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Original article

Histochemical detection of glycoconjugates in the parotid salivary gland of Malayan pangolin (*Manis Javanica*)

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Abstract Study revealed the distribution of glycoconjugates in parotid salivary gland of malayan pangolin *(Manis javanica)* was detected by means of lectins histochemical and enzyme neuraminidase digestion staining methods. The results indicated that tubuloacinar gland and secretory endpieces were characterized by the presence of seromucous cells. Mucous cells showed the great number of acid and vicinal - diol groups and acid with neutral GCs, whereas indicated a few or even absence in serous cells. After digestion with neuraminidase and staining with AB pH2.5, the presence of sialic acid residues.The results of lectins staining, the presence of mannose, N - acetylgalactosamine, Galactosyl (β1 $\longrightarrow$4) N - acetylglucosamine, fucose and N - acetylglucosamine residues in serous cells, whereas mucous cells showed the presence of N - acetylgalactosamine and N - acetylglucosamine residues. After neuraminidase digestion revealed the presence of terminal sialic acid linked to Galactosyl (β1 $\longrightarrow$3) N - acetylgalactosamine in mucous cells. Many GCs found in parotid salivary gland function for protection cells from invasion of pathogenic organisms in oral cavity. **Chiang Mai Veterinary Journal 2010;8(1): 17 - 24**

Keywords: parotid salivary gland, glycoconjugates, histochemical, Malayan pangolin

Introduction

Foot and mouth disease (FMD) is a highly contagious The malayan pangolin *(Manis javanica)* or scaly anteaters are strikingly unique creatures. The digestive system of *Manis javanica* shows peculiar structure because of the insect eating adaption habit. They have extremely long tongues, which covered with a sticky saliva secreted from the large salivary glands [(1)]. The sense of smell of *Manis javanica* is good at the location of termite and ant nests [(2)]. The parotid salivary glands of other mammals is mainly serous cells, secrete a number of proteins, fluid and electrolytes important in glandular secretion, digestion proteins and hydration of mucins. Generally, glycoconjugates appear to play important roles in many functions of salivary

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glands, including the defense against chemical and mechanical damages, as well as microbial invasion in the oral cavity [3,4].

The morphology and histochemistry of parotid salivary glands in mammals has been the object of numerous reports [5,6,7]. However, there has been no report available on the lectin histochemistry of the glycoconjugates in the parotid salivary gland of *Manis javanica*. The present study aims to reveal the distribution of complex carbohydrates in the parotid salivary glands of *Manis javanica* by using conventional, lectins histochemical staining and enzyme neuraminidase digestion methods.

Materials and Methods

Two adult female *Manis javanica*, weighing between 2500 - 3000 g, were obtained from Khao Prathup Chang Wildlife Breeding Research Station, Ratchaburi Province, Thailand. The animals were deeply anesthetized with sodium pentobarbital (20 mg/kg) to overdose by abdomen injection and under' gone operation for collecting tissue of parotid salivary gland as quickly as possible. After sample collection, the specimens were fixed for gross anatomical study. The parotid salivary gland were fixed in buffered formalin, dehydrated, cleared and embedded. Blocks were cut at 3 - 4 µm, mounted on albumin coated slides and stained with AB pH 2.5, PAS, AB pH 2.5 - PAS. Lectins applied were Con A, DBA, PNA, RCA - I, SBA, UEA - I and WGA staining, sometimes in combination with neuraminidase enzyme treatment.

Enzyme digestion

Enzymatic digestion with neuraminidase (from *Vibrio cholerae*) was performed prior to stain with AB pH 2.5 and lectin PNA. Sections were incubated in 0.1 M sodium acetate buffer (pH 5.5) containing 1 unit/ml of the enzyme and 0.04 M $CaCl_2$ at 39 - 41 C° for 12 - 16 h [8]. The control specimens for enzyme digestion were also done by exposing to neuraminidase free buffer under the same experimental conditions.

The sections were observed and photographed under light microscope (LM). This is study a test of eyesight observation and staining of positive sites was assessed subjectively by numbers indicate staining from 0, unstained to 4, very strong.

