SOMATOSTATIN RECEPTORS SSTR2 AND SSTR5 ARE EXPRESSED IN THE HUMAN THORACIC DUCT

H. Jiang, X.F. Deng, C.M. Duan, C. Chen, J.L. Xiang, Y.L. Lu, Q.F. Ma

College of Life Sciences & Bioengineering, Beijing Jiaotong University (HJ), Department of Cardiothoracic Surgery, Affiliated Hospital of Academy of Military Medical Sciences (XFD,JLX,QFM), and Beijing Institute of Basic Medical Sciences (CMD,CC,YLL), Beijing, China

ABSTRACT

Somatostatin and its analog octreotide have been used successfully to treat postoperative chylothorax, and it has been shown that octreotide binds with high affinity to somatostatin receptor (SSTR) subtypes 2 and 5. Therefore, we investigated expression of SSTR2 and SSTR5 in the human thoracic duct by immunohistochemistry. Normal rat pancreas was used as a positive control for antibodies against SSTR2 and SSTR5, and Factor VIII-related antigen, SMA, actin, elastin, or collagen type II, III, IV or V antibodies were used to identify cell types and structures within the human thoracic duct. The antibodies against SSTR2 and SSTR5 worked well and yielded positive staining in control rat islets. In the human thoracic duct, SSTR2 was present in smooth muscle cells and some scattered structures which were stained by antibodies against Factor VIII-related antigen, SMA, actin, elastin or collagen type II, III, IV or V. SSTR5 was also present in smooth muscle cells. The presence of SSTR2 and SSTR5 in the human thoracic duct sheds light on the mechanism of somatostatin and octreotide use in the successful treatment of chylothorax and offers new molecular pathways to explore for potential future therapies.

Keywords: Somatostatin receptors, human thoracic duct, octreotide, chylothorax

Postoperative chylothorax is a serious and life-threatening clinical entity with an incidence of 0.5 to 2.0% in pulmonary resections and 2.2% in esophagectomies (1). Clinical problems associated with chylothorax include dyspnea and prolonged loss of fluid, calories, and lymphocytes caused by the accumulation of chyle in the pleural space, leading to dehydration, malnutrition and immunosuppression (2). When thoracic duct ligation was first introduced in 1948, the mortality associated with chylothorax was reduced from 50 to 15% (3). Today, approximately 20–50% of patients require surgical treatment of thoracic duct ligation, with a mortality of 15-21% (1,3). However, in clinical practice, when conservative treatment fails, surgical intervention may be inappropriate in patients with underlying advanced cancer or serious post-operative complications.

In 1990, Ulibarri first used somatostatin (SST) to treat a patient with lymphorrhagia from ruptured thoracic duct after supraglottic laryngectomy for carcinoma of the larynx (4). Since then, there have been many reports of the successful use of somatostatin or its synthetic analog octreotide (OCT) for treatment of chylothorax (3,5-11). The precise mechanisms responsible are still not well understood. SST is a 14- or 28-amino acid peptide that was originally discovered in 1973 as a hypothalamic neuroendocrine hormone (12). SST is expressed throughout the body and has important physiological effects; it

inhibits pancreatic secretion, neurotransmission in the central nervous system, gut motility, and the release of a number of peptide hormones, such as growth hormone, which is released from the anterior pituitary gland (13). The actions of SST in these tissues are mediated by specific SST receptors (SSTR).

It is unknown whether lymphatic vessels contain somatostatin receptors. In blood vessels of the splanchnic area, SST causes constriction which reduces lymph production (14). Therefore, it is possible that SST could exert a direct effect on the lymphatic system by reducing lymph production (15), or by also constricting lymphatic vessels to prevent transport, or by other unknown mechanisms. To the best of our knowledge, neither regulation of the constriction of lymphatic vessels by SST/OCT nor the expression of SSTR subtypes in lymphatic vessels or thoracic duct has been reported. Accordingly, we investigated expression of somatostatin receptors SSTR2 and SSTR5 in human thoracic ducts from patients with esophageal cancer.

