely that systemic micrometastases are present outside the surgical field at diagnosis. To improve the prognosis of advanced esophageal cancer, neoadjuvant chemotherapy (NACT), which is expected to eradicate systemic micrometastases, followed by surgery is a promising treatment strategy. Several recent studies have reported successful results with NACT [2,3]. Although NACT has been shown to improve the prognosis of patients that experience greater than a 50% decrease in the size of the primary tumor assessed by radiologic imaging, patients with less than a 50% decrease not only suffer from its side effects but also lose precious time to take advantage of alternative treatment options [2,4,5]. In some reports, the prognosis of nonresponders might be worse than those treated with a primary surgical approach [2,4,5]. Despite intensive efforts to identify predictors of response before initiating chemotherapy, there are currently no clear candidate predictors that can be used in daily clinical practice. Therefore, a reliable method that predicts response to chemotherapy is considered to be crucial for using NACT to treat advanced esophageal cancer going forward.

Activation of coagulation pathways frequently occurs in patients with cancer. Such hypercoagulopathy is considered the result of upregulation of tissue factor (TF) in cancer cells, which binds to coagulation factor VII (FVII) and its active form (FVIIa), thus initiating the coagulation cascade *via* the extrinsic pathway. In addition to its role in coagulation, accumulating evidence suggests that TF regulates intracellular signaling pathways that play a crucial role in inflammation, angiogenesis, tumor development, and metastasis [6–8]. Indeed, a high expression of TF is correlated with tumor grade, metastasis, and poor prognosis in various types of cancers [9–11]. In addition, a hypercoagulable state has been reported to be associated with chemoresistance [12]. In esophageal cancer, we previously reported that pretreatment plasma D-dimer levels, a marker of hypercoagulopathy, can be used as a predictor of chemosensitivity [13]. However, there have been few studies analyzing the correlation between TF and chemosensitivity. The present study investigates the association between TF expression levels in pretreatment biopsy samples and response to NACT in patients with advanced esophageal cancer.

2. Materials and methods

2.1. Patients

Between January 2004 and December 2010, a total of 288 patients with squamous cell carcinoma of the thoracic esophagus underwent esophagectomy at our hospital. Of these 288 patients, 176 patients underwent surgery without neoadjuvant treatment, 87 patients underwent NACT (FAP therapy (5-fluorouracil [5-FU] + Doxorubicin hydrochroride + cisplatin [CDDP]), $n = 78$; 5-FU + CDDP, $n = 9$) followed by surgery, and 25 patients underwent neoadjuvant chemoradiotherapy followed by surgery. Of the 78 patients who underwent neoadjuvant FAP therapy, we were able to collect biopsy samples containing tumor cells from 67 patients. All patients underwent esophagoscopy and computed tomography (CT) from the neck to the abdomen for tumor staging based on the seventh edition of the Union for International Cancer Control Tumour, Node, Metastases classification system [14].

2.2. Treatment protocol and follow-up

The FAP regimen consisted of CDDP at a dose of 70 mg/m^2 and Adriamycin at a dose of 35 mg/m^2 by drip infusion on day 1. On days 1 through 7, 5-FU was administered at a dose of 700 mg/m^2 daily by continuous infusion. Two cycles of chemotherapy were given, separated by a 3-wk interval [15–17]. Patients were scheduled for surgery approximately 4 wk after the last day of chemotherapy. Surgical therapy consisted of *en bloc* esophagectomy *via* right thoracotomy with two- or three-field lymphadenectomy and reconstruction using the stomach, jejunum, or colon. After surgery, patients were surveyed every 3 mo by physical examination and measurement of serum tumor markers (squamous cell carcinoma antigen and carcinoembryonic antigen), every 6 mo by CT and abdominal ultrasonography, and annually by endoscopy until tumor recurrence was evident.

2.3. Evaluation of the response to chemotherapy

The effect of chemotherapy was evaluated using two different methods. The clinical response to chemotherapy was evaluated based on the difference in tumor size between the CT scan 2 wk after completion of chemotherapy and the scan before chemotherapy. The largest area of the primary tumor was measured in two dimensions, the greatest diameter and the greatest perpendicular distance. The reduction rate was calculated as follows: (tumor area before treatment − tumor area after treatment)/(tumor area before treatment). Patients with more than a 50% decrease in the size of the primary tumor after NACT were defined as responders. The histologic response to chemotherapy was evaluated by the proportion of viable cancer cells in the entire tumor, based on hematoxylin and eosin–stained sections of surgical specimens. Criteria established by the Japanese Society for Esophageal Diseases [18]: grade 0, no histologic effect; grade 1, viable cancer cells accounted for more than one-third of the tumor tissue; grade 2, viable cancer cells account for less than one-third of the tumor tissue; and grade 3, no residual viable cancer cells.

