

MORPHOLOGICAL AND HISTOCHEMICAL STUDY OF GUINEA PIG DUODENAL SUBMUCOSAL GLANDS

A. A. MOHAMMADPOUR

Department of Basic Sciences, Faculty of Veterinary Medicine,
Ferdowsi University of Mashhad, Mashhad, Iran

Summary

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The duodenum is largely responsible for the breakdown of food in the small intestine, using enzymes. Duodenal submucosal glands, which in general produce a mucous secretion, exist in all mammalian species. These glands are located in the submucosa of the proximal duodenum. The study aimed to demonstrate the morphological and histochemical properties of duodenum and duodenal submucosal glands in the small intestine of the guinea pig. The duodenum of 10 adult healthy animals constituted the material of the study. After dissecting them, three parts of duodenum (cranial, descending and ascending parts) were determined. For histological studies, after tissue preparation, duodenal tissue layers and duodenal submucosal glands in tunica submucosa were measured using the micrometre method. All parameters between the three parts of duodenum were analysed and compared using the ANOVA test. We concluded that duodenal wall thickness was variable in the three parts. It decreased from the cranial ($1306.81 \pm 132.80 \mu\text{m}$) to the ascending part ($1026.92 \pm 80.01 \mu\text{m}$) and in the cranial part was very distinctive. Duodenal or Brunner's glands were composed of only mucous acini densely packed within the submucosa. The glands were well developed in the cranial part of duodenum. There was a significant difference in the thickness of duodenal submucosal glands in all parts of the duodenum that decreased from the cranial to the ascending part ($P < 0.001$) from $147.52 \pm 22.80 \mu\text{m}$ to $38.25 \pm 12.30 \mu\text{m}$ respectively. Histochemical examination revealed that the mucous glands reacted positively with Alcian blue pH 2.5 and Periodic Acid-Schiff (PAS) stains.

Key words: duodenal submucosal glands, duodenum, guinea pig, histochemistry

INTRODUCTION

Although the duodenum is a tiny fraction of the small intestine, it is the site of most of the breakdown of the food passing through it. The duodenum is lined with duodenal submucosal glands, which secrete an alkaline mucus that supports the intestinal enzymes and aids in the absorption of nutrients. The pancreatic duct, which introduces bile and pancreatic juice into the small intestine, is directly connected to the descending duodenum. Pancreatic juice contains enzymes that help

break down food, while bile aids the digestion and absorption of fats. The duodenum is responsible for secreting hormones that trigger the pancreatic duct to release pancreatic juice and bile. In addition, the mucus secreted by duodenal submucosal glands helps protecting the duodenum from the acidity, making the duodenum much less sensitive than the rest of the small intestine to the acidic chyme. Therefore, the duodenum protects the rest of the small intestine by neutralizing the

chyme to some extent before it passes into the jejunum (Cunningham & Klein, 2007).

The general morphology of the duodenum of some species was examined in former studies. However, in few species the distribution and morphology of the duodenal submucosal glands deviated from the general pattern. In the South American opossum, as has also been reported for *Didelphis virginiana* (Schumacher & Krause, 1995), the duodenal submucosal glands were confined to a very narrow region, immediately distal to the pyloric sphincter. In comparison with the other mammals examined, the secretory units of duodenal submucosal glands in the raccoon were more dilated and lined with flatter cells. Similar observations regarding the luminal size of duodenal submucosal glands in the mouse have been reported (Krause, 1981). The acini of duodenal submucosal glands in the raccoon were relatively dilated in comparison with these of other mammals. In the duodenum, goblet cells stained with Periodic Acid-Schiff (PAS) and Alcian blue at both pH 1.0 and 2.5, while duodenal submucosal glands stained only with PAS. The histology of the gastroduodenal junction of the opossum differed from other mammals. In opossums, duodenal submucosal glands were concentrated in a very short portion of the submucosa, extending from the distal part of the pyloric region of the stomach to the proximal duodenum (Schumacher *et al.*, 2004).

The existence of duodenal submucosal glands in the duodenum is uncontested (Botros *et al.*, 1990; Bloom & Fawcett, 1994; Burkitt *et al.*, 2000). However, there remain doubts as to their exact location along the full extent of the duodenal wall, given that the existing opinions in the specialized literature are often incomplete or difficult to interpret (Coutinho *et al.*,

1996; Gartner & Hiatt, 2003). Various studies dealing with the mucosubstance histochemistry of duodenal submucosal glands, pyloric glands and goblet cells in a large number of mammals show marked inter-species and even within-species variation (Poddar & Jacob, 1979). There are incomplete data about the duodenum and duodenal submucosal glands of guinea pigs.

The aim of this study was to examine the normal appearances of duodenum, to measure the inner diameter of duodenal submucosal glands in the three parts of the duodenum and to compare them each with the other. Also, it aimed to determine the histochemical properties of guinea pig duodenal submucosal glands.