MATERIALS AND METHODS

Tissues

Rat pancreas was obtained from a Wistar rat with a weight of approximately 250g. Thoracic ducts from four patients who suffered from esophageal carcinoma and underwent esophagectomy were studied. All patients had a complete mediastinal lymph node dissection, and a supradiaphragmatic ligation of the thoracic duct was performed during operation to inhibit postoperative chylothorax. Patients did not receive any treatment by SST or OCT. Signed consent was obtained from each patient, and the research protocol was approved by the Ethics Committee of the Affiliated Hospital of Academy of Military Medical Sciences.

Specimens were fixed in phosphate-buffered 4% formalin for a minimum of 24h. After dehydration through graded ethanols to xylene, tissue was embedded in paraffin, and five micron sections were stained with hematoxylin and eosin.

Antibodies Against SSTRs and Immunohistochemical Markers

For immunohistochemistry, rabbit polyclonal anti-SSTR2 and anti-SSTR5 antibodies were obtained from the Beijing Boisynthesis Biotechnology Company (Beijing, China). Immunohistochemical staining of pancreas and thoracic duct was performed using available antibodies, such as SSTR2 (Bois, BA1406), SSTR5 (Bois, bs-1139R), insulin (Zhongshan, ZM0155), SST (Zhongshan, ZA0232), glucagon (Zhongshan, ZA0119), Factor VIII-related antigen (Maixin, RAB-0070), smooth muscle actin (Boster, BM0002), Actin (Zhongshan, ZM-0001), Elastin (Boster, BM0823), Vimentin (Boster, MB01350), Collagen type II (Zhongshan, ZM-0390), Collagen type III (Boster, BA0326), Collagen type IV(Zhongshan, ZM-0081), Collagen type V (Bois, bs-0552R).