2.4. Immunohistochemical staining

Biopsy samples were fixed in 10% formalin and embedded in paraffin using conventional techniques. Serial sections were prepared for hematoxylin and eosin staining to confirm the presence of tumor cells and to perform TF immunohistochemical studies. Immunohistochemistry was conducted as follows: after deparaffinization in xylene and dehydration in

graded ethanol solutions, tissue sections were irradiated in a microwave oven for 5 min in 10 mM citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxide for 20 min. After overnight incubation with 1:50 antihuman TF antibody (American Diagnostica, Stamford, CT) at 4°C, staining was performed using the labeled streptavidin–biotin method. As the negative control, primary antibody was omitted from the immunohistochemical reaction. The positive control was a section of umbilical cord, which is known to stain strongly for TF [19]. The entire biopsy specimen was analyzed for evaluation of TF expression. Staining for TF was considered positive when >20% of the tumor cells were immunoreactive to TF, and negative when ≤20% of the tumor cells were positive. The cutoff point for negative or positive TF was set at the median value of all the study participants except nine patients with no TF immunoreactivity, as previously described in pancreatic cancer [11]. All slides were assessed independently by two pathologists (Y.T. and K.K.). Any discordant findings were resolved through consensus. Both pathologists were blinded to the clinicopathologic data.

2.5. *Statistical analysis*

Statistical analyses were performed using StatView 5.0 J software (SAS Institute Inc, Cary, NC). Relationships between TF expression and clinicopathologic factors were evaluated using the chi-square test or the Mann–Whitney U-test as appropriate. Univariate and multivariate survival analyses were performed using Cox proportional hazards regression models. Survival was calculated using the Kaplan–Meier method and assessed using the log-rank test. A two-tailed $P < 0.05$ was considered significant. The study protocol was approved by the Human Ethics Review Committee of Osaka Medical Center for Cancer and Cardiovascular Diseases.

3. Results

3.1. *Patients and tumor characteristics*

The clinical characteristics of the patients are presented in Table 1. Most patients were clinically node-positive. There were 10 (14.9%) patients with clinical stage II disease, 47 (70.1%) with stage III disease, and 10 (14.9%) with stage IV disease. The median follow-up period was 30.2 mo.

3.2. *TF expression and clinicopathologic characteristics*

Table 1 shows the correlation between TF expression and clinicopathologic characteristics. Of the 67 tumors, 29 (43.3%) were TF-positive, mainly in the cytoplasm of tumor cells (Fig. 1A), whereas the remaining 38 (56.7%) were TF-negative (Fig. 1B). There were no differences in baseline characteristics, including sex, age, tumor location, histologic grade, cT, cN, cM, and cStage. The overall clinical response rate to chemotherapy was 47.8%. The clinical response rate in TF-positive patients was 31.0%, which was significantly lower than that in TF-negative patients (60.5%, $P = 0.0166$). Regarding pathologic factors, there were no differences in pT and pN categories, but compared with TF-negative patients, the histologic response to chemotherapy was significantly worse in TF-positive patients ($P = 0.0484$).

Table 1 – TF expression and clinicopathologic factors.

Characteristics	Total number (n = 67)	TF positive (n = 29)	TF negative (n = 38)	P value
Sex				
Male	55	26	29	0.915
Female	12	3	9	
Age				
≤63 y	32	13	19	0.599
≥64 y	35	16	19	
Location				
Upper	9	4	5	0.503
Middle	25	10	15	
Lower	33	15	18	
Histologic grade				
Grade 1	12	4	8	0.169
Grade 2	40	21	19	
Grade 3	15	4	11	
cT				
T1–2	14	4	10	0.503
T3–4	53	25	28	
cN				
N0–1	38	19	19	0.204
N2–3	29	10	19	
cM				
M0	57	25	32	0.82
M1	10	4	6	
cStage				
I, II	10	4	6	0.82
III, IV	57	25	32	
Clinical response				
Responder	32	9	23	0.0166
Nonresponder	35	20	15	
pT				
T0–2	19	7	12	0.5
T3–4	48	22	26	
pN				
N0–1	40	18	22	0.73
N2–3	27	11	16	
Histologic response				
Grade 0	7	4	3	0.0484
Grade 1	48	23	25	
Grade2	11	2	9	
Grade 3	1	0	1	

