Localization of Sugar Residues in the Stomach of Three Species of Monkeys (*Tupaia glis*, *Nycticebus cocang* and *Callithrix jacchus*) by Lectin Histochemistry

Localización de los Residuos de Azúcar en el Estómago de Tres Especies de Monos (*Tupaia glis*, *Nycticebus cocang* y *Callithrix jacchus*) por Histoquímica de Lectina

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SUMMARY: The stomach of three species of non-human primates was investigated by lectin histochemistry to clarify the staining affinity and distribution patterns of their sugar residues. All gastric regions, with little differences between the deep and superficial parts of the same region, were rich in N-acetylglucosamine and/or neuraminic acid. Although, the superficial regions of the gastric mucosa were scanty in N-acetylgalactosamine, α-D-glucose and α-D-mannose, the deep parts of the gastric mucosa were rich in these sugars. In conclusion, there is a difference among the mucosubstances of surface and foveolar mucous cells, mucous neck cells, and gastric gland cells. This indicates heterogeneous composition of gastric mucus, or mucus molecules with variations in the degree of glycosylation of their oligosaccharide chains in the different cells which suggest that lectin binding affinity in the gastric mucosa correlated mostly to the degree of cellular differentiation.

KEY WORDS: Histochemistry; Lectins; Non-human primates; Stomach.

INTRODUCTION

Non human primates present a unique animal model for human disease because of their phylogenetic closeness to human, their convenient size, rather than omnivorous nature of diet and their ability to stay healthy in captivity. Previous investigations showed that, the oligosaccharides side chain of the complex carbohydrate in numerous mammalian glycoconjugates terminates with different sugar residues (Kornfed & Kornfed, 1976; Horowitz & Pigman, 1977; Montereuil, 1980). Those of the gastrointestinal tract are composed of four sugars, galactose (Gal), N-acetylgalactosamine (GalNAc), N-acetylglucosamine (GlcNAc) and α-L-Fucose (α-L-Fuc) present in very constant molecular ratio (Allen & Snary, 1972; Clamp *et al.*, 1981 and Forster *et al.*, 1982). These sugars play a key role in the normal functions of these glycoconjugates. Such function includes regulation of protein conformation, protection of protein from proteolytic attack, cell-cell interaction

(Montereuil), and ion transport across the membrane (Vandeheede *et al.*, 1972). These sugars have been histochemically localized in the mucus secreting cells of human (Aoki *et al.*, 1993), carnivorous (Mayer & Tsukise, 1995), camel (Fayed & Makita, 1997), in rats (Tatematsu *et al.*, 1989), in fish (Pedini, *et al.*, 2005) and in experimental animals (Ishihara *et al.*, 1996; Yang *et al.*, 1996). Recent lectin-histochemical investigations have shown that several lectins are able to label gastrointestinal mucus secreting cells selectively besides the distribution and characterization of their glycoconjugates (Watanabe *et al.*, 1981; Fischer *et al.*, 1984a; Madrid *et al.*, 1989). In pursuit of characterization of lectin binding patterns in normal monkey gastric mucosa, we have systematically studied the distribution of the sugar residues and their suggested biology in the monkey gastric mucosa using lectins as a probe and horseradish peroxidase (HRP) as visualant.

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MATERIAL AND METHOD

Animals. The samples were collected from three species of non-human primates, monkeys, obtained from department of Anatomy, united graduated school of Veterinary Medicine, Yamaguchi University, Yamaguchi, Japan. All investigations were completed in the department of Anatomy and Histology, Faculty of Veterinary Medicine, Kafer El-Sheikh University, Egypt. A total of nine animals were used for this experiment. Among these animals, three were of common treeshrew monkeys (*Tupaia glis*), three were of slow loris monkeys (*Nycticebus cocang*) and three of common marmoset monkeys (*Callithrix jacchus*).

Sample collection and tissues preparation. The approval of the protocol for this research project has been previously arranged by professor Dr. Makita, T. (info@afvt.jp) and so the strict animal welfare regulations and the rules of the animal ethics committee in Japan were followed.

