Kasetsart J. (Nat. Sci.) 41 : 186 - 191 (2007)

Histology and Glycoconjugates Histochemistry in the Small and Large Intestinal Epithelium of the Malayan Pangolin, *Manis javanica*

Apinun Suprasert*, Maleewan Liumsiricharoen, Pakawadee Pongket, Teerasak Prapong, Apantree Doungern and Nathanan Prompa

ABSTRACT

Histology and glycoconjugates histochemistry in the small and large intestinal epithelium of the Malayan Pangolin (*Manis javanica*) were studied by means of histological and histochemical techniques. The staining procedures employed were Hematoxylin-Eosin (H&E), Alcian Blue (AB) pH 2.5, Periodic Acid-Schiff (PAS), AB pH 2.5-PAS and lectin including penut agglutinin (PNA), *Ulex europeus* agglutinin-I (UEA-I), wheat germ agglutinin (WGA) and *Maackia amurensis* (MAL-II).

The intestinal canal displayed no divivsion between small and large intestine, other than a continuous enlargement, and there was no cecum. The mucous membrane of the pangolin small intestine was thrown into high villi and long crypt of Lieberkuhn. While, the mocosa of large intestine lied in a series of irregular fold, not villi. The mucosa of both intestine was covered by simple columnar epithelium in which 2 type of cells were encountered columnar and goblet cells. The mucous granule of goblet cells in small intestine were found to contain primarily neutral glycoconjugates with galactose and sialic acid residues. On the contrary, the mucous epithelium of large bowel involved predominantly acid sulfated glycoconjugates with N-acetylglucosamine, fucose and sialic acid residues. Furthermore, the nature of glycoconjugates in mucous epithelium of both intestine was found to change during the upward migration from deep to superficial part.

Key words: Epithelium, glycoconjugates histochemistry, histology, intestine, pangolin

INTRODUCTION

Malayan pangolin, *Manis javanica*, (Figure 1) is a unique and interesting mammal found in tropical rain forest of Asia. Their digestive system show peculiar structures as adaptation to their toothlessness in oral cavity and insect eating habit. In addition, theirs intestinal tract is characterized by a short intestine where no division between small and large bowel.

A series of previous histological and biochemical studies of mammalian gastrointestinal tract has revealed that glycoconjugates is one of the most important key substance for physiological function of digestive tract. Such glycoconjugates has been shown to be biochemically and heterogenous entity being composed of high molecular weight molecule. Previous histochemical studies have revealed that the secretory glycoconjugates of the intestinal tract were varied

Department of Anatomy, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand.
* Corresponding author, e-mail : fvetais@ku.ac.th

Received date : 02/08/06 Accepted date : 22/01/07

in property from species to species, in addition to regional variations within a particular species (Sheahan and Jervis, 1967; Freeman *et al*., 1980). However, no informations were available as to histology and glycoconjugates histochemistry in the intestinal epithelium of the Malayan pangolin.

The present study was designed to clarify histology and glycoconjugates components in the small and large intestine of Malayan pangolin. The results obtained here were though to involve hitherto unknown aspects of the histology and histochemical architecture of the pangolin intestinal epithelium and to give a better understanding on the functional activities performed by this animal intestinal tract in general.

MATERIALS AND METHODS

From the donor animals, mid part of small and large intestine were dissected out under anesthesia and then fixed by immersion with 10% formalin containing 2% calcium acetate (Leppi, 1968). The tissues were dehydrated in graded ethanol series and embedded in paraffin wax. On rotary microtome, sections were cut at a thickness of 3 μm, deparaffinized in xylene, hydrated in graded ethanol and then subjected to stain with conventional and lectin staining procedures.

Conventional staining procedures

1. Haematoxylin and eosin (H&E) for the general observation of histological structure.
2. Periodic Acid-Schiff (PAS) for vicinal diol containing glycoconjugates (Pearse, 1968).
3. Alcian Blue (AB) pH 2.5 for acidic glycoconjugates (Spicer *et al*., 1967).
4. AB pH 2.5-PAS for demonstrating of acidic and neutral glycoconjugates (Spicer *et al*., 1967).
5. High Iron Diamine (HID) for sulfated glycoconjugates (Spicer, 1965).

