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The Histological Structure and Location of Substance P in the Digestive Tract of the Siberian Tiger (*Panthera tigris altaica*)

[1]Yunhai Zhang, [1]Yunsheng Li, [2]Zhizhong Zhang, [1]Zubing Cao, [1]Shudong Jiang, [2]Jing Yang, [1]Xiaorong Zhang and [1]Fugui Fang
[1]Provincial Laboratory of Animal Genetic Resources Protection and Breeding, College of Animal Science and Technology, Anhui Agricultural University, No. 130 of Changjiang West Road, Hefei, 230036 Anhui, P.R. China
[2]Hefei Wild Animal Park, Hefei, 230061 Anhui, China

Abstract: Substance P (SP) belongs to the tachykinin family and plays important roles in many physiological processes. The distribution of SP has not been investigated in the digestive tract of the tiger. The present study was performed to describe the histological structure and location of SP in the digestive tract which included the esophagus, stomach, small intestine and large intestine of the Siberian tiger at 8 days of age. The tissues of the digestive tract were collected from three 8 days old Siberian tigers that died of cold weather. The weight was recorded and the histological sections were made and stained with HE. The localization of SP in tissue was detected by immunohistochemical two-step PV-9000. The results show that the length of the digestive tract was approximately 215.6 cm with an average weight of 48.95 g. The wall of the digestive tract consisted of mucosa, submucosa, unica muscularis and tunica adventitia. The epithelium of the esophagus was a stratified squamous type without beginning surface cornification and the unica muscularis consisted of skeletal muscle. The lamina propria of the stomach was located by many glands but did not reach the muscularis mucosa. SP-immunoreactive cells were identified primarily in the epithelium and the cardiac gland of the stomach. SP-immunoreactive cells were also found in the intestinal gland of the lamina propria of the cecum and the colon. The current study preliminarily revealed the histological features and the distribution of SP in the digestive tract of an 8 days old Siberian tiger which requires further investigation.

Key words: Siberian tiger, digestive tract, histological structure, substance P, cardiac gland, China

INTRODUCTION

The Siberian tiger (*Panthera tigris altaica*) which is also known as the Amur, Altaic, Korean, North Chinese or Ussuri tiger is a subspecies of tigers. The dual threats of illegal poaching and the destruction of its habitat have brought this species to the brink of extinction with a population of 450 in the world and 20 in China (Hu *et al*., 2006).

A few studies have reported on tiger reproductive performance, genetics and cloning (Putranto *et al*., 2007; Gjorret *et al*., 2002; Hu *et al*., 2006; Liu *et al*., 2010) However, no studies have identified the histological structure and cytological characteristics of SP in the digestive tract of the Siberian tiger. With the restricted population of the tiger, it would be a challenge for scientists to learn and familiarize themselves with the morphologic characteristics of SP in the tiger.

SP is an 11 amino acid peptide that acts as a tachykinin and interacts with the Neurokinin (NK) receptor family which includes several subtypes (NK-1, NK-2, NK-3) of G-protein coupled receptors (Goto and Tanaka, 2002). The NK-1 receptor (NK-1R) has the highest affinity for SP (Goto and Tanaka, 2002). After binding to NK-1R, SP regulates many biological functions such as pain perception (Greco *et al*., 2008), reinforcement (Kertes *et al*., 2009), anxiety and stress (Ebner and Singewald, 2006), neurogenesis (Park *et al*., 2007), vasodilation (Miike *et al*., 2009), breath controlling (Bonham, 1995), vomiting (Diemunsch *et al*., 2009) and intestinal epithelial migration (Turner *et al*., 2007). Previous studies have analyzed the physiological processes of the digestive tract (Felderbauer *et al*., 2007; Turner *et al*., 2007). SP and its receptor are widely distributed in the gastrointestinal tract of many animals in a regionally specific pattern in the gastrointestinal tract

Corresponding Author: Fugui Fang, College of Animal Science and Technology, Anhui Agricultural University, No. 130 of Changjiang West Road, Hefei, 230036 Anhui, P.R. China

(Agungpriyono *et al*., 2000; Godlewski and Kaleczyc, 2010; Holzer *et al*., 1980; Yamada *et al*., 1999). However, the regional distribution of SP in the digestive tract of Siberian tiger remains unclear.