Location, histologic grade, cT, cN, cM, cStage, pT, and pN were based on the seventh edition of the Union for International Cancer Control Tumour, Node, Metastases classification guidelines. Histologic response was based on the Japanese Society for Esophageal Diseases Guidelines for the Clinical and Pathologic Studies on Carcinoma of the Esophagus, 10th edition.

3.3. *Association between clinical response to chemotherapy and pretreatment factors*

Because clinical response to chemotherapy can be evaluated before surgery and has been previously demonstrated to be a surrogate marker for prognosis [15–17], we performed univariate and multivariate analyses to investigate which variables are associated with a clinical response to chemotherapy.

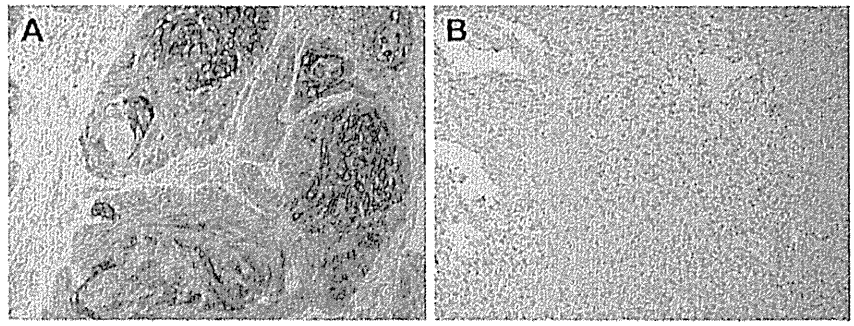

Fig. 1 – Representative examples of immunohistochemical staining for TF. (A) TF-positive esophageal squamous cell cancer. Note that the staining is mainly in the cytoplasm of tumor cells (magnification, ×200). Staining was performed using the labeled streptavidin-biotin method. (B) TF-negative esophageal squamous cell cancer. Note almost no appreciable staining of tumor cells (magnification, ×200).

Among the pretreatment variables examined in the univariate analysis, including sex, age, tumor location, T category, N category, M category, histologic grade, and TF expression, only sex and TF expression were significantly associated with the clinical response to chemotherapy. Multivariate analysis of sex and TF expression revealed that both factors were significantly associated with the clinical response to chemotherapy ($P = 0.0254$ and 0.0431, respectively; Table 2).

3.4. *Association between survival and pretreatment factors*

Among clinical factors that are assessable before chemotherapy, including sex, age, tumor location, T category, N category, M category, histologic grade, and TF expression, TF expression was significantly associated with progression-free survival (PFS) in the univariate analysis. When multivariate analysis for PFS was performed using sex, T category, N category, M category, and TF expression, only TF expression was identified as a prognostic factor ($P = 0.0353$; Table 3). TF-positive patients had significantly worse survival than TF-negative patients ($P = 0.0434$; Fig. 2).

Table 2 – Univariate and multivariate analysis of clinical response to NACT.

Variables	Univariate analysis	Multivariate analysis		
	P value	HR	95% CI	P value
Sex				
Male vs female	0.0065	6.54	1.26–34.5	0.0254
Age, y				
≥64 vs ≤63	0.866	NI		
Location				
Upper vs middle or lower	0.8305	NI		
cT				
T3-4 vs T1-2	0.171	NI		
cN				
N2-3 vs N0-1	0.756	NI		
cM				
M1 vs M0	0.878	NI		
Histologic grade				
Grade 3 vs grade 1–2	0.923	NI		
TF expression				
Negative vs positive	0.0166	3.01	1.04–8.77	0.0431

CI = confidence interval; HR = hazard ratio; NI = not included in the multivariate model.