Immunohistochemistry

After embedding, the sections were dewaxed in xylene and rehydrated through a graded series of ethanols. Antigen retrieval was performed using microwave treatment for 20 min at 98°C using 0.01 M citric acid buffer (pH 6.0). Endogenous peroxidase activity was quenched by incubating sections in 3% hydrogen peroxide for 10 min at room temperature. Non-specific binding sites were blocked by pre-incubation with 1% bovine albumin in PBS for 30 min at room temperature. Reacted tissue sections were then incubated with antibodies for 18 h at 4°C. Antigen-antibody complexes were subsequently visualized using the Polink-2 Plus® Polymer HRP Detection System kit /DAB (Zhongshan Goldenbridge Co, Beijing, China) and counterstained with hematoxylin. Positive control experiments included normal
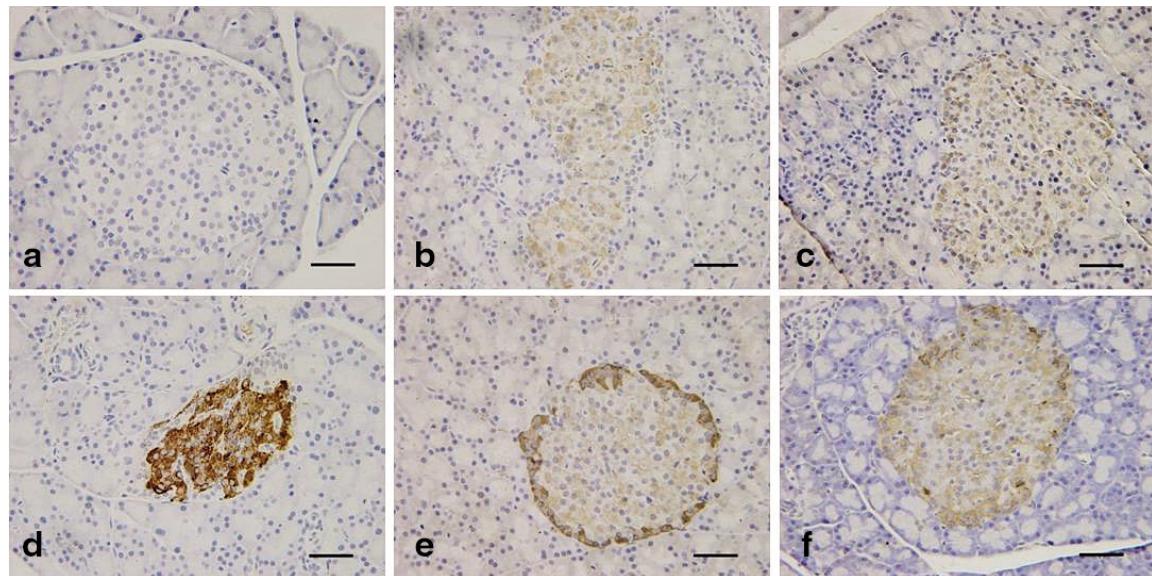
Fig. 1. Immunohistochemical staining of rat pancreas. Sections stained without primary antibody were used as a negative control (a). Antibody staining against SSTR2 (b) demonstrated many positively stained cells present in the center and at the periphery of the islet while SSTR5 staining (c) identified the majority of the islet cells. Insulin-producing cells accounted for nearly 80% of the islet (d) and glucagon-producing cells are located both around the islet mantle and scattered within the islets (e). SST-producing cells are identified as a small population of cells sparsely distributed in both central and peripheral regions of the islet (f). Scale bar=50μm.
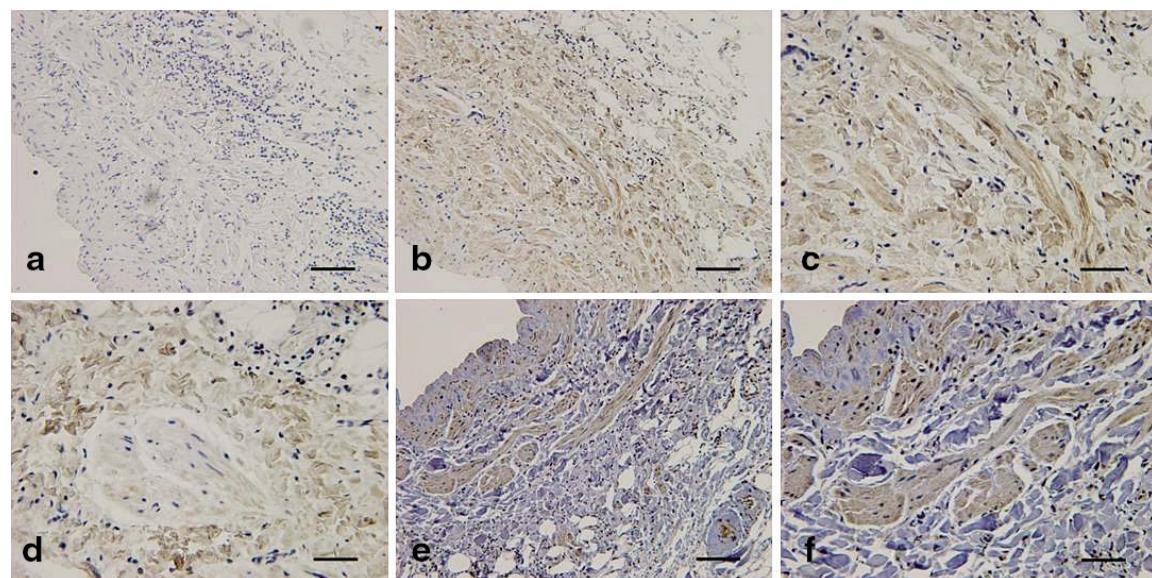