Lectin staining procedures

1 Peanut agglutinin (PNA) for detection galactose residues of glycoconjugates (Stoward, 1980).

2 Wheat germ agglutinin (WGA) for detection of N-acetyl glucosamine of glycoconjugates (Goldstein and Hayes, 1978).

3 Ulex europeus agglutinin I (UEA-I) for detection of fucose residues of glycoconjugates (Goldstein and Hayes, 1978).

4 *Maackia amurensis* MAL-II for detection of sialic acid residues of glycoconjugates (Martin *et al*., 2002).

Control staining for lectin

Lectin containing a particular sugar was performed as control staining procedures. In order to detect the activity of endogenous peroxidase in tissue, some control tissue sections were reacted with diaminobenzidine (DAB) only.

RESULTS

The intestinal canal displayed no division between small and large intestine, other than a continuous enlargement and there was no cecum (Figure 2). The mucous membrane of the pangolin small intestine was thrown into high villi and long crypt of lieberkuhn. While the mucosa of large intestine lied in a series of irregular fold, not villi. The mucosa of both intestine was cover by simple columnar epithelium in which 2 types of cells were encountered columnar and goblet cell. Submucosa composed of loose and dense irregular connective tissue containing blood vessels, lymphoid tissue and nerves. The external muscular layer consisted of inner circular and outer longitudinal.

In the small intestine of the pangolin, the goblet cells and striated border of columnar cells were PAS-positive and were stained bright red (Figure 3). They were all stained weakly blue with AB pH 2.5. In the combined AB pH 2.5-PAS staining procedures, most of the goblet cells in

mucous epithelium of pangolin small intestine were deep blue (Figure 4) and some other were reddish-purple. When stained with the HID, goblet cells and striated border of columnar cells turned weakly to moderately black. All conventional staining result of the goblet cells revealed that the intensity of stain was increasing from crypt part to superficial part.

In mucous epithelium of large intestine, the mucous granules of goblet cell and striated border of columnar cell exhibited positive reaction with AB pH 2.5 (Figure 5), PAS, AB pH 2.5-PAS (Figure 6) and HID. Even mucous epithelium of large and small intestine presented the same result, though large intestine showed more intensity than small intestine.

With regard to the binding patterns, nearly all crypt and surface goblet cells displayed a positive reaction with the applied lectins. The binding affinity tend to increase from small to large intestine in case of UEA-I (Figure 7), WGA and MAL-II (Figure 8).This trend was most prominent with UEA-I . However, this was not true for PNA in which the staining intensity was the same both in small and large intestine.

All the results obtained from

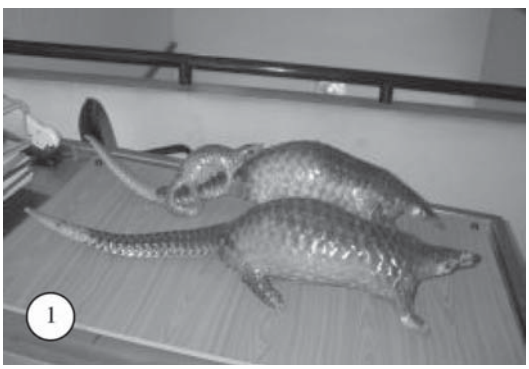

Figure 1 The Malayan pangolin, *Manis javanica,*. Stuff specimens.

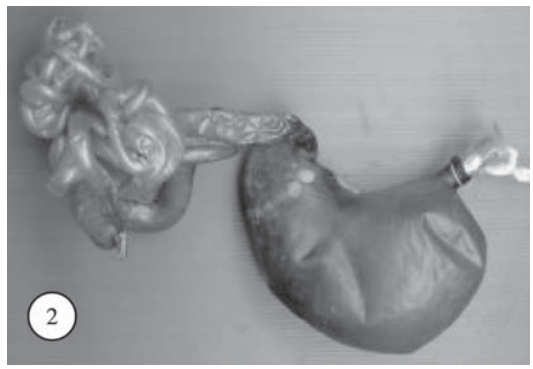

Figure 2 Stomach, small and large intentine of the pangolin. Air dried specimen.