4. Discussion

This is the first study demonstrating that TF expression in the pretreatment biopsy specimen is a useful marker of chemosensitivity in esophageal cancer. One of the underlying mechanisms of this association between TF expression and chemosensitivity may be related to TF's ability to promote angiogenesis by increasing vascular endothelial growth factor (VEGF) expression [20–22], which has been reported to be a predictive marker for poor response to chemotherapy [23–26]. Foekens *et al.* [23] reported that high levels of VEGF were predictive of a poor response to chemotherapy in patients with breast cancer. Dirix *et al.* [24] showed that serum VEGF levels were higher in progressive disease compared with responsive disease in patients with metastatic cancer of various origins treated with chemotherapy. Another possible mechanism may be that the TF/FVIIa complex prevents apoptosis. Fang *et al.* [27] reported that expression of the antiapoptotic protein Bcl-2 was induced in neuroblastoma cell lines overexpressing TF/FVIIa, resulting in resistance to chemotherapy. Jiang *et al.* [28] demonstrated that the TF/FVIIa complex prevents apoptosis in human breast cancer cells. Further studies are necessary to clarify mechanisms involved in TF expression and chemosensitivity.

Hypercoagulability has been reported to be associated with chemoresistance [12,13]. TF may play a role in this association. In this study, venous thromboembolism during NACT was occurred in two of the 29 TF-positive patients (6.9%), whereas none of the 38 TF-negative patients developed venous thromboembolism. Venous thromboembolism occurred more frequently in TF-positive patients, but this difference was not statistically significant ($P = 0.10$). In 62 patients, pretherapeutic levels of D-dimer, a marker of

Table 3 – Univariate and multivariate analysis of PFS.

Variables	Univariate analysis	Multivariate analysis		
	P value	HR	95% CI	P value
Sex				
Male vs female	0.061	2.21	0.815–6.90	0.113
Age				
≥64 vs <63 y	0.407	NI		
Location				
Upper vs middle or lower	0.215	NI		
cT				
T3-4 vs T1-2	0.0595	2.16	0.819–5.71	0.12
cN				
N2-3 vs N0-1	0.123	1.73	0.792–3.80	0.169
cM				
M1 vs M0	0.0859	1.15	0.460–2.88	0.765
Histologic grade				
Grade 3 vs grade 1–2	0.809	NI		
TF expression				
Negative vs positive	0.0472	2.16	1.02–4.41	0.0353

CI = confidence interval; HR = hazard ratio; NI = not included in the multivariate model.
Location, histologic grade, cT, cN, and cM were based on the seventh edition of the Union for International Cancer Control Tumour, Node, Metastases classification guidelines.

hypercoagulopathy, tended to be higher in TF-positive patients (TF-positive, 0.49 ± 0.09 μg/mL; TF-negative, 0.35 ± 0.05 μg/mL; $P = 0.16$). One of the reasons for this insignificant association between TF expression and D-dimer levels may be the relatively small sample size. Several studies have shown that chemotherapy combined with anticoagulation therapy, such as unfractionated heparin (UFH) and low-molecular-weight heparin (LMWH), may improve the efficacy of chemotherapy or prolong survival of cancer patients [29,30]. In a randomized clinical trial of 272 patients with small cell lung cancer (SCLC), Lebeau *et al.* [29] compared the effects of combination chemotherapy with *versus* without

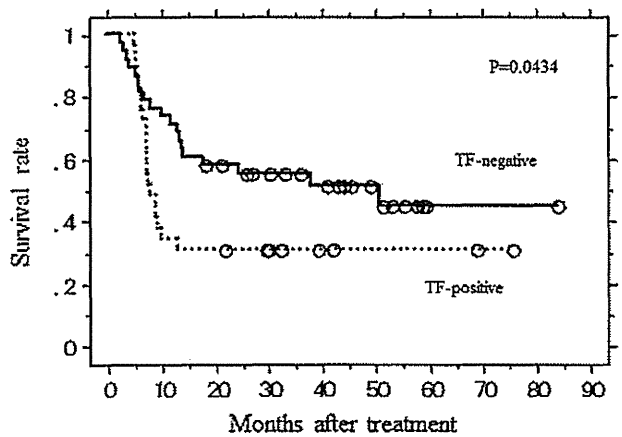

Fig. 2 – Kaplan–Meier PFS curves according to TF expression in biopsy samples. TF-positive patients had significantly worse survival than TF-negative patients ($P = 0.0434$).

