Changes of gastrointestinal argyrophil endocrine cells in the osteoporotic SD rats induced by ovariectomy

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The regional distributions and frequencies of argyrophil endocrine cells in gastrointestinal (GI) tract of osteoporotic Sprague-Dawley rat induced by ovariectomy were studied using Grimelius silver stain. The experimental animals were divided into two groups, one is non-ovariectomized group (Sham) and the other is ovariectomized group (OVX). Samples were collected from each part of GI tract (fundus, pylorus, duodenum, jejunum, ileum, cecum, colon and rectum) at 10th week after ovariectomy or sham operation. In this study, argyrophil cells were detected throughout the entire GI tract with various frequencies regardless of ovariectomy. Most of these argyrophil cells in the mucosa of GI tract were generally spherical or spindle in shape (open type cell) while cells showing round in shape (close type cell) were found occasionally in gastric and/or intestinal gland regions. The regional distributions of GI argyrophil endocrine cells in OVX were similar to those of Sham. However, significant decreases of argyrophil cells were detected in OVX compared to those of Sham except for the pylorus, jejunum and cecum. In pylorus and jejunum, argyrophil cells in OVX dramatically decreased compared to those of Sham but significances were not recorded. In addition, argyrophil cells in cecum of OVX showed similar frequency compared to that of Sham. The endocrine cells are the anatomical units responsible for the production of gut hormones that regulate gut motility and digestion including absorption, and a change in their density would reflect the change in the capacity of producing these hormones and regulating gut motility and digestion. Ovariectomy induced severe quantitative changes of GI argyrophil endocrine cell density, and the abnormality in density of GI endocrine cells may contribute to the development of gastrointestinal symptoms in osteoporosis such as impairments of calcium and some lipids, frequently encountered in patients with postmenopausal osteoporosis.

**Key words:** Ovariectomy, rat, argyrophil, endocrine, frequency

**Introduction**

Osteoporosis is caused by an imbalance between bone resorption and bone formation, which results in bone loss and fractures after mineral flux. The frequency of fractures significantly increases in osteoporosis, and hip fractures in senile patients are a very serious problem because it often limits the patients quality of life. The postmenopausal osteoporosis model using ovariectomized rat is useful for evaluation of osteoporetic drugs, because several parameters clearly decrease by the ovariectomy within 4 weeks after operation [27]. In addition, the ovariectomized rat bone loss model is suitable for studying problems that are relevant to postmenopausal bone loss, because ovariectomy that induced bone loss in the rat and postmenopausal bone loss share many similar characteristics including decreased intestinal absorption of calcium [12].

Gastrointestinal (GI) endocrine cells dispersed in the epithelia and gastric glands of the digestive tract synthesized various kinds of gastrointestinal hormones and played an important role in the physiological functions of the alimentary tract [2]. Until now, the investigation of gastrointestinal endocrine cells is considered to be an important part of a phylogenic study [5] and the endocrine cells are regarded as the anatomical units responsible for the production of gut hormones, and a change in their density would reflect the change in the capacity of producing these hormones [8]. Silver techniques have been regarded as a general method for detecting GI endocrine cells and Grimelius positive cells are classified as argyrophil cells [10,11].

The changes of distribution and frequency of GI argyrophil endocrine cells in some diseases are also well
demonstrated especially in some cancer status [9,22], gastritis including *Helicobacter pylori* [1,17] and some GI tract disorder [7]. In addition, these GI argyrophil cells are also changed after treatment of some drugs such as ebrotidine [21] and omeprazole [4]. The distribution and frequency of GI endocrine cells were varied with feeding habits [23] and osteoporetic patients and/or animals show quite different feeding habits [25]. However, there was no report dealing with the changes of GI argyrophil endocrine cells at osteoporotic status in spite of some clear disorder of gastric absorption of calcium ion [13], lipids [15] and GI argyrophil endocrine cells showed somewhat different feeding habits, and osteoporosis induced by ovariectomy or post-menopause is directly related to some endocrine system especially to estrogen [19,20].

The purpose of this study is to observe the changes of regional distribution and frequency of GI argyrophil endocrine cells in a postmenopausal osteoporotic rat induced by ovariectomy. In this study, each part of GI tract is sampled at 10th week after ovariectomy or sham-operation.

Material and Methods

Experimental animals

Twenty Sprague-Dawley (SD) female rat (6-wk old upon receipt, Charles River, Japan) were used after acclimatization for 7 days. Animals were allocated 5 per polycarbonate cage in a temperature (20-25°C) and humidity (30-35%) controlled room. Light: dark cycle was 12 hr: 12 hr and feed (Samyang, Seoul, Korea) and water were supplied free to access. Half rats were ovariectomized group (OVX) and remainders were sham-operated group (Sham).

Bilateral ovariectomy

All rats were anesthetized with Ketamine hydrochloride (60 mg/2 ml/kg) and Xylazine hydrochloride (2.5 mg/2 ml/kg) combination and subjected to operation. Bilateral ovariectomy was performed by removing both ovaries in the abdominal cavity, and sham operation (ovary identification) was performed in case of sham.

