Research Paper

Histochemical Study of Intestinal Mucins in Adult Male New Zealand Rabbit

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ABSTRACT

Introduction: The rabbit is an important laboratory species for various experimental studies in the gastrointestinal tract. A detailed knowledge of the histological and chemical structure of the rabbit small intestine is important for identifying pathogens and applying appropriate treatment methods. This study aims to investigate the mucin secreted by goblet cells and Brunner glands in different parts of the rabbit small intestine.

Methods: Five adult male New Zealand rabbits were used for this study. Different parts of the small intestine, including the duodenum, jejunum, and ileum, were collected according to a systematic, uniform random procedure. Samples were processed, embedded in paraffin, and cut into 5-µm sections. Sections were stained for histochemical examination with periodic acid-schiff (PAS) and Alcian blue (AB) (pH 1.0 and 2.5) and PAS-AB (pH 2.5) and aldehyde-fuchsin (AF-AB) (pH 2.5) staining techniques.

Results: The results showed that the goblet cells of the small intestine responded positively to PAS staining, whereas no positive response was observed in the secretory units of Brunner's glands. The secretory units of goblet cells and Brunner's glands responded positively to AB at pH 1 and 2.5, representing sulfated and carboxylic acid mucin, respectively. PAS-AB Staining with (pH 2.5) showed that in the goblet cells of the small intestine, the content of neutral mucin was higher than acidic mucin, while the secretory units of Brunner's glands contained only acidic mucin. In the AF-AB (pH 2.5) staining of the goblet cells, the content of sulfated mucin was higher than that of carboxylate mucin. In Brunner's glands, the amount of carboxylate mucin was higher than that of sulfate mucin.

Conclusion: In this study, the histochemical mucin characteristics of rabbit small intestine are described in detail. The results of the present study reveal similarities and differences with other mammals.

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1. Introduction

The small intestine is a part of the digestive system located between the stomach and the large intestine, where most food digestion and absorption occur [1]. The small intestine has three sections: The duodenum, jejunum, and ileum. Histologically, the small intestine consists of a mucosal, submucosal, muscular, and serosal layer [2]. Histochemistry is a combination of biochemical and histological techniques used to study the structure and chemical properties of cells and tissues [3]. Mucins are high molecular weight and highly glycosylated (glycoconjugated) proteins produced by epithelial tissues of most animal species. Mucins perform functions such as lubrication, cell signaling, and chemical barrier formation [4]. Mucin is one of the major glycoproteins that make up mucus. This molecule, composed partly of various sugars and partly of proteins, is composed of repeating units containing proline, threonine, and serine [5]. Mucin is secreted by goblet cells, leukocytes, and cells of the digestive tract and plays a role in regulating ion transport and, in other cases, a role as a receptor [6].

Goblet cells are found in the cells of epithelial tissue that play a role in mucin secretion [7]. Goblet cells are found on the surface of both villi and crypts [8]. They have a nucleus at the base and cytoplasm at the tip, which is filled with mucus and acts like a unicellular gland [9]. The mucus secreted by these cells is an acidic glycoprotein that makes the surface of the cells slippery and has a protective function [10].

Goblet cells mainly use a merocrine secretion method and secrete mucinogen granules into the lumen, but they can also use apocrine methods [11].

Brunner's glands (duodenal glands) are tubular submucosal glands found in the duodenum [12]. The main function of these glands is to produce an alkaline secretion rich in mucus (bicarbonate-containing mucus) to protect the duodenum from the acidic contents entering the duodenum from the stomach, and also to provide alkaline conditions for the activity of intestinal enzymes [13].

The morphology of the intestine among different species reflects dietary habits, absorption, and digestive function and, therefore, varies from species to species [14]. Mucus and mucin play an important role in the small intestine to prevent contact and invasion of bacteria and pathogens [15]. The mucus barrier is one of the most important factors in protecting the gastrointestinal tract, so its role has been studied for decades. However, the mechanism of intestinal protection still needs to be better understood [6].

Therefore, histochemical studies of the small intestinal mucin have been performed in various animal species, such as humans [16], pigs [17], rats [18], mice [19], and Persian squirrels [20]. Detailed histochemical studies of goblet cells and Brunner's gland mucus have not been thoroughly performed in small intestines of rabbits, but there are studies for identifying acidic and neutral mucin in some parts of the intestine, such as the duodenum [21].

The rabbits are an important laboratory species for various experimental research in the field of digestive systems [22]. On the other hand, this species is also important from a veterinary point of view because various diseases such as intestinal obstruction, intestinal ulcers, dysautonomia, bacterial and viral infections, parasites, and toxins can affect the intestine [23].