The present study was the first observation of the structure and localization of SP in the digestive tract in the neonatal male Siberian tiger. The results that are presented here will help us understand the biological characteristics of this endangered species.

MATERIALS AND METHODS

Animals and sample collection: The samples were collected from three male Siberian tigers that died of cold weather at the Hefei Wildlife park in Anhui province in China. No abnormalities were found. The tigers were 8 days of age. After death, the abdominal cavity was opened and the digestive organs were dissected and immediately brought to the laboratory in saline at 4°C. Subsequently, the weight and length of the digestive organs were examined.

Preparation of sections: A small tissue sample of each digestive organ was collected and fixed for 24 h in 4% paraformaldehyde (Klinipath, Geel, Belgium) at 4°C. After fixation, all tissue samples were rinsed and embedded into paraffin wax using standard procedures (Ren *et al*., 2007). Total 5μ-thick sections were cut, placed on poly-L-lysine-coated glass slides and dried for 1 h at 60°C for histological and immunohistochemical analyses.

Histological evaluation: The sections were stained with Hematoxylin and Eosin (HE). Morphological structures of the digestive organs were observed and photographed under a microscope.

Immunohistochemical procedure: Immunohistochemistry was performed using standard procedures (PV-9000 method) with rabbit anti-substance P (1:100 dilution, code BA1619, Zhongshan Goldenbridge Biotechnology Co., Ltd. Beijing, China) as the primary antibody. The secondary antibody was biotinylated goat anti-rabbit IgG (GBI, USA). Samples were developed using standard DAB reagents (Zhongshan Goldenbridge Biotechnology Co., Ltd. Beijing, China). Pictures were captured using a microscope. The negative controls were produced by substituting the primary antibody with 0.01 M PBS.

Acquisition and analysis of data: Representative tissue was obtained using 8-10 random sections. The thicknesses of the pipe wall and the mucosa of each organ in the digestive duct were measured using morphological analysis software (Images Advanced 3.2, MOTIC, Hong Kong, China). The data was expressed as the mean±SD.

RESULTS AND DISCUSSION

The structure of the digestive duct: The digestive duct consisted of the esophagus, the stomach, the small intestine and the large intestine. The four layers of the wall of the digestive tract from the internal to the external sides of the digestive tract are the mucosa, the submucosa, the unica muscularis and the tunica adventitia.

Esophagus: The average length and weight of the esophagus was 14.80 cm and 1.14 g, respectively (Table 1). The thickness of the wall of esophagus and mucosa was 707.28 and 245.77 μm (Table 2). The layers of the esophagus were clearly discernable. The mucosa consisted of the epithelium, the lamina propria and a thin muscularis mucosa (Fig. 1). The epithelium of the esophagus was a stratified squamous type with beginning surface cornification (Fig. 1a). The lamina propria contained dense, irregular connective tissue under the surface epithelium (Fig. 1a).

The submucosa consisted of dense, irregular connective tissue that was found between the muscle of the mucosa and the thicker external muscle layers (which were vascularized), a neural plexus and an esophageal gland (Fig. 1b). The mucosa and submucosa formed many folds (Fig. 1). The unica muscularis consisted of skeletal

Table 1: The length and mass of each organ in the digestive duct of the 8 days old Siberian tiger

Names	Length (cm)	Mass (g)
Body length and weight	44.35±1.350	3052.50±67.50
Esophagus	14.80±0.200	1.14±0.040
Stomach	10.00±0.800	8.17±0.330
Duodenum	11.10±0.100	1.82±0.070
Jejunum	176.00±18.00	31.81±7.970
Ileum	2.75±0.250	0.47±0.110
Caecum	3.25±0.750	1.22±0.500
Colon	10.75±4.750	4.33±0.950
Rectum	4.75±0.250	1.05±0.120

Table 2: The thickness of the wall and the mucosa of each organ in the digestive duct of the 8 days old Siberian tiger