Fig. 2. Immunohistochemistry of SSTR2 and SSTR5 in human thoracic ducts. Sections stained without primary antibody were used as a negative control (a). Positive SSTR2 staining was seen in smooth muscle cells (b,c) with negative endothelium and some scattered regions composed of unidentified cell types (d). SSTR5 is present in smooth muscle cells (e,f). Scale bar=50μm (c,d,f) and 100μm (a,b,e).
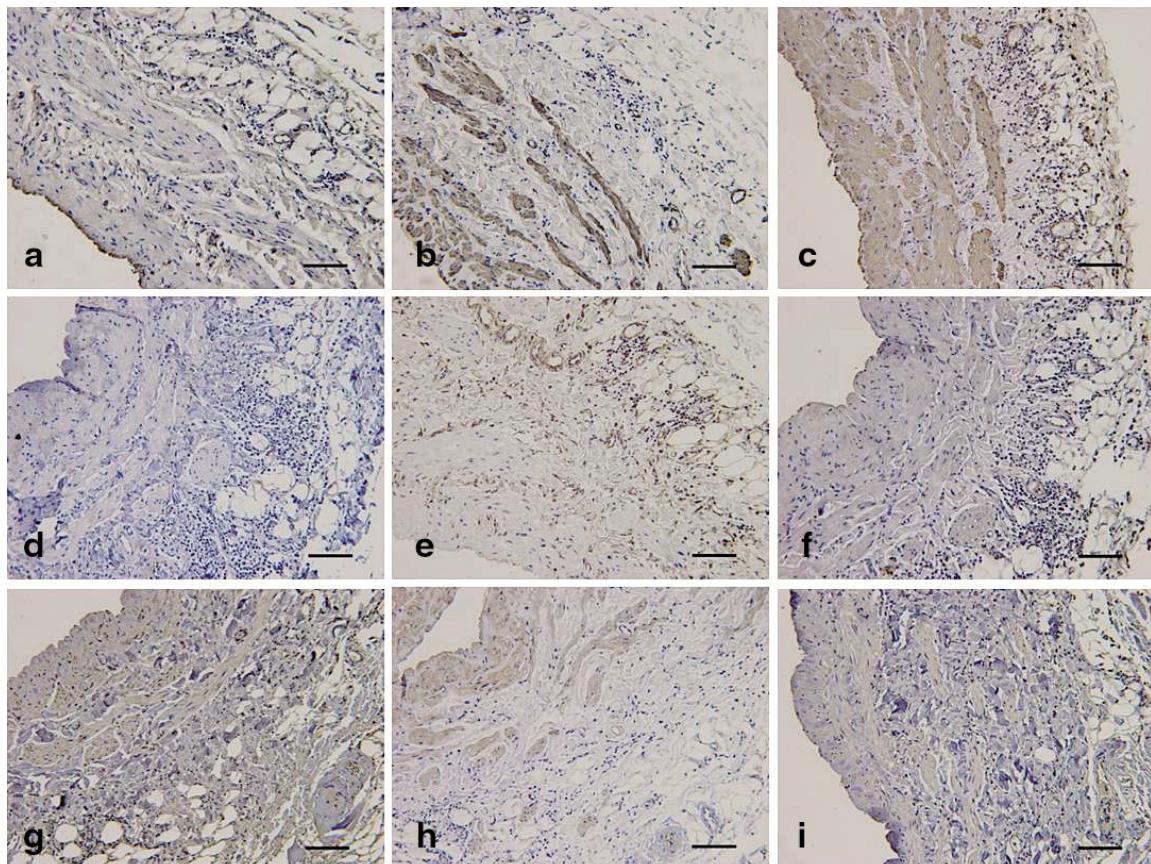
Fig. 3. Structures of the human thoracic duct revealed by immunohistochemical staining: The endothelium was stained with an antibody against Factor VIII-related antigen (a), smooth muscle cells stained with antibodies against smooth muscle actin (SMA) (b) and actin (c), elastic fibers (stained by elastin) are scattered among the inner and middle layers of the thoracic duct (d), connective tissue (stained by vimentin) is present among the smooth muscle cells and in the outer layer of thoracic duct (e), and a few fibers composed of collagen type II (f) and many fibers composed of collagen type III (g), IV (h) and V (I) are present among the smooth muscle cells. Scale bar=100μm.

rat pancreas. For negative controls, the primary antibody was omitted, and normal goat serum was used.