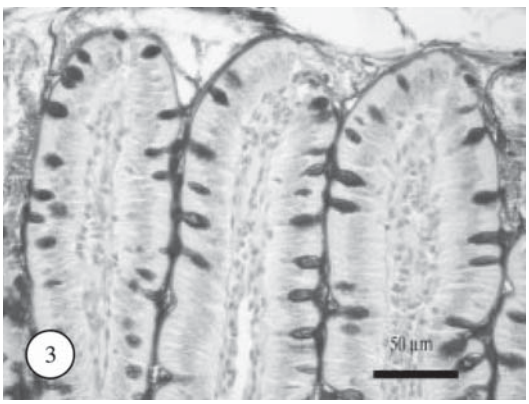

Figure 3 The mucous granules of goblet cells and striated border of columnar cells in the small intestine were strongly reactive. PAS staining.

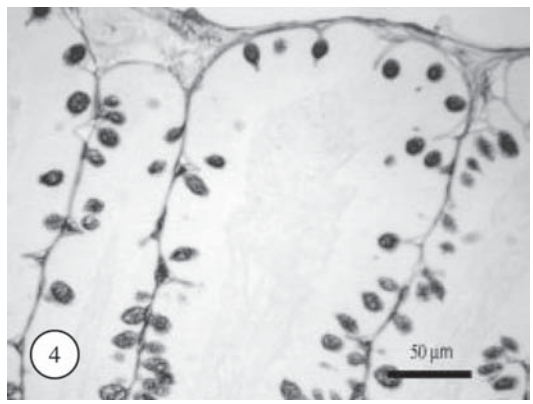

Figure 4 AB pH 2.5-PAS staining in the mucous epithelium of small intestine.

conventional and lectin staining procedures were summarized in Table 1.

DISCUSSION

In agreement with some previous studies on animal and human gastrointestinal epithelium (Sheahan and Jervis, 1976; Freeman *et al*., 1980; Boland *et al*., 1982; William and Linda, 2000), our investigation revealed characteristic differences in the binding pattern of some conventional staining of normal pangolin intestine. The small intestinal epithelium of the pangolin contained predominantly with neutral glycoconjugates. In contrast, the large intestinal epithelium involved predominantly with acid

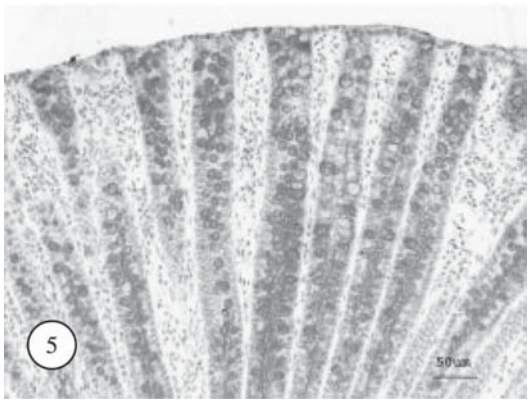

Figure 5 The mucous granules of goblet cells in large intestine exhibited moderately positive reaction. AB pH 2.5 – Hematoxyline staining.

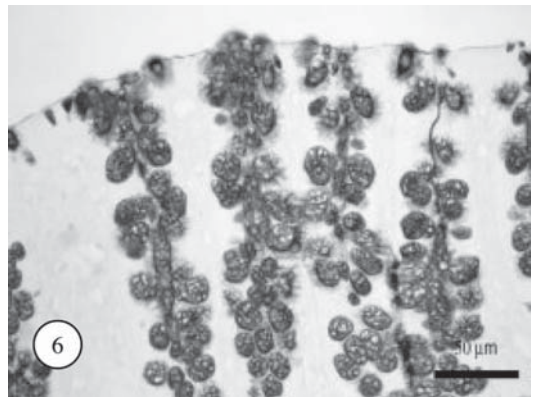

Figure 6 In the mucous epithelium of large intestine, the dual staining with AB pH 2.5-PAS resulted in deep blue coloration at mucous granule of goblet cells.