Names	Wall (μm)	Mucosa (μm)
Esophagus	707.28±110.02	245.77±73.18
Cardia	978.37±149.78	273.16±68.54
Pyloric	836.14±121.79	298.13±45.18
Fundus	736.18±72.890	280.21±51.25
Duodenum	885.69±5.7300	628.74±22.87
Jejunum	1186.17±18.000	581.81±17.57
Ileum	1135.43±12.570	606.14±41.34
Caecum	753.93±11.350	340.99±11.31
Colon	810.29±56.540	410.33±21.55
Rectum	750.41±23.370	320.05±12.21

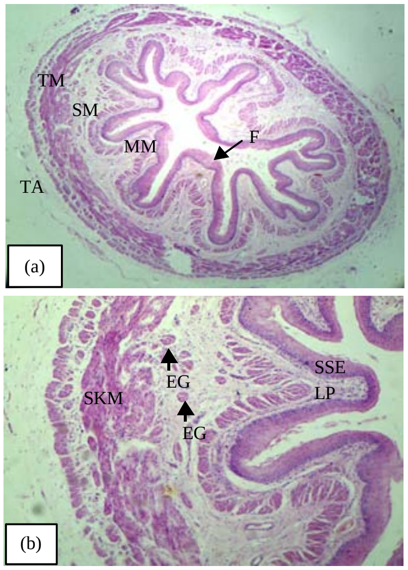

Fig. 1: Histological structure of esophagus in the 8 days old Siberian tiger using the HE method. a: overall esophagus tissue structure (x40) and b: local esophagus tissue structure under high magnification (x100). Structures in the pipe wall of the esophagus are shown as Mucous Membrane (MM), Submucosa (SM), Tunica Muscularis (TM), Tunica Adventitia (TA), Fold (F), Stratified Squamous Epithelium (SSE), Lamina Propria (LP), Esophageal Gland (EG) and Skeletal Muscle (SKM)

muscle with an inner circular and an outer longitudinal layer (Fig. 1b). The tunica adventitia contained loose connective tissue (Fig. 1b).

Stomach: The average length and mass of the stomach was 10.00 cm and 8.17 g, respectively (Table 1). The epithelium of the stomach was a simple columnar type that dipped into many pits (Fig. 2a). The lamina propria contained many glands, including the cardiac gland, the fundic gland and the pyloric gland. However, not all of the glands reached the muscularis mucosa. The cardiac gland was a tubular type with a large lumen and the cell was a cubical type (Fig. 2b). The fundic gland was a branched tubular type. The lumen was small and contained the gastric glands and the majority of cell types that are involved in gastric function (Fig. 2c). Many parietal cells and a few chief cells were clearly observed in the fundic gland. The parietal cell was large, circular in shape and positive for eosinophilic staining. The chief cell was pyramidal in shape (Fig. 2c). The pyloric gland contained many branches and columnar cells (Fig. 2d).

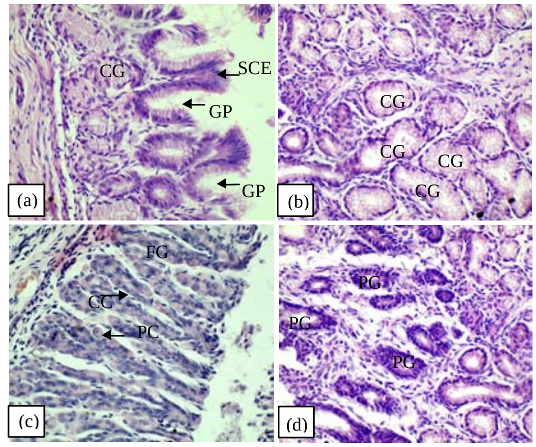

Fig. 2: The histological structure of the stomach in the 8 days old Siberian tiger using the HE method. a: Simple Columnar Epithelium (SCE) and Gastric Pit (GP), b: Cardiac Gland (CG), c: Fundic Gland (FG) and d: Pyloric Gland (PG). Cells in the fundic gland are shown as the Chief Cell (CC) and the Parietal Cell (PC). Magnification at x400

