Short Communication

Developmental changes in distribution of the mucous gel layer in rat small intestine

Y Iiboshi[1] MD, PhD, R Nezu[2] MD, J Khan[1] MBBS, H Yoshida[1] MD, K Sando[1] MD, PhD, M Fukuzawa[1] MD, PhD, S Kamata[1] MD, PhD, Y Takagi[1] MD, PhD and A Okada[1] MD, PhD

Department of Pediatric Surgery[1] and First Department of Surgery[2], Osaka University Medical School, Osaka, Japan

The intraluminal mucous gel layer across the small intestine of rats aged 3 days, 1, 2, 4 and 6 weeks (n=10x5) was studied to investigate its postnatal development. Celloidin stabilisation of dried cryostat sections of small intestine, with the luminal contents, preserved the intraluminal mucous gel layer for staining by the periodic acid-Schiff reaction. Morphological differences in the mucous gel, between the villi of the small intestine, in rats of several postnatal ages were observed, most notably after the age of 2 weeks. The adhesive mucous gel layer, covering the intestinal epithelium in the small intestine, appears to undergo rapid development after weaning.

Key words: Small intestine, jejunum, ileum, development, mucous gel layer, unstirred layer, absorption, intestinal barrier, macromolecules, bacteria, intestinal closure

Introduction

The mucous gel layer covering the intestinal epithelium seems to play an important role in absorption and as a barrier[1-4]. First, the mucus is thought to contribute to the intestinal unstirred layer that controls the absorption or permeability of various substances[5-12]. Second, mucin, the main component of mucus, maintains the mucosal surface pH under 7[1], which is important in the absorption of dipeptides[13] and in the defence against bacteria[2,3]. The gradient of H^+ is utilized for the transport of dipeptides through the apical membrane of the intestinal epithelial cell. Moreover, the low surface pH seems to prevent bacterial overgrowth. But little is known about the intraluminal distribution of mucus in the small intestine *in vivo*, or its development, although the age-related changes in chemical composition and physical properties of mucus glycoproteins have been reported[14].

In this study, we examined the morphological changes in the intraluminal mucous gel layer across the small intestine in rats at several postnatal ages to understand how this layer might alter the absorption and barrier functions of the developing small intestine. We stabilised mucus in dried cryostat sections with celloidin.

Materials and Methods

Young male Sprague-Dawley rats (Keari Co, Osaka, Japan), bred under standard conditions in our laboratories, and aged 3 days, 1 week, 2, 4, 6 weeks (n=10x5) were used for these experiments. Before sacrifice, the 3-day-, 1,and 2-week-old rats were separated from their mothers for 2 hours and the 4 and 6-week-old rats (separated from their mothers at 3 weeks) were deprived of food for 2 hours. Under inhalation anesthesia with diethyl ether, segments of jejunum and ileum including their luminal contents were ligated and excised. Samples were tied to wooden sticks, powdered with talcum, and frozen in liquid nitrogen. The specimens were mounted in Tissue-Tek (Miles, Elkhart, IN) and sectioned in a cryostat (Sakura Co, Tokyo, Japan) at -25°C into 10 μm cross-sections across the lumen. Sections were placed on poly-L-lysine-coated slides (Muto Co, Osaka, Japan). Small intestinal sections were dried at room temperature (21° to 22°C) and placed in 0.2% celloid in solution for 3 minutes to preserve the mucous gel. Subsequently, the slides were air dried again for 5 to 10 minutes and hardened in 80% alcohol for 3 minutes. Sections were then fixed in 10% formalin, rinsed twice in distilled water, and stained by the periodic acid-Schiff reaction (PAS) for light microscopy. Sections were placed in 0.5% periodic acid for 5 minutes, rinsed twice in distilled water, and placed in Schiff solution for 15 minutes. Subsequently, sections were placed three times in sulfurous acid for 3 minutes each, rinsed with water for 3 minutes, and placed in hematoxylin solution for 3 minutes. Sections were then dehydrated in alcohol, covered with Eukitt (O Kindler GmbH & Co, Freiburg, Germany), and topped with a coverslip.