The monkeys were sacrificed under deep anaesthesia with an intravenous injection of pentobarbital sodium (50 mg/kg). The samples were rapidly removed and pieces of its tissue were fixed in phosphate buffer saline (PBS) contain 4% paraformaldehyde for 72 hours and thoroughly rinsed in the same buffer. A total of 10 sections of 4mm thickness from each gastric regions for each histochemical procedures were examined. The section were deparafinized in xylene and dehydrated in gradual ethanol series. One section from each gastric regions stained with Gill's hematoxylin (Gill et al., 1974) and eosin (H & E) for general histological assessment. The other 9 sections were subjected to the histochemical staining procedures summarized in Table I. for detection of the binding sites of the sugar residues.

HRP Lectin technique. The processing and staining procedure with the various lectins (Table I) was similar to that described by Schulte & Spicer (1983), Bancroft & Stevenes (1996) and Rhodes & Milton (1998). Briefly, after

hydration the sections were treated with 0.3% hydrogen peroxide (H_2O_2), rinsed in distilled water and washed in 1% bovine serum albumen (BSA) in 0.1M PBS pH 7.4. The sections were incubated for 12 hours at 4 °C in HRP-lectin (Sigma Chemical Co. St. Louis, Mo, USA), dissolved in 0.1M PBS pH 7.4 (contains 0.1 M NaCl, 0.1mM $CaCl_2$, $MgCl_2$, and $MnCl_2$) and then rinsed three times in PBS. The optimal concentration used with each lectin which allowed maximum staining with minimum background was as listed in Table I. Visualization of the sites containing HRP-lectin was obtained by incubating the slides with PBS containing 3', 3' diaminobenzidine tetrahydrochloride (DAB) (25 mg/100 ml and 0.003% H_2O_2) for 10 min, at room temperature. Slides were rinsed in distilled water, dehydrated using gradual ethanol solutions, cleared in xylene and mounted in DPX.

Hapten sugars inhibition. Every lectins was preincubated with the corresponding hapten sugars listed in Table I. The hapten sugar inhibitors were employed from 0.05M to 1M and complete elimination of the staining was obtained at 0.2M.

RESULTS

Histological Observations. Using the light microscope, the results of the current investigation were similar in the three examined species of monkey either in general histological assessment or lectin histochemistry and no significant differences could be noticed between them. In histological sections, the gastric mucosa appeared lined by a simple columnar epithelium that invaginated to form the gastric pits which were continuous with the gastric glands and received their secretory products. The gastric wall contained aggregated lymphoid nodules at the cardiac region distributed as a band-like region along the cardio-esophageal junction (Fig. 1a and b) in the submucosa however; few of them were located in the deep layer of the mucosa, lamina

Table (1): Carbohydrate binding specificity of lectins used in this study.

Taxonomic name	Common name	Abbreviations	Label	Concentration $\mu g/ml$	Major sugar specification	Sugar binding inhibitor
<i>Arachis hypogaea</i>	Peanut	PNA	HRP	100	Gal- β -(1-3)-GalNAc	Gal
<i>Canavalia ensiformis</i>	Jake bean	Con-A	HRP	20	-D-Man, -D-Glc	- methyl-D-Man
<i>Triticum vulgaris</i>	Wheat germ	WGA	HRP	6	(β -(1-4)-D-GlcNAc) ₂ , NeuNAc	NeuNAc

Symbol: Gal=Galactose; Glc=Glucose; GalNAc=N-acetylgalactosamine; GlcNAc= N-acetylglucosamine; Man=Mannose; NeuNAc=N-acetyl neuraminic acid (sialic acid); HRP = horseradish peroxidase

propria in between the gastric glands. The mucosal folds were broader in these regions than where there were no aggregated lymphoid nodules. The cardiac glands characterized by shallow gastric pits and short simple coiled tubular glands extended in the lamina propria that contained little amount of connective tissue filling the space in between the glands. The fundic gland region showed long straight glands with shallow gastric pits. The fundic glands filled

the lamina propria almost completely with very little amount of connective tissue in between them (Fig. 1c and d). Besides the surface mucous secreting cells and the mucous neck cells, the main two cells observed in the fundic glands were the chief and parietal cells. The pyloric glands were similar to cardiac glands except that, their gastric pits were markedly deep and there were no lymphoid nodules any where in this region.

Fig.1. The gastric mucosa at the cardiac and fundic gland regions. A; low power of the gastric wall at the cardiac region showing the mucosa (M), submucosa (SM) containing the lymphoid nodules (L) and the tunica muscularis (TM). Note the broad mucosal fold at the region of lymphoid nodules. B; higher magnification to the same region showing the mucosa (M) and the lymphoid nodules in the submucosa (L). C; higher magnification to the fundic glands showing the shallow gastric pits (GP) and the long straight fundic glands (FG) with its chief cells (C) and parietal cells (P). D: higher magnification to the fundic glands (FG) and its chief cells (C) and parietal cells (P); SM, sub mucosa. H&E stain. Bar = 100 mm in A, B and C.