Histology and quantity analyses

After phlebotomy, each region of GI tract, fundus, pylorus, duodenum, jejunum, ileum, cecum, colon and rectum were detected with 75.90 ± 19.26, 56.20 ± 9.98, 12.20 ± 3.16, 67.90 ± 15.60 cells, respectively. In OVX, argyrophil cells were numerously detected in the stomach and large intestine compared to that of the small intestine regardless of sham (Fig 1a–l) and OVX (Fig 2a–j). Quantity of argyrophil cells: Among 1000 parenchymal cells, argyrophil cells in sham were detected in the fundus, pylorus, duodenum, jejunum, ileum, cecum, colon and rectum with 291.70 ± 71.74, 68.70 ± 14.06, 21.80 ± 3.68, 11.10 ± 2.47, 11.70 ± 2.11, 12.40 ± 3.24, 51.20 ± 12.33 and 10.30 ± 3.16 cells, respectively. In OVX, argyrophil cells were detected in the fundus, pylorus, duodenum, jejunum, ileum, cecum, colon, and rectum, respectively. In most regions of GI tract, argyrophil cells showed significant (*p* < 0.001 or *p* < 0.05) decrease in OVX compared to that of sham except for the pylorus, jejunum and cecum. In pylorus and jejunum, argyrophil cells in OVX dramatically decreased compared to those of Sham but significances were not recorded. In addition, argyrophil cells in cecum of OVX showed similar frequency compared to that of Sham but significances were not recorded. In pylorus and jejunum, argyrophil cells showed significant (*p* < 0.05) decrease in OVX compared to that of sham.

Discussion

It is generally accepted that osteoporosis is metabolic and...
hormonal disorder that is clearly related to estrogen [19,20] and also osteoporotic patients and/or animals show quite different feeding habits [25]. Silver techniques have been regarded as a general method for detecting GI endocrine cells and Grimelius positive cells are classified as argyrophil cells [10,11]. The GI endocrine cells were generally divided into two types, one was round to spherical shaped close type cells which were located in the stomach regions, and the other was spherical to spindle shaped open type cells which were situated in the intestinal regions. In addition, the endocrine cells are regarded as the anatomical units responsible for the production of gut hormones, and a change in their density would reflect the change in the capacity of producing these hormones [8].

In the present study, the changes of the argyrophil endocrine cells in the GI tract of SD rat after ovariectomy were observed by silver technique, the Grimelius method. The general distribution of the argyrophil cells in the GI tract...
of OVX showed quite similar patterns compared to that of sham. As results of ovariectomy, argyrophil cells significantly ($p < 0.01$ or $p < 0.05$) decreased throughout the entire GI tract except for the pylorus, jejunum and cecum. In pylorus and jejunum, argyrophil cells in OVX dramatically decreased compared to those of Sham but significances were not recorded. In addition, argyrophil cells in cecum of OVX showed similar frequency compared to that of Sham. In the fundus and rectum, most dramatical changes were demonstrated.

It was generally accepted that the changes of argyrophil cells were clearly related to digestive status of animals. In *Helicobacter pylori* infection, hyperplasia of argyrophil cells were demonstrated [17] and they also increased in patients with ulcerative colitis and Crohn's disease [7], atrophic gastritis [1], hypergastrinemia [3] and pernicious anemia [26]. It has been postulated that the changes in the GI endocrine cells are a selective process to meet the new demands exerted by the dramatic decrease in intestinal

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**Fig. 2.** Argyrophil endocrine cells in the GI tract of OVX; Most of argyrophil cells were dispersed in the mucosa of the fundus (a), pylorus (b), duodenum (c), jejunum (d), ileum (e), cecum (f and g), colon (h and i) and rectum (j). a~f, h and j: 150; g and i: 500, Grimelius method.
absorption [6] and osteoporotic patients and/or experimental animals shows impairment of absorption of calcium ion [13,18] and increase of absorption of cholesterol and other lipids [15]. Therefore, the decrease of GI argyrophil endocrine cells may be responsible for the malabsorption of calcium and lipids that occur in patients with postmenopausal osteoporosis and these decrease of endocrine cells are also detected with aging especially to cells that release the hormone regulating GI motility [16].

In conclusion, ovariectomy induced severe quantitative changes of GI argyrophil endocrine cell density, and the abnormality in density of GI endocrine cells may contribute to the development of gastrointestinal symptoms in osteoporosis such as impairments of calcium and some lipids, frequently encountered in patients with postmenopausal osteoporosis. However, the target or individual changes of GI endocrine cells are not clear and the change of argentaffin cells that are stained by other silver technique and generally used in GI endocrine researches [14] is also unknown. Elucidation of the changes of individual GI endocrine cells using immunohistochemistry [24] and/or change of argentaffin cells using another silver technique will provide mechanisms for understanding GI disorder that occurs in various disease. Further detailed studies with immunohistochemical and another silver techniques will be needed.

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