Accurate knowledge of the tissue and chemical structure of the intestine is important to identify pathogens and apply appropriate treatment methods. Therefore, in this study, the histochemical profile of rabbit small intestinal mucus is investigated using the periodic acid-schiff (PAS) and Alcian blue (AB) techniques (pH 1.0 and 2.5), as well as PAS-AB (pH 2.5) and aldehyde-fuchsin- AB (pH 2.5).

2. Methods

Five adult male New Zealand rabbits were used for this study. The animals were purchased from the Pasteur Institute of Iran. They were fed by standard food and water ad libitum. The animals were euthanized with chloroform. The abdominal cavity was dissected, the small intestine was separated, and its various parts, including the duodenum, jejunum, and ileum, were removed. Each part of the intestine along the antimesenteric border was cut, opened, fixed on balsa wood, and immersed in a 4% buffer formalin solution. Then, according to the method of systematic random selection, 8-10 pieces for each part (duodenum, jejunum, and ileum) were selected and immersed in 4% buffer formalin for further fixation. After routine tissue processing, the specimens were embedded in paraffin, and tissue sections with a thickness of 5 µm were prepared using the microtome.

Mucin secreted by goblet cells and Brunner's glands in the small intestine of rabbits was analyzed by a series of histochemical tests. Periodic acid-schiff (PAS) was used to detect neutral mucin [21]. Sulfate and carboxylic acid

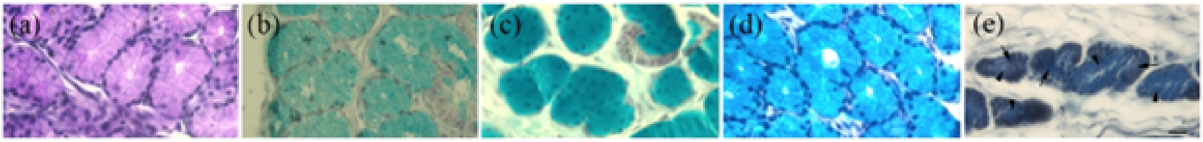

Figure 1. Histochemical characteristics of goblet cells in the small intestine of New Zealand rabbits

a) Periodic acid-Schiff staining of goblet cells, b) Alcian blue staining (pH 1) of duodenal goblet cells, c) Alcian blue staining (pH 2.5) of jejunal goblet cells, d) Periodic acid schiff alcian blue staining (pH 2.5) of jejunal goblet cells, e) Duodenal goblet cells stained with aldehyde fuchsin alcian blue (pH 2.5) (scale bar 40 µm)

Note: Many goblet cells in the small intestine are stained reddish purple, indicating that the secreted mucins are of mixed type and the content of neutral mucin is higher than that of acidic mucin (arrowhead), and a small number of cells are stained bluish purple, which is the expression of mixed mucin in which the content of acid mucin is higher than that of neutral (arrow).

mucins were detected with the stain AB (pH 1 and 2.5) [24]. In addition, the combination of PAS and AB (pH 2.5) was used to detect acidic and neutral mucin [25]. Furthermore, AF-AB (pH 2.5) was performed to detect the type of sulfated or carboxylated mucin [26].

3. Results

The characteristics of the mucins secreted by the mucosal secretions of rabbit small intestine are summarized in Table 1. Goblet cells reacted positively with PAS and showed a red color due to the presence of neutral mucins (Figure 1a), and the highest intensity of the reaction was observed in the duodenum (Table 1), while no positive reaction was observed in Brunner's glands (Figure 2a).

The secretory units of goblet cells and Brunner's glands showed a positive reaction with AB at pH 1 and 2.5, representing sulfated mucin and carboxylic acid mucin, respectively (Figure 1b, 1c and Figure 2b, c). The highest intensity of goblet cell reaction with AB (pH 1) was observed in the ileum and with AB (pH 2.5) in the jejunum (Table 1).

PAS-AB (pH 2.5) to compare the content of acidic and neutral mucin showed that many goblet cells in the small intestine were stained reddish purple, indicating mixed-type mucin secretion, in which the content of neutral mucin is higher than that of acidic mucin, and that a small number of cells were bluish-purple in colour, indicating mixed-type mucin in which the content of acidic mucin is higher than that of neutral (Figure 1d). The jejunum showed a stronger response to PAS-AB (pH 2.5) than other parts of the small intestine (Table 1), although the secretory units of Brunner's glands were seen only in blue colour and contained acidic mucin (Figure 2d).

The study of the reaction of the secretory units of the small intestine with AF-AB (pH 2.5), performed to compare the content of carboxylic acid and sulfated mucin, showed that in the goblet cells, the content of sulfated mucin (purple colour) was higher than the content of carboxylmucin (blue colour) (Figure 1e). Different parts of the small intestine showed the same reaction with AF-AB (pH 2.5) (Table 1). In the secretory units of Brunner's glands, the content of carboxylated mucin was higher than that of sulfated mucin (Figure 2e).