Small intestine: The small intestine consisted of three segments as follows: duodenum, jejunum and ileum. The length of the three segments was 11.10, 176.00 and 2.75 cm, respectively (Table 1). The thickness of the wall was 885.69 μm for the duodenum, 1186.17 μm for the jejunum and 1135.43 μm for ileum. The mucosa thickness was 628.74 μm for the duodenum, 581.81 μm for the jejunum and 606.14 μm for ileum (Table 2). The epithelium of the small intestine was a simple columnar type and dipped into the simple tubular small intestinal gland in the lamina propria (Fig. 3a and b). There were three orders of folding at the microscopic level. The first order of folding was the plicae which are the folds of the mucosa that each contained a submucosal core. The next level of folding was the intestinal villus. Each fingerlike villus contained a core of lamina propria (Fig. 3c). There were a central lacteal in the core of the lamina propria of the intestinal villus (Fig. 3d). The next order was comprised of the striated border which contained columnar cells with apical specializations (Fig. 3d). Different structures were found in the intestinal villus of the three segments as follows: a foliaceous type in the duodenum (Fig. 3a and b), a fingerlike type in the jejunum (Fig. 3c and d) and a pyramidal type in the ileum. The most goblet cells were presented in the epithelium and the small intestinal gland of ileum (Fig. 3e and f). The duodenal gland was not observed in the submucosa (Fig. 3b).

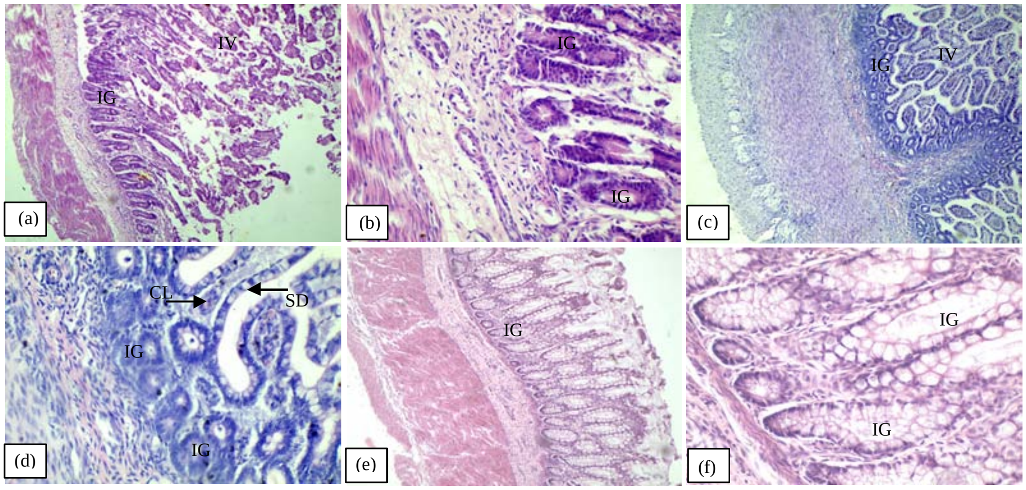

Fig. 3: The histological structures of the small intestine in the 8 days old Siberian tiger using the HE method. a and b: duodenum, c and d: jejunum and e and f: ileum. a-c: magnification at x100 and e-f: magnification at x400. Structures in the wall of the small intestine are shown as the Intestinal Gland (IG), the Intestinal Villus (IV), the Striated border (SB) and the Central Lacteal (CL)

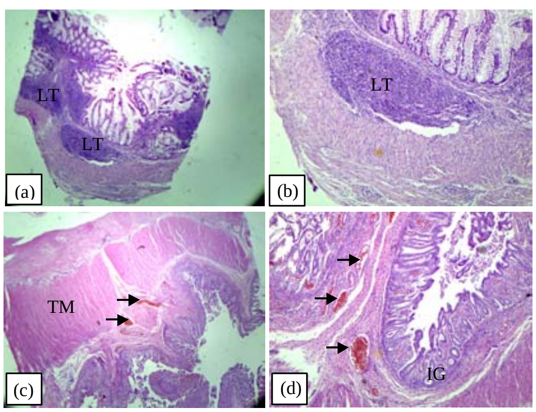

Fig. 4: The histological structures of the large intestine in the 8 days old Siberian tiger using the HE method. a and b: cecum, c and d: colon. a and c: magnification at x40 and b and d: magnification at x100. The structures in the wall of the large intestine are shown as Lymphoid Tissue (LT), the Intestinal Gland (IG) and the Tunica Muscularis (TM). Arrow indicates blood vessels