RESULTS

Specificity of SSTR2 and SSTR5 Antibodies

We first checked the activity of antibodies against SSTR2 and SSTR5 by immunohistochemical staining in rat pancreas (*Fig. 1*). Antibodies against SSTR2 and SSTR5 worked well and yielded positive staining in islets. SSTR2 was expressed by many cells in the center of the islets as well as at the periphery (*Fig. 1b*), whereas SSTR5 was localized in the majority of islet cells (*Fig. 1c*). As expected, ß cells were identified using the insulin antibody and accounted for about 80% of the islet endocrine cells (*Fig. 1d*). Glucagon-producing cells were much less numerous than ß cells and were located mainly around the islet mantle but were also scattered within the islets (*Fig. 1e*). SST-producing cells were identified as a small population of cells distributed sparsely throughout the islet (*Fig. 1f*).

Expression of SSTR2 and SSTR5 in the Human Thoracic Duct

We analyzed the expression of SSTR2 and SSTR5 in four thoracic duct samples (*Fig. 2*). The two SSTR subtypes were present in each sample and were not located along the endothelium (*Fig. 2b,c,e,f*). SSTR2 was present in smooth muscle cells (*Fig. 2c*) and some scattered regions composed of unidentified cell types (*Fig. 2d*). SSTR5 was present in smooth muscle cells (*Fig. 2e,f*). Most of the specimens showed a mixed sub-cellular distribution pattern for the receptors with strong staining in the cytoplasm and weaker staining at the membrane.

Structure of the Human Thoracic Duct

In an attempt to identify cell types in the scattered, SSTR2-positive regions in the human thoracic duct, we used antibodies against Factor VIII-related antigen, SMA, actin, elastin and collagens type II, III, IV and V (*Fig. 3*). Staining with these antibodies was positive, but the scattered structures were not identifiable. The staining clearly shows the structures of the human thoracic duct. The inner layer was endothelium, identified by staining with the antibody against Factor VIII- related antigen (*Fig. 3a*); in the middle there were many circular and longitudinal smooth muscle cells stained by smooth muscle actin (*Fig. 3b*) and actin (*Fig. 3c*). Many thin elastic fibers stained by elastin were sparsely distributed throughout the inner and middle layers of the thoracic duct (*Fig. 3d*), and connective tissue (positive for vimentin) was present within the smooth muscle and in the outer layer of the thoracic duct (*Fig. 3e*). Among the smooth muscle cells there were a few fibers composed of collagen type II (*Fig. 3f*) and many fibers composed of collagens type III (*Fig. 3g*), IV (*Fig. 3h*) and V (*Fig. 3i*).

DISCUSSION

The five known SSTRs belong to a family of seven transmembrane domain G-protein coupled receptors and are encoded on five different chromosomes. These SSTR subtypes are distributed throughout the body in regions including the central nervous system (16), pancreas (17), vascular tissue (18), skin (19), prostate (20) and cardiac myocytes (21). Using immunohistochemistry, Taniyama and colleagues examined the systemic localization of all SSTR subtypes in normal human organs, including the parotid, thyroid, and parathyroid glands, bronchial and esophageal nodes, stomach, duodenum, small intestine, colon, rectum, liver, pancreas and kidney (22). In all human tissues examined, various SSTR subtypes were detected not only in parenchymal cells, but also in various other cells such as lymphocytes, fibroblasts and endothelial cells. These findings demonstrate the potentially broad systemic actions of somatostatin in non-endocrine cells (22). The various actions of SST are mediated through specific membrane receptors, which have been found in various regions of many tissues and organs (23). Indeed, distinct but often overlapping patterns in the expression of these subtypes have been demonstrated (23).