MATERIALS AND METHODS

Animals

Ten healthy adult male guinea pigs (*Cavia porcellus*), long-haired breed, aged 8–10 months, weighing 500–550 g were used for the study. The animals were obtained from the Razi institute in Mashhad, Iran and feed with regular food made of pelleted alfalfa grains. The animals were anaesthetised with sodium pentobarbital and then euthanized by an overdose of the same drug.

Tissue samples

The small intestines, which were removed from the body of the animals by the immersion method following the dissection of the abdominal cavity were examined histochemically. Part of the tissue samples were first fixed in 10% neutral buffered formalin and then subjected to routine tissue processing for light microscopy. The resultant blocks were cut into sections 5 µm thick and stained with haematoxylin

and eosin for general histological examination. Periodic Acid-Schiff (PAS) and Alcian blue (pH 2.5) were used for neutral and for acidic mucosubstance, respectively. In PAS staining technique, the sections were oxidized for 5 min in 1% aqueous periodic acid, washed under running tap water for 5 min, rinsed in distilled water, and then treated with Schiff's reagent for 15 min. Afterwards, the sections were washed with water for 10 min and counterstained with haematoxylin. Finally, the sections were dehydrated in alcohol, cleared in xylene, and mounted in a resinous mountant. In Alcian blue method (pH 2.5), sections were stained in freshly filtered 1% Alcian blue 8 GX in 3% acetic acid (pH 2.5) for 30 min and washed in water. The sections were dried with fine filter paper and were dehydrated in alcohol, cleared in xylene, and mounted in a resinous mountant.

Statistical analysis

For histological studies, after tissue preparation, duodenal tissue layers and duodenal submucosal glands in tunica submucosa were measured using the micrometre method. All parameters between the three parts of the duodenum were analysed and compared using ANOVA test.

RESULTS

In the guinea pig, duodenal submucosal glands are compound, tubuloalveolar, composed only of mucous acini densely packed within the submucosa. The cross-section of each gland comprised six to ten cells around a narrow lumen and the cells had a truncated pyramidal form. In these cells the nucleus was round, located near the basement membrane, and with a distinct nucleolus. The glands were well developed in the cranial part of the duode-

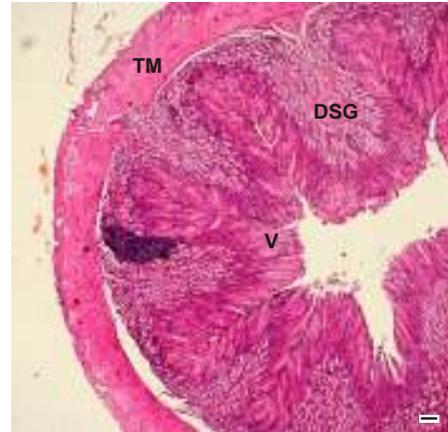


Fig. 1. Optical photomicrograph of the cranial part of duodenal wall. Note the numerous plicae circularis in this part, duodenal submucosal glands (DSG) are numerous, occupy the entire duodenal submucosa and extend near to the base of villi (V). The tunica muscularis (TM) was composed of thick inner and thin outer layers. H&E staining, bar=100 µm.

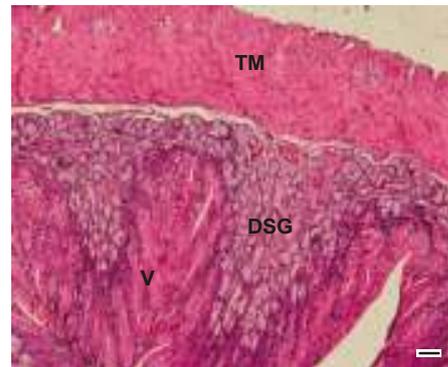


Fig. 2. Optical photomicrograph of the cranial part of duodenal wall. Note the duodenal submucosal glands (DSG) located in tunica submucosa. The glands are only mucous and tubuloalveolar. Tunica muscularis (TM) and Villi (V) are labelled. H&E staining, bar=100 µm.

num (Fig. 1). The cranial part of duodenum presented large quantities of duodenal submucosal glands in all of the fields examined. In this part, intestinal wall was

thicker than descending and ascending parts. Plicae circularis were also well developed and detectable. The descending part also showed the presence of duodenal submucosal glands in the entire field examined, albeit in smaller quantities than in the cranial part (Fig. 2). In the ascending part, the number of duodenal submucosal glands in submucosa was variable and there was a minimal quantity of glands. In some of these sections, duodenal submucosal glands were few and poorly developed. In ascending part plicae circularis were not detected and the intestinal wall was thinner than the other two parts (Fig. 3).