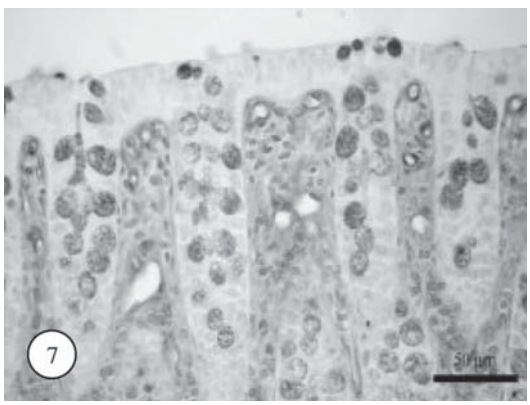

Figure 7 The mucous epithelium of large intestine were strongly reaction. The difference in staining intensity between upper goblet cells at the surface and lower goblet cell at the crypt were clearly seen. UAE-I staining.

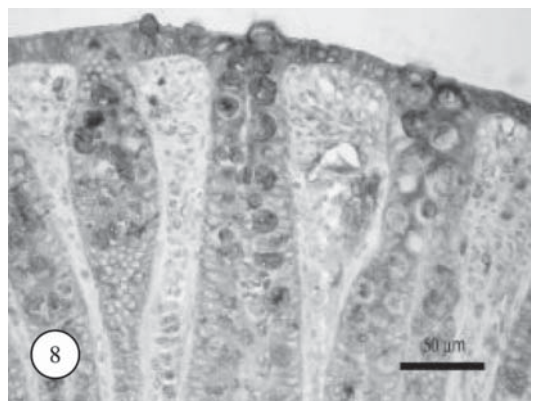

Figure 8 In large intestine, the surface goblet cells exhibited strong reaction, whereas crypt goblet cells showed a weak or moderate reaction. MAL-II staining.

sulfated glycoconjugates. In view of staining specificities of AB pH 2.5, PAS, AB pH 2.5-PAS and HID (Spicer, 1965; Spicer *et al*., 1967; Pearse, 1968) the present results can be comphrehened consistently in mucous granule of goblet cell and striated border of columnar cell of small and large intestine of pangolin, glycoconjugates were provided with acid sulfated and vicinal diol groupings. Such acid sulfated glycoconjugates in the superficial part appeared to be higher in acidity as compared with those in the crypt. The acid sulfated glycoconjugates in large intestinal epithelium also higher than in small intestinal epithelium as judged from intensity of such conventional staining technique.

Change in quality and quantity of glycoconjugates between small and large intestine might be depend on physiological and environmental factors in both intestinal parts. In addition, change in histochemical activity of goblet cell mucous granules during upward migration along the crypt in both intestine suggest that the surface epithelial cells are constantly exposed to stimuli, subjected to a continuous mucous production and a rapid cellular renewal to protect themselves against various harmful substances (Fox, 1979).

Glycoconjugates histochemistry, in general allows demonstration of hexose with periodate reactive vicinal diol groups, and carboxyl and sulfate group with selective affinities for various cationic reagents as judged from routine studies with Alcian Blue and Periodic Acid-Schiff. A new approach in the study of secretory glycoconjugates can be achieved by the use of lectins as histochemical markers of specific saccharides residues. If the staining mechanism underlying the positive staining reaction of UEA-I, WGA, PNA and MAL-II in all mucous granule of goblet cell and striated border of columnar cell were taken into consideration (Golstein and Hayes, 1978). It can be concluded in this study that carbohydrates in the mucous epithelium are glycoconjugates with fucose, galactose, N-acetylglucosamine and sialic acid residues. The hexose residues of glycoconjugates in large intestinal epithelium are also higher than in small intestinal epithelium as judged from the result of intensity of such lectin staining technique.