Hemangiogenesis and lymphangiogenesis are thought to occur in parallel during embryonic development. It is thus essential to understand the development and regulation of blood vessels to understand the biology of the lymphatic vessels (24). The lymphatic muscle cells have some functional similarities to blood vascular smooth muscle cells (25). The smooth muscle layer in blood vessels controls the contractile tone of the vessels in response to vasoactive substances (24). If the mechanism of constriction of smooth muscle cells in lymphatic vessels is the same as that in blood vessels, the effect of SST or OCT on the contraction of blood vessels may be similar to its action on the contraction of lymphatic vessels (including the thoracic duct).

In previous experiments, SST was able to cause contraction of vascular structures. In

isolated canine cerebral arteries (26) and human saphenous vein (27), SST or its analogs elicited a significant contraction. Moreover, it has been demonstrated that these peptides cause constriction of the human dorsal hand vein *in vivo* (28). These variable effects of SST may be related to the presence of different types of SSTRs in different vascular structures and different species (29). By immunohistochemistry, SSTR1-5 antigens were found to localize predominantly in the smooth muscle cells of the rat aorta after angioplasty (30). However, all 5 SSTRs are expressed in rat splanchnic blood vessels (31). Human blood vessels expressed high levels of SSTR1 and significantly lower levels of SSTR2 and 4 with normal veins and arteries, as well as atherosclerotic arteries, expressing predominantly SSTR1 in endothelial but not vascular smooth muscle cells (18). The expression of different SSTRs in blood vessels may reflect different mechanisms for regulation of constriction of smooth muscle cells.

SST has been demonstrated to cause contraction of cultured rat aortic vascular smooth muscle cells, and this effect is likely to be mediated via SSTR4 because only SSTR4 was found to be present in these cells by RT-PCR (29). SST and several SST analogs have been shown to cause contraction of the human saphenous vein by what appeared to be a direct effect on the smooth muscle. Their relative potencies suggest that their effects were mediated by a SSTR that is like the recombinant SSTR2. The receptor transduction mechanism appears to involve activation of L-type calcium channels and entry of extracellular calcium (32).

The SSTRs can be divided into two subgroups. The first subgroup of receptors, which includes SSTRs 2, 3 and 5, has a high affinity for SST-14, SST-28 and SST analogs such as OCT. The second group, which includes SSTRs 1 and 4, has a high affinity for SST-14 but a lower affinity for the majority of available SST analogs (18,23). All five SSTR subtypes bind SST-14 and SST-28 with similar high affinities, but there are differences in the binding affinities of the structural analogs of SST (23). OCT, the first SST analog introduced for clinical use, inhibits the release of growth hormone, glucagon and insulin in monkeys 45, 11, and 1.3 times more powerfully, respectively, than does SST-14 (23). Its elimination half-life after subcutaneous administration is two hours, and rebound hypersecretion of hormones does not occur (23). In clinical treatment of chylothorax, OCT is more commonly used than SST because of its long half-life and ease of use. OCT binds with high affinity to SSTR 2 and SSTR 5 and with moderate affinity to SSTR 3 but does not bind to SSTR 1 and 4 (23). It remains unknown if there may be other receptors besides SSTR2 and SSTR5 responding to SST or OCT for treatment of chylothorax.

In the present study, we detected immunoreactive SSTR2 in smooth muscle cells and some scattered structures composed of unknown cell types in the human thoracic duct. SSTR5 was also present in smooth muscle cells. Both SSTR2 and SSTR5 were primarily present in the cytoplasm and stained weakly at the membrane. Although SSTRs are membrane-associated receptors, a significant amount of staining was seen in the cytoplasm, perinuclear region, and the nucleus (in a few immunoreactive cells). Our data were similar to those found in the human prostate tissue by Montironi et al (20). The interpretation, based on molecular studies suggesting nuclear accumulation of SST analogs mediated by SSTRs, is that, after binding their ligand, SSTR-ligand complexes undergo cellular internalization with progressive intracytoplasmic and intranuclear translocation (20). Cytoplasmic SSTR is generally considered to represent either newly synthesized or internalized membrane receptor protein (33). However, Le Romancer's results suggested that nuclear translocation of SST analogs is mediated by p86-Ku and not by SSTRs (20). Further investigation is required to elucidate the

biological and/or clinical significance of this cytoplasmic localization of SSTRs.