subcutaneous UFH. Patients receiving UFH had significantly better response rates and survival. Altinbas *et al.* [30] reported that the response to chemotherapy and survival was significantly better in SCLC patients treated with chemotherapy plus LMWH compared with those receiving chemotherapy alone. The effects of UFH or LMWH on the clinical course of patients with cancer have not been clearly defined. One hypothesized mechanism of action is *de novo* activation of TF pathway inhibitor, an inhibitor of the TF/FVIIa complex [31]. Furthermore, Fang *et al.* [27] found that the inhibition of endogenous TF expression in a TF-overexpressing neuroblastoma cell line with small interfering RNA resulted in the downregulation of Bcl-2 and sensitization to doxorubicin-induced apoptosis. Thus, agents that have an inhibitory effect on TF may improve the efficacy of chemotherapy, particularly in patients with high levels of TF expression.

The association between TF expression and malignancy is complex. TF synthesis may be influenced by several tumor-related phenomena, including activation of oncogenes, inactivation of tumor suppressor genes, and changes in the tumor microenvironment such as hypoxia and inflammation. In colorectal cancer, mutations in the *K-ras* proto-oncogene and *p53*, which result in the loss of p53 function, result in constitutive activation of the MAPK and PI3K signaling pathways, thus leading to enhanced TF expression [32]. In the present study, we did not evaluate any other prognostic molecular markers and therefore further studies are required to clarify the mechanisms that regulate TF expression in esophageal squamous cell cancer.

In this study, sex was also an independent factor for the clinical response to chemotherapy. Several investigators have described sex-based differences in the treatment efficacy of chemotherapy. We have previously reported that in patients with advanced esophageal cancer treated with NACT followed by surgery, the clinical response rate was significantly higher in women [15]. Singh *et al.* [33] found that female SCLC patients treated with chemotherapy had significantly better outcomes with respect to both the response and overall survival. These results are consistent with the present study. One reason why chemotherapy is more efficacious in women may be related to differences in the clearance of chemotherapeutic agents. Indeed, Milano *et al.* [34] reported that the capacity to clear 5-FU is lower in women. Dobbs *et al.* [35] showed that among patients with normal liver biochemistry, men had a higher rate of doxorubicin clearance than women.

Among the clinical factors evaluable before NACT, TF expression was the only independent factor associated with PFS. In a multivariate analysis involving factors evaluable before NACT and clinical response to NACT, clinical response was identified as the only independent prognostic factor ($P = 0.0098$), whereas TF expression was not an independent prognostic factor ($P = 0.38$, data not shown). The attenuation of the effect of TF expression in the multivariate analysis was principally the result of adjusting for clinical response. To clarify whether TF expression is a prognostic marker, patients who undergo esophagectomy without preoperative treatment should be analyzed.

Recently, positron emission tomography (PET) has become a useful modality for predicting responses during the early phases or shortly after neoadjuvant therapy in patients with

esophageal or esophagogastric cancer [36–38]. Weber et al. demonstrated that compared with nonresponders, responders had a significantly higher reduction in fluorine-18–labeled deoxyglucose uptake by tumors after 14 d of preoperative chemotherapy. Because PET evaluates changes in metabolic activity before and after treatment, this approach is unable to predict responses before therapy. The principal advantage of molecular analysis of pretreatment biopsy specimen over PET is that unnecessary therapy may be avoided altogether.

The limitation of this study is that TF expression detected in endoscopic biopsy specimens may not be representative of the entire tumor, because of tumor heterogeneity. In a preliminary analysis, we evaluated TF expression in 10 surgically resected samples of esophageal squamous cell carcinoma. TF expression was predominantly expressed on the invasive front of the tumor, as reported in pancreatic cancer [11]. On the superficial aspect of the tumor, where biopsy samples are usually obtained, TF expression was mostly homogeneous. Therefore, the heterogeneity of TF expression between different biopsy samples from the same patient can be considered to be low.

In this study, a tumor expressing high levels of TF was defined as positive staining in >20% of cells. When the cutoff value was set at 30%, the clinical response rate in TF-positive patients was 36.6%, which was significantly lower than that in TF-negative patients (65.4%, $P = 0.0215$). On the other hand, there was no significant difference in PFS between the two groups ($P = 0.347$). When the cutoff value was set at 10%, there was no significant difference in either the clinical response rate or PFS between the two groups ($P = 0.0773$ and 0.0543, respectively). Thus, the cutoff value of 20% was the most clinically significant for evaluating the clinical response rate and survival. When the intensity of TF expression was classified into four degrees (0% negative, >0% and <20% positive tumor cells, ≥20% and <40% positive, and ≥40% positive), TF expression tended to be associated with the clinical response ($P = 0.087$). One reason for statistically insignificant association between TF expression and clinical response may be the relatively small sample size.