Large intestine: The large intestine consisted of the cecum, the colon and the rectum. The lengths of the cecum, the colon and the rectum were 3.25, 10.75 and 4.75 cm, respectively (Table 1). The wall thicknesses of the cecum, the colon and the rectum were 753.93, 810.29 and 750.41 μm, respectively. The mucosa thicknessess of the cecum, the colon and the rectum were 340.99, 410.33 and 320.05 μm, respectively (Table 2). The epithelium of the large bowel was a simple columnar epithelium that contained crypts but no villi (Fig. 4). The goblet cell was the predominant cell of the epithelium and the intestinal gland of the large intestine (Fig. 4). Many lymphatic tissue were found in the submucosa of cecum (Fig. 4a and b). There were blood vessels in the submucosa and the thickened unica muscularis in the colon (Fig. 4c and d).

Location of substance P in the digestive tract: In the digestive tract, SP-Immunoreactive (SP-IR) cells were mainly localized in the epithelium (Fig. 5a), the cardiac gland (Fig. 5b) of the stomach and the intestinal glands of the lamina propria of the cecum (Fig. 5c) and the colon (Fig. 5d). SP-IR cells were round or oval. The number of SP-IR cells in the colon was greater than that of the cecum. However, SP staining was not observed when the SP antibody was replaced with PBS (Fig. 5e and f).

For the first time, the current study showed the structural details and the distribution of substance P in the digestive tract including the esophagus, the stomach, the small intestine and the large intestine of the 8 days old Siberian tiger. Many folds and thick submucosa were evident in the esophagus of the 8 days old Siberian tiger. The esophagus uses these folds to expand and devour food. The unica muscularis of the esophagus consisted of skeletal muscle which is evident in dogs and ruminants. Abundant glands were distributed in the mucosa of the stomach which is different from dogs, pigs and horses

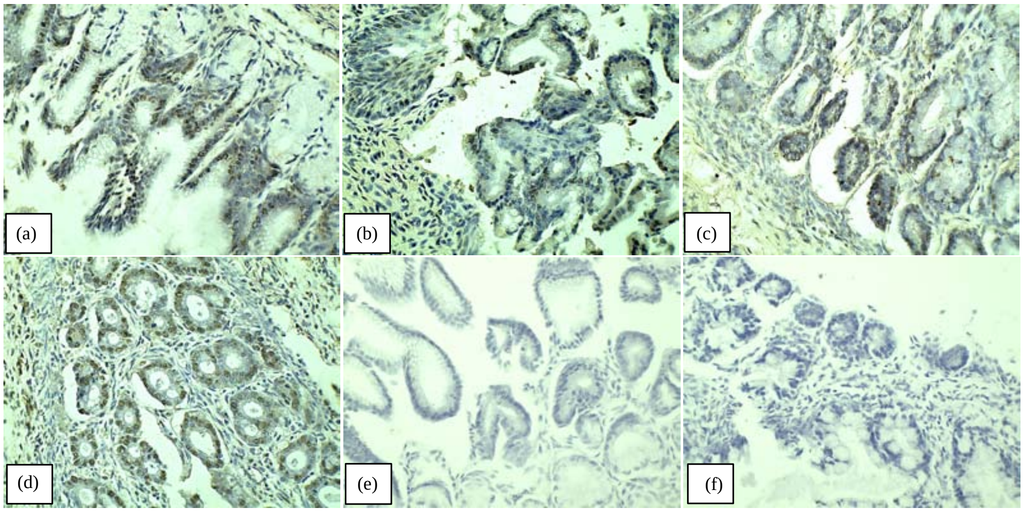

Fig. 5: The distribution of SP in the stomach and the large intestine of the 8 days old Siberian tiger using the PV-9000 method. Images are shown at the magnification x400. a: immunoreactive cells in the mucosal membrane epithelium of the stomach, b: SP-IR cells in the cardiac gland, c: immunoreactive cells in the intestinal gland of the cecum, d: immunoreactive cells in the intestinal gland of the colon, e: the negative control for the mucosal membrane epithelium of the stomach and f: the negative control for the intestinal gland of the cecum