4. Discussion

The general characteristics of the histochemical profile of rabbit small intestinal mucus in this study are consistent with those described for other mammals, with some differences.

Mucin secretion from rabbit small intestinal goblet cells contained neutral mucin and carboxylic and sulphatic acid mucins. The results obtained in this study were similar to histochemical studies performed in 13 mammalian species, including rats, voles, guinea pigs,

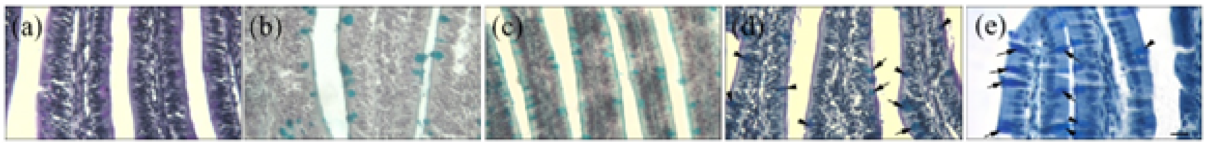

Figure 2. Histochemical characteristics of mucin-secreting cells in Brunner's glands of New Zealand rabbits

a) Periodic acid-Schiff staining showing no positive reaction in the secretory units of Brunner's glands, b) Alcian blue staining (pH 1) showing the positive reaction of the secretory units of Brunner's glands, c) Alcian blue staining (pH 2.5) in the secretory units of Brunner's glands, d) Periodic acid Schiff-Alcian blue staining (pH 2.5), e) Aldehyde fuchsin alcian blue stain (pH 2.5) showing carboxylmucin (arrowhead) and sulfatmucin (arrow) (scale bar 40 µm)

Note: The secretory units of Brunner's glands are stained blue only and contain acidic mucin.

Table 1. Histochemical characteristics of the mucin-secreting units (goblet cells and Brunner' glands) in the small intestine of New Zealand rabbits

Staining	Goblet Cells			Brunner's Glands
	Duodenum	Jejunum	Ileum	
PAS	+++	+	++	-
AB (pH 1)	+	+	++	+
AB (pH 2.5)	++	+++	+	++
PAS-AB (pH 2.5)	++	+++	++	+++
AF-AB (pH 2.5)	++	++	++	++

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Abbreviations: PAS: Periodic acid-Schiff; AB: Alcian blue, AF: Aldehyde fuchsin.
-Negative staining, +Weak staining, ++Moderate staining, +++Intense staining.

and rabbits [21]. Moreover, similar results were observed in the goblet cells of the small intestine of Persian squirrel [20] and the duodenum of mice [27]. These observations suggest that the mucins secreted by goblet cells have been conserved with respect to their charge during mammalian evolution [28].

This study showed that Brunner's glands are composed of mucous and serous cells. Brunner's glands in horses consist of mucous and serous cells [29]. In Persian squirrel [20], moose, elk, bison, and white-tailed deer [30], they consist of mucous cells only. Brunner's glands are located in the rabbit's small intestine at the beginning of the duodenum, consistent with most other mammals [31]. Interestingly, in horses, these glands are located in the jejunum region [32].

This study showed that Brunner's glands secrete carboxylic acid mucin and sulfate acid mucin in rabbits. In bison, deer, and guinea pigs, these glands also contain sulfated and carboxylic acid mucins [33]. Brunner's glands produce acidic and neutral mucin in mice [27] and Persian squirrels [20]. In humans, rhesus and Japanese macaques, cats, raccoons, rats, and opossums, Brunner's glands contain neutral mucin [21].

In cattle, the Brunner' glands of the small intestine contain neutral and acidic mucins that appear as lobules, indicating specific digestive processes in ruminants [34]. In horses, the Brunner's glands secrete acidic mucin. The acidic mucin secreted in the equine small intestine may play an important role in cellulose metabolism or in the digestion of bacterial microflora from the stomach of herbivores [35]. The results show that the Brunner' glands of 27 bat species that fed differently produced only neutral mucins [36]. It was also found that the histochemical profiles of the different species could not be assigned to one order or another [21]. The protective role of these glands has been demonstrated in several studies [21, 24], and perhaps the location of Brunner's glands in the initial region of the duodenum in rabbits suggests the presence of a damaging factor to the epithelium in this region.

5. Conclusion

Considering that the rabbit is important as a laboratory animal for various studies on the gastrointestinal tract and is also kept as a domestic animal, the mucin secreted by the goblet cells and Brunner's glands in different parts of the small intestine of this species was studied. The results showed that the goblet cells of the small intestine secrete neutral and acidic mucin, while the Brunner's glands are involved in acidic secretion.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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