A major limitation of this study is that our immunohistochemistry was not accompanied by a molecular investigation. It is important to determine which subtypes of the SSTRs exert their effects on which cells of the human thoracic duct. However, it is nearly impossible to obtain precise data on the distribution and location of SSTR subtypes when employing molecular methods such as Northern blots, RT-PCR and real-time PCR; all of these methods investigate the tissue as a whole (20). We also did not have the capability to double or triple stain the samples, which would have allowed co- localization of multiple markers.

The present results have important functional and therapeutic implications. This is the first description of immunohisto-chemically detected SSTR2 and SSTR5 proteins at the cellular level in the smooth muscle cells of the human thoracic duct, and it provides a possible mechanism to explain why OCT is successful in treating chylothorax. Additionally, these findings provide the basis for further experiments to firmly establish a link between SST/OCT and the contraction of lymphatic vessels through binding of specific SSTRs which may correlate with different pathways in SST/OCT treatment of chylothorax.

ACKNOWLEDGMENT

REFERENCES

1. Mafe, JJ, JM Caravajal, B Baschwitz, et al: Bilateral chylothorax after thymectomy via median sternotomy and resolution through conservative treatment. Eur. J. Cardiothorac. Surg. 24 (2003), 466-468.

2. Kalomenidis, I: Octreotide and chylothorax. Curr. Opin. Pulm. Med. 12 (2006), 264-267.

3. Demos, NJ, J Kozel, JE Scerbo: Somatostatin in the treatment of chylothorax. Chest 119 (2001), 964-966.

4. Ulíbarri, JI, Y Sanz, C Fuentes, et al: Reduction of lymphorrhagia from ruptured thoracic duct by somatostatin. Lancet 336 (1990), 258.

5. Rimensberger, PC, B Muller-Schenker, A Kalangos, et al: Treatment of a persistent postoperative chylothorax with somatostatin. Ann. Thorac. Surg. 66 (1998), 253-254.

6. Kelly, RF, SJ Shumway: Conservative management of postoperative chylothorax using somatostatin. Ann. Thorac. Surg. 69 (2000), 1944-1945.

7. Collard, JM, PF Laterre, F Boemer, et al: Conservative treatment of postsurgical lymphatic leaks with somatostatin-14. Chest 117 (2000), 902-905.

8. Mikroulis, D, V Didilis, G Bitzikas, et al: Octreotide in the treatment of chylothorax. Chest 121 (2002), 2079-2081.

9. Hamdan, MA, ML Gaeta: Octreotide and low-fat breast milk in postoperative chylothorax. Ann. Thorac. Surg. 77 (2004), 2215-2217.

10. Gabbieri, D, L Bavutti, F Zaca, et al: Conservative treatment of postoperative chylothorax with octreotide. Ital. Heart J. 5 (2004), 479-482.

11. Mincher, L, J Evans, MW Jenner, et al: The successful treatment of chylous effusions in malignant disease with octreotide. Clin. Oncol. 17 (2005), 118-121.

12. Brazeau, P, W Vale, R Burgus, et al: Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science 179 (1973), 77-79.

13. Epelbaum, J, P Dournaud, M Fodor, et al: The neurobiology of somatostatin. Crit. Rev. Neurobiol. 8 (1994), 25-44.

14. Lim, KA, SH Kim, J Huh, et al: Somatostatin for postoperative chylothorax after surgery for children with congenital heart disease. J. Korean Med. Sci. 20 (2005), 947-951.

15. Carcoforo, P, G Soliani, U Maestroni, et al: Octreotide in the treatment of lymphorrhea after axillary node dissection: A prospective randomized controlled trial. J. Am. Coll. Surg. 196 (2003), 365-369.