Results

The conventional and lectins histochemical staining methods demonstrated positive reactions of various intensities for different glycoconjugates in the secretory endpieces of the parotid salivary gland of malayan pangolins (*Manis javanica*). The main findings of this work were summarized in Table 1. Our study through light microscopy (LM), the stain with H&E showed tubuloacinar gland and secretory endpieces was characterized by the presence of a large number

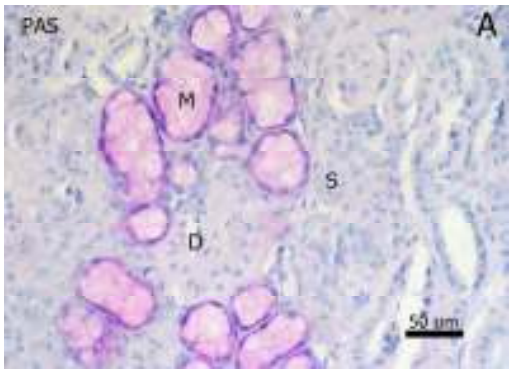
of serous cells and a few mucous cells (seromucous cells).

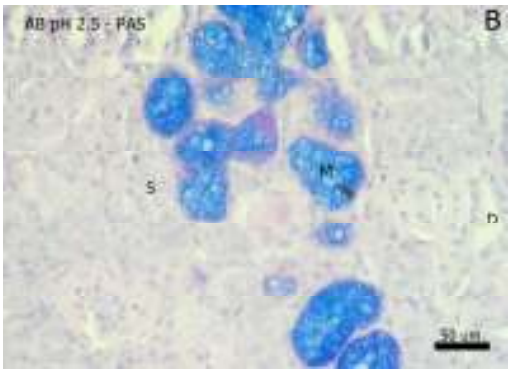
The histochemical results (Figure 1), the serous cells showed a weak positive stain to PAS, while mucous cells showed strong stain (A). The majority of serous cells showed pale positive stain to AB pH 2.5 - PAS, while mucous cells showed intensive stain (B). The mucous cells showed strong staining with the AB pH 2.5 but serous cells did not stain (C). After treatment with neuraminidase and staining by AB pH 2.5, both mucous cells and serous cells exhibited weak positive reaction (D). The serous cells disclosed very strong reaction with lectins Con A, whereas mucous cells showed pale staining (E). The serous cells showed weak to moderate staining with lectins RCA - I, SBA and UEA - I (F). In contrast, the mucous cells were unreactive. The lectins DBA strongly made the serous cells, whereas the mucous cells were moderately stained (G). The lectins WGA moderately stained the serous cells and strongly stained the mucous cells (H). The lectins PNA strongly marked the serous cells, whereas the mucous cells showed negative staining (I). After treatment with neuraminidase and staining by lectins PNA, mucous cells showed strong stain and serous cells showed moderate reaction (J).

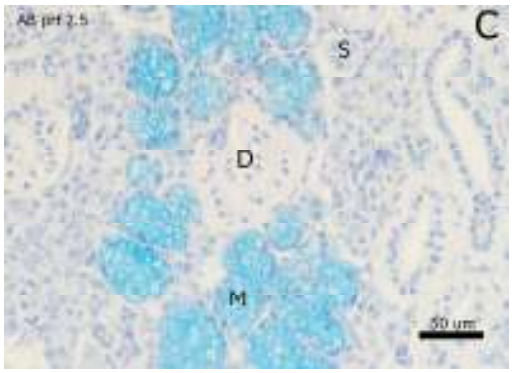
Table 1. A Summary of the results from the conventional and lectin histochemical tests to demonstrate the parotid salivary gland of Malayan pangolin (*Manis javanica*)

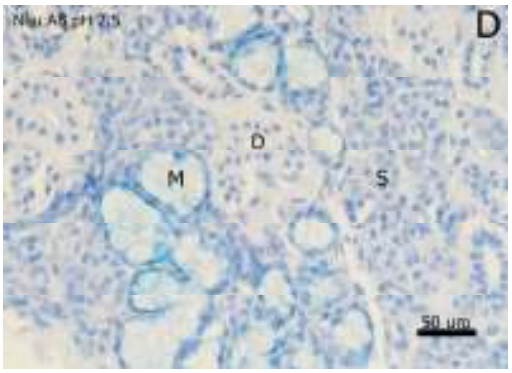
Staining procedures	Acronym	Specificity	Color of positive reaction	
			Serous cells (S)	Mucous cells (M)
Alcian Blue pH 2.5	AB pH 2.5	acidic GCs	0	4
Neuraminidase AB pH 2.5	Neu AB pH2.5	sialic acid	0	1
Periodic acid - Schiff	PAS	vicinal - diol group GCs	1	2
AB pH 2.5 - PAS	AB pH 2.5 - PAS	acidic and neutral GCs	1	4
Concanavalin A	Con A	mannose	4	1
Dolichos biflorus agglutinin	DBA	N - acetylgalactosamine	4	2
Ricinus communis agglutinin-I	RCA - I	Galactosyl (β1 $\rightarrow$4) N-acetylglucosamine	1 - 2	0
Soy bean agglutinin	SBA	N - acetylgalactosamine	2	0
Ulex europaeus agglutinin-I	UEA - I	fucose	2	0
Wheat germ agglutinin	WGA	N - acetylglucosamine	2	3
Peanut agglutinin	PNA	Galactosyl (β1$\rightarrow$3) N - acetylgalactosamine	3	0
Neuraminidase PNA	Neu PNA	sialic acid	2	3