The widespread occurrence of sialic acid in gastrointestinal tract of the pangolin indicated that a variety of biological functions can be associated with this sugar. As for mucins, they form a continuous lining on the inside surface of hollow organs, thereby, virtue of their viscous and elastic properties; they function as protective

Table 1 Histochemical staining in goblet cells of small and large intestinal epithelium of the pangolin.

Staining method	Small intestine		Large intestine	
	Crypt goblet cells	Surface goblet cell	Crypt goblet cells	Surface goblet cell
ABpH 2.5	1B	1-2B	2-3B	3B
PAS	1M	2-3M	3M	3-4M
ABpH2.5-PAS	1-2MB	2-3MB	2-3BM	3-4BM
HID	1Bl	2Bl	2-3Bl	3Bl
UEA-I	1Br	1Br	2Br	2-3Br
WGA	1Br	1-2Br	2Br	3Br
PNA	1-2Br	2Br	1Br	1-2Br
MAL-II	1Br	1-2Br	1-2Br	2-3Br

Abbreviation

B = Blue M = Magenta Bl = Black Br = Brown

1-4 = Number indicates intensity of staining reaction

agents and lubricants. In addition, as integral components of the plasma membrane of gastrointestinal epithelium they are involved in transport, secretion and other metabolic processes (Werner *et al*., 1982; Martin *et al*., 2002)

LITERATURE CITED

Boland, C. R., C. K. Montgomery and Y. S. Kim. 1982. Alterations in human colonic mucin occurring with cellular differentiation and malignant transformation. **Proc. Natl. Acad. Sci**. U.S.A. Washington 79: 2051-2055.

Freeman H. J, R. Lotan and Y. S. Kim.1980. Application of lectins for detection of goblet cells glycoconjugate differences in proximal and distal colon of the rat. **Lab. Invest**. 40: 405-4123.

Fox, R. A. 1979. Membrane glycoproteins shed in defence of the cells of the gastrointestinal tract. **Med. Hypoth.** 5: 669-68.

Goldstein, I. J. and C. E. Hayes. 1978. The Lectins : Carbohydrate-binding proteins of plant and animal. **Adv. Carbohydr. Chem. Biochem**. 35: 127-340

Leppi, T. J. 1968. Morphochemical analysis of mucous cell in the skin and slime Gland of hagfishes. **Histochemie**. 15: 68-78.

Martin L. T., J. D. Marth, A. Varki and N. M. Varki. 2002. Genitically altered mice with different sialyltransferase deficiencies show tissue-specific alterations in sialylation and sialic acid 9-0-acetylation. **J. Biol. Chem**. 277(36): 32930-32938.

Pearse, A. G. E. 1968. The periodic acid-Schiff technique, pp. 659-660. *In* **Histochemistry, theoretical and applied**, 13 rd ed. Edinburgh, London, New York : J & A Churchill.

Sheahan D. G. and H. R. Jervis. 1976. Comparative histochemistry of gastrointestinal mucosubstances. **Am. J. Anat**. 146: 103-132.

Spicer, S. S. 1965. Diamine method for differentiating mucosubstances histochemically. **J. Histochem. Cytochem**. 13: 211-234.

Spicer, S. S., R. G. Horn and T. J. Leppi 1967. Histochemistry of connective tissue mucopolysaccharides, pp. 251-303. *In* B.M. Wagner and D.E. Smith (eds.). **The Connective Tissue**. Baltimore: William & Wilkins.

Stoward, P. S., S. S. Spicer and R. T. Miller. 1980. Histochemical reactivity penut Lectin-horseradish peroxidase conjugate. **J. Histochem. Cytochem**. 28: 979-990.

Werner, R., K. Eckart, B. Christian and G. Wolfgang. 1982. Biological significance of sialic acid, pp. 263-305. *In* R. Schauer (eds.). **Sialic acid : Chemistry, Metabolism and Function, Cell Biology Monographs**. 10th ed. Springer Verlag, Wien, New York, USA.

William J. B. and M.B. Linda. 2000. **Color atlas of Veterinary Histology**. 2nd ed. Lippincott. William & Wilkin. USA. 121 p