Histochemical Observations. Marked differences in the staining pattern with lectin conjugates used in the current study could be noticed, not only between the different gastric regions but also among the various microanatomical structures of the same region particularly between the base, middle and upper parts of the gastric glands.

The cardiac gland region showed strong positive reaction to HRP-conjugated *Triticum vulgaris* agglutinine

(HRP/WGA). This positive reaction included the surface mucous cells, the gastric pits and the gastric glands (Fig. 2). At the beginning of the cardiac gland region, antral region, the glands showed positive reaction along their whole length even in the portions surrounded the lymphoid nodules located at this region (Fig. 2c). Later on, while directing backward, the positive reaction disappeared from the base of the glands and restricted to the surface mucous cells and the upper

Fig. 2. Cardio-esophageal junction of monkey stomach stained by HRP/WGA lectin histochemistry. A; is a low power magnification to the junction showing the strong positive reaction to these lectins in the surface mucous cells (S), the gastric pits (arrows) in the cardiac glands; L, lymphoid nodules, SM, submucosa, TM, tunica muscularis, and E, esophageal epithelium. B; higher magnification to the junction, S, surface mucous cells, arrow, gastric pits, L, lymphoid nodules, E, esophageal epithelium. C; is a higher magnification to the deep layer of cardiac glands showing lymphoid nodules (L) may extend to the lamina propria and surrounded by the positively stained cardiac glands. Bar = 100 mm in A, B and C.

Fig. 3. Different regions of the stomach of monkey stained by the HRP/WGA lectin histochemistry. A; Low power to the cardiac gland region (CG) showing the positive reaction in the entire length of the glands; Sm, submucosa. Note also the strong positive reaction in the mucus covering the gastric mucosa. B; Transition zone between the cardiac gland (CG) and the fundic gland (FG). Note here the base of the glands in both regions is negative (arrows). C and D; Higher magnifications to the cardiac gland (CG) and fundic glands (FG) respectively, showing the negative reaction at the bases of the gastric glands (broad arrows). E; Pyloric gland region (PG) showing the positive reaction in some cells of the lower part of the glands only (arrow heads). Bar = 100 mm in A, B, C, D and E.

portions of the cardiac glands, isthmus and body regions (Fig. 3). A similar distribution of HRP/WGA lectin binding sites was seen in the fundic gland region, where the positive reaction was limited to the upper portion of the glands and disappeared from their bases. The opposite pattern of staining was noticed in the pyloric gland region. The surface mucous cells and the gastric pits were negative while some cells in the bodies and the bases of the glands were moderately stained (Fig. 3e). The secreted mucous, covering the gastric mucosa, was strongly positive to HRP/WGA in the cardiac and fundic gland regions and negative in the pyloric gland region.

The results obtained by HRP-conjugated / Concanavalina ensiformis agglutinine (HRP/Con-A) in the cardiac gland and pyloric gland regions were similar. In the both regions, the surface mucous epithelium and the foveolar

epithelium showed negative reaction, while the bodies and the bases of the glands gave a strong positive reaction to HRP/Con-A. In the cardiac gland region, the positive reaction extended to the region surrounding the lymphatic nodules (Fig. 4). Almost similar reaction could be noticed in the fundic gland region, where the surface mucous cells and the foveolar cells were negative, the bases of the glands showed negative reaction too. The body of the fundic gland was the only part with positive reaction with HRP/Con-A.

Similar results could be noticed in the staining affinity of different regions of gastric mucosa in case of HRP-conjugated Peanut agglutinine (HRP/PNA). In the pyloric gland region, strong positive reaction was noticed in some cells at the base of the glands only, while the surface mucus cells, cells lining the foveolae and the body of the pyloric glands showed negative reaction (Fig. 5).

Fig. 4. The HRP/Con-A lectins histochemistry in the cardiac (CG) in a; in fundic (FG) in b; and in pyloric glands (PG) in c. Note that the positive reaction at the cardiac gland region extended to the deep layer of the glands even in the portions surrounded the lymphoid nodules (L). At the fundic gland the positive reaction was limited to the body region while the positive reaction at the pyloric gland region located in the body and base of the glands with no reaction in the surface mucous cells and the gastric pits. Bar = 100 mm.