5. Conclusions

TF expression levels in pretreatment biopsy samples are useful for predicting the response to NACT in advanced esophageal cancer. Further studies of the mechanisms underlying the association between TF expression and chemosensitivity are needed. Chemotherapy combined with agents that inhibit TF, such as UFH or LMWH, may improve the efficacy of chemotherapy, particular in patients with high levels of TF expression.

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Disclosure

The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.

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Prognostic Significance of ^{18}F-fluorodeoxyglucose Positron Emission Tomography (FDG-PET)-Positive Lymph Nodes Following Neoadjuvant Chemotherapy and Surgery for Resectable Thoracic Esophageal Squamous Cell Carcinoma

Takushi Yasuda, M.D.[1], Masahiko Yano, M.D.[2], Hiroshi Miyata, M.D.[3], Makoto Yamasaki, M.D.[3], Shuji Takiguchi, M.D.[3], Yoshiyuki Fujiwara, M.D.[2], and Yuichiro Doki, M.D.[3]

[1]Department of Surgery, Faculty of Medicine, Kinki University, Osaka-Sayama, Osaka, Japan; [2]Department of Gastroenterological Surgery, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan; [3]Department of Gastroenterological Surgery, Graduate School of Medicine, Osaka University, Osaka, Japan

ABSTRACT

Purpose. Patients with resectable thoracic esophageal squamous cell cancer (TESCC) and positron emission tomography (PET)-positive lymph nodes (PET-N positive) are likely to have ≥3 pathological lymph node metastases (pLNMs) and show a higher rate of postoperative recurrence despite curative resection than PET-N-negative TESCC patients. We examined the prognostic significance of ^{18}F-fluorodeoxyglucose uptake into lymph node metastases after neoadjuvant chemotherapy (NAC) for PET-N positive TESCC and aimed to propose the optimal NAC response criteria for these patients.

Methods. Fifty-one patients with PET-N positive TESCC underwent two courses of NAC followed by surgery. Metabolic responses of primary tumors and LNs were prospectively evaluated and associations with clinicopathological data and patient survival assessed by univariate and multivariate analyses.

Results. After NAC, 21 patients were post-treatment (post-) PET-N positive and 30 post-PET-N negative. A significantly ($p < 0.001$) high proportion of the post-PET-N-negative group had ≤2 pLNMs than the post-PET-N positive group (86.7 vs. 28.6 %). The PET-N negative group also had a significantly lower distant metastasis rate (23.3 vs. 75.0 %) and higher 5-year relapse-free survival (RFS) rate (69.0 vs. 20.0 %). Univariate and multivariate Cox's proportional hazard regression analyses identified post-PET-N negative status as the only significant favorable predictive factor for low postoperative recurrence ($p = 0.015$) independent of the primary tumor response.

Conclusions. PET-N negative status predicts ≤2 pLNMs and longer RFS in resectable TESCC patients even after NAC. Therefore, post-PET-N status, not the effects on the primary tumor, is a critical NAC treatment response criterion for evaluating prognosis and guiding subsequent treatment.

Esophageal cancer with multiple lymph node metastases (LNMs) has a dismal prognosis.[1–5] In a previous study, we reported that resectable thoracic esophageal squamous cell carcinoma (TESCC) patients with positron emission tomography (PET)-positive LNs were more likely to exhibit ≥3 pathological LNMs (pLNMs) and a higher rate of postoperative distant recurrences, resulting in a much lower 5-year relapse-free survival (RFS) rate compared with patients without PET positive LNs (29.6 vs. 75.1 %, respectively).[6] Aggressive neoadjuvant chemotherapy (NAC) was performed for those patients with PET-positive LNs to suppress postoperative recurrence, reducing the number of pLNMs and improving 5-year RFS twofold, but the difference was not significant compared with the historical PET-positive group without NAC.[7] The benefits of NAC are limited in the responders; thus, it is important to individualize therapy based on response criteria that best predict future outcome.

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T. Yasuda, M.D.
e-mail: tyasuda@surg.med.kindai.ac.jp

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