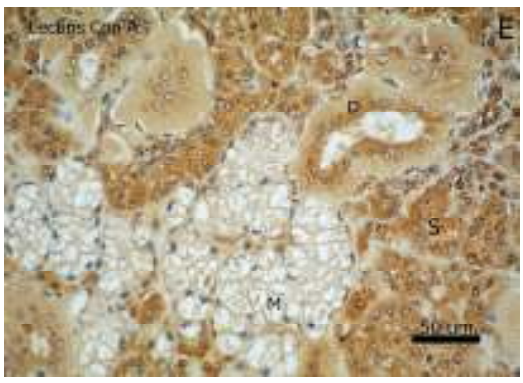
Note : 0 = unstained, 1 = weak, 2 = moderate, 3 = strong, 4 = very strong, Neu = Neuraminidase
GCs = Glycoconjugates, S = Serous cells, M = Mucous cells, D = Striated ducts

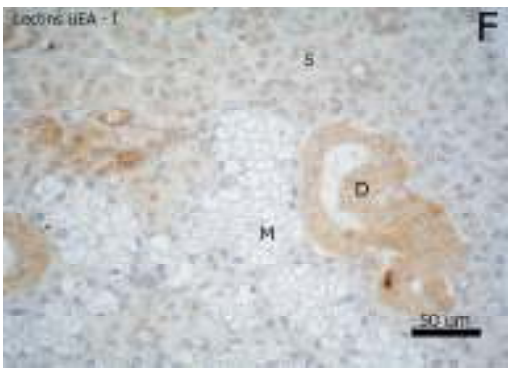

PAS
A
M
S
D
50 um

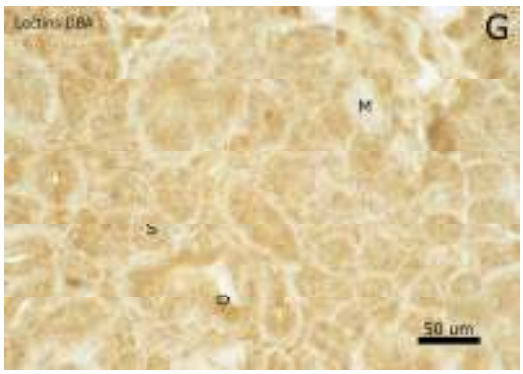

AB pH 2.5 - PAS
B
S
M
D
50 um

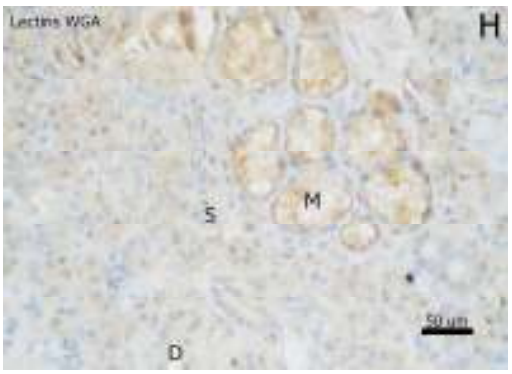

AB pH 2.5
C
S
D
M
50 um


Neu AB pH 2.5
D
D
M
S
50 um


Lectins Con A
E
D
S
M
50 um


Lectins UEA - I
F
S
D
M
50 um


Lectins DBA
G
M
S
D
50 um


Lectins WGA
H
M
S
D
50 um

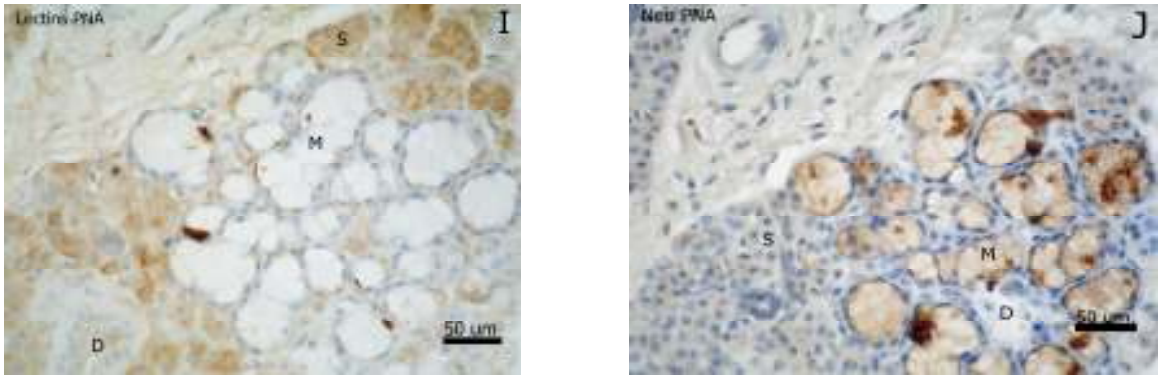


Figure 1. Photomicrographs of the parotid salivaly gland of malayan pangolin (*Manis javanica*). PAS staining (A), AB pH 2.5 - PAS staining (B), AB pH 2.5 staining (C), Neu AB pH 2.5 staining (D), Lectins Con A (E), Lectins UEA - I (F), Lectins DBA (G), Lectins WGA (H), Lectins PNA (I), Neu PNA (J).

Discussion

The malayan pangolins (*Manis javanica*) is an insectivorous mammals with peculiar structures of digestive system. Since there is no teeth in oral cavity of *Manis javanica*, there are a lot of salivary glands and sticky saliva in a rod - shaped tongue. The parotid salivary gland of *Manis javanica*, is located at the backside of the ear, while other mammals it is in front of the ear. The lymph node is located between parotid salivary gland and mandibular salivary gland. The parotid salivary gland of *Manis javanica* consists of mainly serous cells and a few of mucous cells [9], similar to these of goat, cat [5] and vampire bat [10] .

The analysis of the histochemical results in *Manis javanica* obtained with AB pH 2.5, PAS, AB pH 2.5 - PAS staining in the present work, the mucous cells indicate a rich supply of acid and vicinal diol groups with acid and neutral glycoconjugates, respectively. In contrast, the serous cells a lesser or absence acid and vicinal diol groups, similar to those of Hoary bamboo rat [7] with acid and neutral glycoconjugates. The significance of acid glycoconjugates in secretory endpieces, prevent damage to the gut epithelium [11] and neutral glycoconjugates could be transport of macromolecules through the membranes[12]. In addition, the acid glycoconjugates are thought to contain terminal sialic acid residues since AB pH 2.5 reaction decreased in intensity after treatment with neuraminidase[13]. The glycoconjugates distribution in secretory endpieces of parotid salivary gland (*Manis javanica*) has been studied using different labeled lectins. Because lectins can recognize specific carbohydrate residues in the cells [14].