2000). The gastric gland of the Siberian tiger had the same features as that of typical carnivorous animals. The bottom of the gastric gland did not touch the muscular layer of the gastric mucosa which protects the gastric wall from being punctured by pieces of bone. The mean length of the intestinal canal of the Siberian tigers was 5-7 times longer than their body length, 2.77-2.90 times longer than the length of *Felis manul* or 2.5 times longer than the length of the *Felis manul* and ocelots. The structure of the small intestine and cecum of the Siberian tigers was the same as the structure of the *Felis manul* and ocelots. The nutrition was absorbed mainly through the duodenum and the jejunum as indicated by the liberal duodenal villi, the long jejunal villi and the short ileal villi which aid absorption. The duodenum of the Siberian tigers did not have a duodenal gland which was different from those of other animals but similar to that of the ursus arctos. However, further investigation is required. The cecum of the Siberian tigers was conical, stubby and sac-like which was different from those of the *Felis bieti*, the *Felis manul*, the lesser pandas, wolves and pandas that lack a cecum. In the Siberian tigers, the mucosa of the colon was thick. Zhang *et al*. (2009a, b) reported that the tube wall and the mucosa of the colon of the wolf were thicker than that of dogs. These data may correlate with the rate of reabsorption in the large intestine because carnivorous animals in the wild maximize the absorption of water and electrolytes from food which is useful in the event of food shortages. The main reasons for the differences in the Siberian tigers alimentary canal were that they had different feeding habits and ways of feeding. By comparing the relationship between the morphology of alimentary organs and the feeding habits of 19 species of South African rodents, (Perrin and Curtis, 1980) concluded that feeding habits were a primary contributor to the evolutionary differences in the morphology of alimentary organs in different species of rodents.

The gastrointestinal tract is a large and complex endocrine system which secretes many hormones such as SP and plays an important role in regulating SP secretion. SP was identified in the gastric mucosal epithelia, the cardiac glands, the cecum and the colon. These data confirmed the findings that have been reported by (Zhang *et al*., 2009, b) showing that the distribution density of SP was high in the colon of young *Meriones unguiculatus*. The main functions of SP include the excitation of smooth muscles, strengthening the group movement of the colon and the excitation of the mucosa in the colon to secret water and electrolytes to regulate gastrointestinal motility. However, researchers did not identify SP in the small intestine or the rectum of Siberian tigers which was different from the SP pattern in other mammals (Agungpriyono *et al*., 2000; Yamada *et al*., 1999). SP is found in the rectum at 11 weeks and in the colon at 14 weeks in the development of human embryos.

At the same gestational age, the numbers of SP in the rectum are higher than that in the colon (Hong and Liang, 2003; Kitamura *et al*., 1985) has shown that the density of SP is high in the rectum and the duodenum in cows (>1 years old) and calves (<3 months old).

These data indicate that the distribution of SP is species-specific and diverse. The evidence that the distribution of SP in the intestinal tract changed with age is supported by the age-related changes with SP in the human fetus (Lehy and Cristina, 1979), *Meriones unguiculatus* (Zhang *et al*., 2009a) and the rat (Ferrante *et al*., 1991). Therefore, the function of SP in the intestinal tract is related to age. The distribution of SP in young Siberian tigers is related to high metabolic rates and nutritional requirements. The distribution of SP had a close relationship to the digestive function of the intestinal tract in the colon and the rectum based on gestational age. SP may serve as a nutritional factor for immunocytes and help improve and perfect the immune system in the intestinal tract (Hong and Liang, 2003). SP in young Siberian tigers may have similar functions that are discussed earlier.

CONCLUSION

In summary, researchers reported for the first time the histological structures and the distribution of SP in the intestinal tract of male Siberian tigers aged at 8 days old. However, these results were based on histology. Further research is required to understand the physiology at the cellular level.

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