DISCUSSION

The current study revealed that there was an aggregated lymphoid nodule in the gastric wall especially at the cardiac region. In an agreement with this result Wang (2003) reported lymphoid nodules distributed as a band like region along the ventral wall of the stomach neck of Bactrian camel. These findings indicate that the stomach of monkey possesses not only a special structure of digestion but also has characteristic immunological morphology and these aggregated lymphoid nodules are an important part of the gut-associated lymphoid tissue.

In the stomach, the mucus plays a key role in the defense mechanism of gastric epithelial cells from the corrosive hydrochloric acid and from pepsin of the gastric juice (Ferri & Liquori, 1992). The composition of the mucins of the digestive tract probably depends on their functions. This microheterogeneity of the mucins as revealed by lectin histochemistry can be attributed to the ability of the mucus to

Fig. 5. Pyloric gland region of monkey stomach stained by the HRP/PNA lectins histochemistry at the (PG) showing the negative reaction in the surface mucous cells, in gastric pits and body of the glands. The only positive reactions found in some cells at the bases of the glands (asterisk). Inset; is a higher magnification to the region at the gland bases showing the positive reaction. Bar = 100 mm for the figure and inset.

entrap a wide variety of microorganisms. Despite the carbohydrate microheterogeneity, the mucins of various origins are very similar in their macromolecular properties and polymeric structure (Madrid *et al.*, 1998).

The results of our study have shown regional and cellular differences in carbohydrate components of the gastric mucosa and have demonstrated the selectivity and the reliability of individual lectins. Similar results concerning the different pattern of lectin binding sites have been reported by many authors (Spicer *et al.*, 1978; Stoward *et al.*, 1980; Suzuki *et al.*, 1982; Kuhlmann *et al.*, 1983; Fischer *et al.*, 1983; Fischer *et al.*, 1984a; Fischer *et al.*, 1984b; Madrid *et al.*, 1990; Ferri & Liquori; Rios-Martin *et al.*, 1993; Jiang *et al.*, 2004; Schumacher *et al.*, 2004).

In the current study, the staining affinity of gastric mucosa to the WGA was strong at the cardio-esophageal junction in the gastric surface mucous epithelial, mucous neck cells and cardiac glands. Then after, this affinity was confined to the surface mucous cell and the foveolar mucous cells in the cardiac and fundic glands. In the pyloric gland region this affinity was restricted to the body and base of the glands while it was absent from both the surface mucous cell, and foveolar mucous cells. The mucous covering of the gastric mucosa was positive in both cardiac and fundic gland regions and negative in the pyloric gland region. In this context Ito *et al.* (1985) mentioned that the mucous neck cells produce acidic mucins that bind WGA. Madrid *et al.* (1990) reported that the mucinous content of the cells lining the surface and pit of gastric mucosa as well as mucous neck cells were moderately reactive to WGA. Rios-Martin *et al.* documented that extracellular mucus was negative for WGA. Fischer *et al.* (1984a) mentioned that WGA binds to gastric mucus secreting cells. In contrast to our results, Suzuki *et al.* mentioned that the mucous granules of the pyloric gland cells and mucous neck cells showed an affinity for WGA as well as surface and foveolar mucous cells bind to WGA. The intense reaction of gastric mucosa to WGA suggest the presence of N-acetyl-D-glucosamine (GlcNAc) and N-acetyl neuraminic acid residues (Ishida *et al.*, 1988; Bogoeva *et al.*, 2004). The presence of neuraminic acid in gastric mucosa and mucous is important for entrapping a wide variety of microorganisms; defensive function (Madrid *et al.*, 1998).

Our results indicated that the cardiac gland region showed a positive reaction to the Con-A in the deep portions of the glands even in the portions surrounded the lymphoid nodules, while the surface mucous and foveolar epithelium were negative. The same result could be noticed in the pyloric glands, where the surface mucous epithelium and the foveolar epithelium showed negative reaction, the body and the bases of the glands showed a strong positive reaction. Similarly,

Suzuki *et al.* documented that the mucous granules of the pyloric gland cells and mucous neck cells showed an affinity for Con-A, while surface and foveolar mucous cells do not bind to Con-A. These results clearly show the difference among the mucosubstances of mucous neck cells, pyloric gland cells, and surface and foveolar mucous cells. The mucosubstances of the mucous neck cells and pyloric glands cells contain Con-A binding sugar residues, α-D-glucos and α-D-mannos, whereas the mucosubstances of the surface and foveolar mucous cells contain little or none. This suggested that the gastric mucosubstances contain either small amount of mannose or none.