16. Csaba, Z, P Dournaud: Cellular biology of somatostatin receptors. Neuropeptides 35 (2001), 1-23.

17. Kumar, U, R Sasi, S Suresh, et al: Subtype-selective expression of the five somatostatin receptors (hSSTR1-5) in human pancreatic islet cells: A quantitative double-label

immunohistochemical analysis. Diabetes 48 (1999), 77-85.

18. Curtis, SB, J Hewitt, S Yakubovitz, et al: Somatostatin receptor subtype expression and function in human vascular tissue. Am. J. Physiol. Heart Circ. Physiol. 278 (2000), H1815-H1822.

19. Hagströmer, L, L Emtestam, M Stridsberg, et al: Expression pattern of somatostatin receptor subtypes 1-5 in human skin: An immunohistochemical study of healthy subjects and patients with psoriasis or atopic dermatitis. Exp. Dermatol. 15 (2006), 950-957.

20. Montironi, R, L Cheng, R Mazzucchelli, et al: Immunohistochemical detection and localization of somatostatin receptor subtypes in prostate tissue from patients with bladder outlet obstruction. Cell Oncol. 30 (2008), 473-482.

21. Smith, WH, RU Nair, D Adamson, et al: Somatostatin receptor subtype expression in the human heart: Differential expression by myocytes and fibroblasts. J. Endocrinol. 187 (2005), 379-386.

22. Taniyama, Y, T Suzuki, Y Mikami, et al: Systemic distribution of somatostatin receptor subtypes in human: An immunohistochemical study. J. Endocrinol. 52 (2005), 605-611.

23. Lamberts, SW, AJ van der Lely, WW de Herder, et al: Octreotide. N. Engl. J. Med. 334 (1996), 246-254.

24. Jussila, L, K Alitalo: Vascular growth factors and lymphangiogenesis. Physiol. Rev. 82 (2002), 673-700.

25. Muthuchamy, M, A Gashev, N Boswell, et al: Molecular and functional analyses of the contractile apparatus in lymphatic muscle. FASEB J. 17 (2003), 920-922.

26. Shirahase, H, M Kanda, H Shimaji, et al: Somatostatin-induced contraction mediated by endothelial TXA2 production in canine cerebral arteries. Life Sci. 53 (1993), 1539-1544.

27. Luu, TN, AH Chester, GS O'Neil, et al: Effects of vasoactive neuropeptides on human saphenous vein. Br. Heart J. 67 (1992), 474-477.

28. Panconesi, A, PL Del Bianco, G Franchi, et al: Somatostatin: Peripheral venoconstrictive activity and interaction with monoamines in man. Regul. Pept. 18 (1987), 267-276.

29. Torrecillas, G, J Medina, ML Díez-Marqués, et al: Mechanisms involved in the somatostatin-induced contraction of vascular smooth muscle cells. Peptides 20 (1999), 929-935.

30. Khare, S, U Kumar, R Sasi, et al: Differential regulation of somatostatin receptor types 1-5 in rat aorta after angioplasty. FASEB J. 13 (1999), 387-394.

31. Reynaert, H, E van Rossen, N Uyama, et al: Expression of somatostatin receptors in splanchnic blood vessels of normal and cirrhotic rats. Liver Int. 27 (2007), 825-831.

32. Dimech, J, W Feniuk, RD Latimer, et al: Somatostatin-induced contraction of human isolated saphenous vein involves sst2 receptor-mediated activation of L-type calcium channels. J. Cardiovasc. Pharmacol. 26 (1995), 721-728.

33. Hofland, LJ, Q Liu, PM Van Koetsveld, et al: Immunohistochemical detection of somatostatin receptor subtypes sst1 and sst2A in human somatostatin receptor positive tumors. J. Clin. Endocrinol. Metab. 84 (1999), 775-780.

QunFeng Ma, MD
Department of Cardiothoracic Surgery
Affiliated Hospital of Academy of Military
  Medical Sciences
No 8, DongDalie Street
District of FengTai
Beijing, China, 100071
Tel: 861066947155
E-mail: maqunfeng @126.com