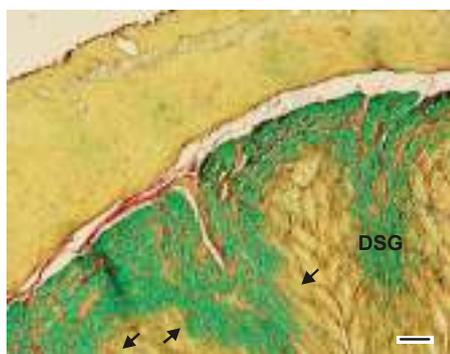


Fig. 3. Optical photomicrograph of the cranial part of duodenum. Secretory unit cells of duodenal submucosal glands (DSG) and goblet cells (arrow) positive in Alcian blue (pH 2.5). Alcian blue staining, bar=100 μ m.

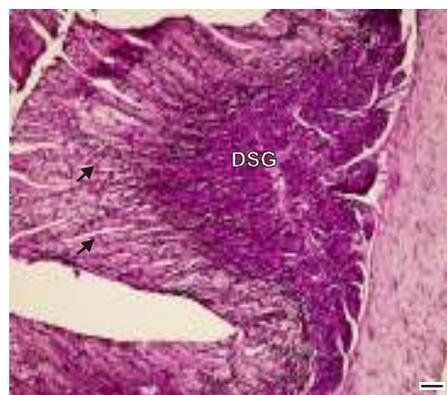


Fig. 4. Optical photomicrograph of the cranial part of duodenum. Secretory unit cells of duodenal submucosal glands (DSG) and goblet cells (arrow) reacted positively with Periodic acid Schiff. Periodic acid Schiff staining, bar=100 μ m.

The inner diameter of duodenal submucosal glands in the three parts of the duodenum was different and larger in the cranial part. There was a significant difference in inner diameters of duodenal submucosal glands in all parts of the duodenum, decreasing from the cranial ($147.00 \pm 22.87 \mu\text{m}$) to the ascending part ($38.00 \pm 12.29 \mu\text{m}$). The thickness of tunica muscularis and total thickness of intestinal wall were reduced from the cranial to the ascending part (Table 1).

Table 1. Histometrical parameters of three parts of duodenum in guinea pig (mean \pm SD; n=10)

Parameters (μ m)	Cranial part	Descending part	Ascending part
Height of villi	785.00 \pm 87.67	850.00 \pm 69.03	828.84 \pm 46.59
Inner diameter of duodenal submucosal gland	147.00 \pm 22.87 ^A	58.00 \pm 14.73 ^B	38.00 \pm 12.29 ^B
Thickness of tunica muscularis	293.18 \pm 37.23 ^A	142.14 \pm 26.07 ^B	105.00 \pm 15.81 ^B
Total thickness of intestinal wall	1306.81 \pm 132.80 ^A	1039.50 \pm 136.06 ^B	1026.92 \pm 80.01 ^B

Different letters within a row indicate difference, significant at $P < 0.001$.



Fig. 5. Optical photomicrograph of the descending part of duodenum. Duodenal submucosal glands (DSG) are present in tunica submucosa; however, their numbers are fewer than in the cranial part of duodenum. Secretory unit cells of DSG and goblet cells (arrow) reacted positively with Alcian blue (pH 2.5). Alcian blue staining, bar=100 μ m.



Fig. 6. Optical photomicrograph of the ascending part of duodenum. Greatly reduced numbers of duodenal submucosal glands (DSG) can be seen in the duodenal submucosa. The glands are only occasionally present in the duodenal submucosa. Secretory unit cells of DSG and goblet cells (arrow) reacted positively with Alcian blue (pH 2.5). Alcian blue staining, bar=100 μ m.

The results of the carbohydrate histochemistry were shown on Fig. 3, 4, 5 and 6. In classic carbohydrate histochemistry,

a positive PAS reaction indicates the presence of neutral carbohydrates, while positive Alcian blue reactions at pH 2.5 indicate the presence of acidic carboxylated residues, respectively. In this research, goblet cells and duodenal submucosal glands were stained with PAS and Alcian blue at pH 2.5.

DISCUSSION

Since the duodenal submucosal glands were discovered by Wepfer in 1679, studies in many animal species were conducted to clarify the extent and the density of their distribution, the types of cells forming the glands by light and electron microscopy. However, there are only few histochemical studies on the composition of the secretion of this gland. In the present study it was elucidated that the duodenal glands in the guinea pig were composed only of mucous cells. The principal function of the glands is thought to be protection of the duodenal mucosa against the erosive effects of the gastric juice by virtue of the mucoid nature of its secretion, its alkalinity, and possibly by the buffering capacity of its bicarbonate content. No enzymatic activity involved in the digestive process has yet been found, although in some species juices collected from the region of duodenal submucosal glands contained a mucolytic enzyme. Secretion of the glands is enhanced by humoral (crude secretion), nervous (parasympathetic), and mechanical stimuli (presence of food or rubbing the mucosal surface). Although the similarities of the duodenal glands of various animals have been emphasized in the past, physiological evidence of significant differences among species were reported in more recent histochemical studies (Farkas *et al.*,

1989; Fuse *et al.*, 1990; Krause, 2000; Morre *et al.*, 2000).