In an accordance Ito *et al.* and Madrid *et al.* (1990), mentioned that the mucous neck cells produce acidic mucins that bind mostly with Con-A. In the same time, Ito *et al.* reported that in human gastric mucosa, the secretory granules of the surface mucous cells showed negative reaction with Con-A. In addition, Fischer *et al.* (1984a), documented that the mucus in the surface mucous cells of gastric mucosa was characterized by the absence of Con-A binding sites, while all cell types lying under the proliferation zone are intensely stained. Similar results could be observed in camel stomach (Fayed & Makita).

We reported here that the gastric mucosa showed various patterns of staining with the PNA. The positive reaction was limited to some cells at the bases of the pyloric glands with negative reaction in the surface mucous cells and in foveolar mucous cells. In contrast, Ito *et al.* reported that in human gastric mucosa, the secretory granules of the superficial mucous cells reveal specific binding with PNA and the mucous neck cells produce acidic mucins that bind PNA. Kuhlmann *et al.* mentioned that large numbers of surface mucous cell, neck and cardiac gland cells stained with PNA. Moreover, Rios-Martin *et al.* documented that labeling with PNA revealed a biphasic pattern (peripheral positively) on mucous droplets in surface and foveolar cells.

In an accord, Fischer *et al.* (1984a) mentioned that the mucus in the surface epithelial cells of gastric mucosa was characterized by the absence of PNA reaction. They added, mucous neck cells and some antral gland showed a high density of PNA lectin binding, while those of deep foveolar and isthmus cells showed moderate reaction to PNA. In addition, Stoward *et al.* found that cardiac glands and mucous neck cells, in the deep but not the superficial portion, of gastric glands stained strongly with PNA. According to McCartney (1986), binding of PNA to surface mucous cells of gastric mucosa occurred in non-secretors but not secretors. The absence of PNA binding sites from the gastric surface epithelium suggested that it is apparently possessed neither terminal galactose residues nor galactose-sialic acid dimmers (Fischer *et al.*, 1984a).

The different binding of labeled lectins to the individual cell types of the normal mucosa suggest differences in glycoprotein composition of the: a) surface epithelial cells showing various degrees of differentiation; b) cell types lying above and below the generative cell zone; c) specific membrane systems of the parietal and chief cells (Fischer *et al.*, 1983). Moreover, the variation in lectin binding sites between the different cell types is an indicative to a reduced glycosylation in mucous neck cells and other glandular epithelial cells in comparison with surface mucous cells (Fischer *et al.*, 1984a).

In conclusion our findings suggest that there is a difference among the mucosubstances of surface and foveolar mucous cells, mucous neck cells, and gastric gland

cells, i.e. heterogeneous composition of gastric mucus, or mucus molecules with variations in the degree of glycosylation of their oligosaccharide chains in the different cells. Besides, lectin binding patterns in gastric mucosa are related, most likely, to the degree of cellular differentiation.

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RESUMEN: El estómago de tres especies de primates no humanos fue investigado por histoquímica de lectinas para determinar la afinidad de tinción y los patrones de distribución de sus residuos de azúcar. Todas las regiones gástricas, con pequeñas diferencias entre las partes profundas y superficiales de la misma región, eran ricas en N-acetilglucosamina y/o ácido neuramínico. Si bien, las regiones superficiales de la mucosa gástrica eran escasas en N-acetilgalactosamina, a-D-glucosa y a-D-manosa, las partes profundas de la mucosa gástrica eran ricas en estos azúcares. En conclusión, existe una diferencia entre las mucosustancias de la superficie y células mucosas foveolares, células mucosas del cuello y células de las glándulas gástricas. Esto indica una composición heterogénea de la mucosa gástrica, o moléculas de moco con variaciones en el grado de glicosilación de sus cadenas de oligosacáridos en las diferentes células, sugiriendo que la afinidad de unión de lectinas en la mucosa gástrica se relaciona principalmente con el grado de diferenciación celular.

PALABRAS CLAVE: Histoquímica; Lectinas; Primates no humanos; Estómago.

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