In this study, lectins Con A, DBA, RCA - I, SBA, UEA - I and WGA staining is positive in serous cells, showed a large number of mannose, similar results were found in fallow - deer [6] and lesser mouse deer [15] N - acetylgalactosamine, similar to those of fallow - deer [6] and Hoary Bamboo Rats [7], Galactosyl ($\beta 1 \rightarrow 4$) N - acetylglucosamine, fucose and N - acetylglucosamine residues, respectively. On the contrary, the negative results in mucous cells after lectins Con A, RCA - I and UEA - I staining, whereas positive result in mucous cells staining with lectins Con A, DBA and WGA, indicating the presence of mannose, N - acetylgalactosamine and N -acetylglucosamine residues. Lectin histochemistry revealed differences in production of glycoconjugates in secretory endpieces. The abundance of serous cells secretes mainly of glycoconjugates. In addition to their role in initiation of infection, such as the function in protection against infectious agents in the oral cavity [16]. In the present digestion with neuraminidase study, the mucous cells showed increase staining with lectins PNA demonstrating the presence of terminal sialic acid linked to Galactosyl ($\beta 1 \rightarrow 3$) N - acetylgalactosamine in mucous cells, while the serous cells (both controls and digestion neuraminidase) showed the presence of Galactosyl ($\beta 1 \rightarrow 3$) N - acetylgalactosamine residues.

In our study, the parotid salivary gland has shown the different of glycoconjugates in secretory endpieces of malayan pangolins (*Manis javanica*). Interestingly, the mucous cells of parotid salivary gland involved a large amount of sialic acid residues. Such residues are believed to coat the mucosal surface so as to provide and environment designed to preserve hydration[17]. Thus, the salivary mucins may be of primary importance in defense against chemical and mechanical damage and microbial invasion in the oral cavity [3,4]. In addition, they may be involved in the processes specific for the oral cavity such as mastication and food bolus formation.

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