Cells, which compose the duodenal submucosal glands, vary with species. These glands were reported to be composed of two types of cells, serous and mucous cells in rabbits and horses (Oduor-Okelo, 1976; Pfeiffer & Dabareiner, 1992; Takehana *et al.*, 1989), while they were composed of only mucous cells in the moose, elk, bison and white-tailed deer (Krause, 1981; 2000).

Histological results in guinea pigs showed that histological layers and rate of duodenal submucosal glands secretion in the three parts of duodenum were variable. In cranial part of duodenum, the submucosa had numerous spiral folds (the plicae circulares), which increase the surface area for maximum absorption. Also, the number of duodenal submucosal glands was higher than those in the other parts and total wall thickness was higher. We suggested that, in the cranial part of duodenum, the acidity of gastric secretion was higher compared to the other parts, thus, more duodenal submucosal glands are needed to neutralize the acidity of food. In guinea pigs, duodenal submucosal glands were observed in the three parts, but their density decreased from the cranial to the ascending part. In most species, duodenal submucosal glands are distributed in an area starting from the gastrointestinal junction and extending to varying distances in the proximal small intestine (Alogninouwa *et al.*, 1996; Krause, 2000; Takehana *et al.*, 2000; Verdiglione *et al.*, 2002), while in humans they extend almost to the level of the papilla of Vater (Treasure, 1978). In mammalian species and in eutherians, duodenal submucosal glands are located within the first few millimetres of the proximal duodenum, just distal to the pyloric sphincter

(Krause, 2000). In horses, duodenal submucosal glands occupy a very large area and extend approximately 6 m caudal to the pylorus. They are known to exist also in the jejunum of pigs and large herbivores (Verdiglione *et al.*, 2002). In rabbits, the distribution of duodenal submucosal glands was determined to start from the pyloroduodenal junction and to extend near the jejunum. In the pony (Takehana *et al.*, 1989) mucous glands were reported to be present along the duodenum, while serous glands were determined to be located in the upper part of the duodenum within the region extending.

Despite their similar morphological appearance in the H&E sections, the PAS and Alcian blue (pH 1.0 and 2.5) staining properties of duodenal submucosal glands showed marked differences in our study. In bison, deer, voles, and cotton-tailed and domestic rabbits, they contain acidic sulphated and carboxylated mucins, whereas in humans, rhesus and Japanese macaques, cats, raccoons, rats and opossums they contain neutral mucins. This variation could not be attributed to either the order or the diet of the mammals (Schumacher *et al.*, 2004).

The general morphology of the duodenum of guinea pig examined in this study was in accordance with that described for mammals in general. In classic carbohydrate histochemistry, a positive PAS reaction indicates the presence of neutral carbohydrate, while positive Alcian blue reactions at pH 1.0 and 2.5 indicates the presence of acidic sulphated and acidic carboxylated residues, respectively (Spicer & Schulte, 1992). In guinea pigs, histochemistry of duodenal submucosal glands showed that secretory unit cells reacted positively with Periodic acid Schiff and Alcian blue (pH 2.5) stains. These results indicated that the secretion of duo-

denal glands in guinea pigs contained neutral sugar and acid carbohydrate with sialic acid. Various studies dealing with the mucosubstance histochemistry of duodenal submucosal glands in large numbers of mammals show marked species variation and variation even within the species (Poddar & Jacob, 1979). Therefore, depending on species, duodenal submucosal glands in guinea pig secreted neutral and acidic carboxylated mucin. In addition, the results showed that the goblet cells in guinea pig contained acidic carboxylated and neutral mucins. These observations suggest that the mucins secreted by goblet cells have been conserved with regard to their charge during the evolution of mammals (Ota *et al.*, 1998). In studies conducted using different techniques, depending on species, duodenal submucosal glands were reported to contain neutral or acidic mucin glycoproteins or the combination of both types of mucin (Crescenzi *et al.*, 1988; Takehana *et al.*, 1989; 1991a, 2000; Krause, 2000; Verdiglione *et al.*, 2002). Generally, the duodenal glands are believed to protect the duodenal mucosa from the gastric hydrochloric acid.

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Correspondence:

A. Mohammadpour
Department of Basic Sciences,
Faculty of Veterinary Medicine,
Ferdowsi University of Mashhad,
Mashhad, Iran
e-mail: a_mopour@yahoo.com