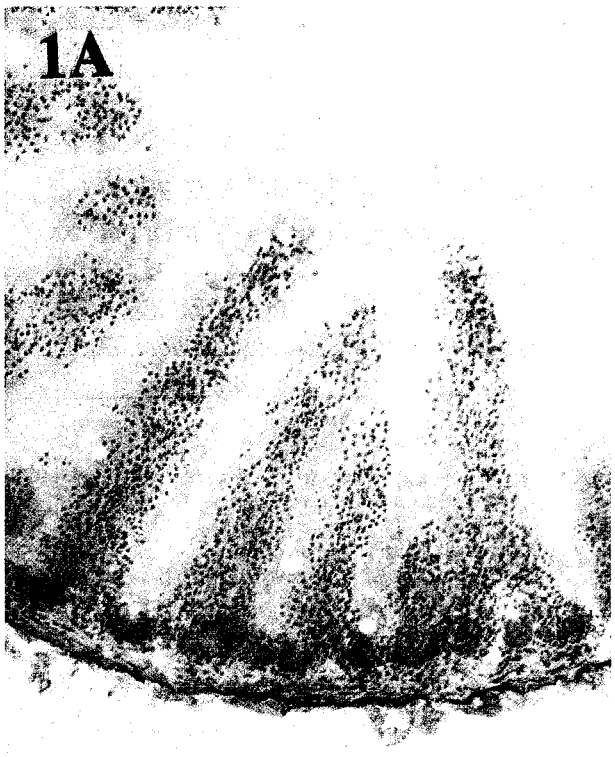
Results

In 3-day- and 1-week-old rats, there was little PAS-positive mucous gel observed between the villi in any section of jejunum and ileum. Intraluminal mucous gel in the jejunum or ileum of 1-week-old rats is shown in Figure 1A and 2A, respectively. In 2- to 6-week-old rats, the spaces between villi were filled with PAS-positive mucous gel in every section of jejunum and ileum.

Intraluminal mucous gel in the jejunum and ileum of 2-week-old rats is shown in Figure 1B and 2B, respectively. In 4- and 6-week-old rats, almost all surfaces of the villi were covered with mucous gel in both jejunum and ileum. The intraluminal mucous gel in the jejunum and ileum of 6-week-old rats is shown in Figure 1C and 2C, respectively. Additionally, an age-related development of crypts was observed in the jejunum and ileum.

Correspondence address: Yasuhiko Iiboshi, MD, PhD, Department of Paediatric Surgery, Osaka University Medical School,2-2 Yamadaoka, Suita, Osaka 565, Japan.
Tel: 06-879-3754; Fax: 06-879-3759

Figure 1. Intraluminal mucous gel in rat jejunum. (A) 1 week old. (B) 2 weeks old. (C) 6 weeks old. Periodic acid-Schiff (PAS), magnification x25. The major changes were an age-related increase in the PAS positive mucous gel layer between villi and an age-related development of crypt.



Discussion

In the present study, little PAS-positive material was observed between villi in the 3-day- or 1-week-old animals, whereas the spaces between villi were filled with PAS-positive material in the 2- to 6-week-old animals. These findings confirm the presence of PAS-positive mucin which is considered the main component of mucus. Qualitatively, the gradual development of the mucous gel layer from a thin diaphanous layer in newborn animals to a very thick, coherent structure in older animals along the entire small intestine was recognized. It is suggested that, especially after weaning, the properties of mucus allow it to form an adhesive gel layer[15] and to cover almost the entire surface of the villi to play an important role in absorption and as a barrier. Indeed, mucin in the newborn rat contains more protein and less carbohydrate than that in the adult rat and differs in buoyant density and mobility on electrophoresis[14]. But, the relation between these phenomena and the functions of mucin contributing to absorption and barrier function are inadequately understood.

The transmission of macromolecules from the intestinal contents to the systemic circulation occurs in many mammalian species, especially during the neonatal period[16]. This transmission ceases or is severely reduced at different developmental stages for different species and is referred to as *intestinal closure*. In rats, this intestinal closure occurs after weaning and is almost coincides with the development of the mucous gel layer. So, it is possible that the development of the mucous gel layer might contribute to intestinal closure.

The epithelial surface of the small intestine is covered with an unstirred water layer in mammalian species[5-12]. It is reported that the unstirred layer thickness of about 1/2 mm determined *in vivo* exceeds the thickness measured *in vitro*: 150 to 200 μm[17]. This phenomenon can be explained by the presence of mucus gel covering intestinal epithelium, because reactive mucus release might occur in the perfused rat small intestine. We have shown that the reactive mucus release changes the permeability of small intestine under irritative conditions like perfusion[18].

Figure 2. Intraluminal mucous gel in rat ileum. (A) 1 week old. (B) 2 weeks old. (C) 6 weeks old. Periodic acid-Schiff (PAS), magnification x25. The major changes were an age-related increase in the PAS positive mucous gel layer between villi and an age-related development of crypt.

The effective surface area of the diffusion barrier in the intestine has also been shown to correct appropriately the unstirred layer resistance which leads to low permeability coefficients for passive transport process and high Km values for active transport processes[6]. Using three separate experimental and mathematical approaches, this surface area was found to vary from 1.02 cm^2 to 14.24 cm^2 per 100 mg dry weight of rat intestine. These values are very much lower than the 1226 cm^2 and 696 cm^2 per 100 mg area of the microvillus membrane in the jejunum and ileum, respectively. The presence of a mucous gel layer covering the intestinal epithelium of rats after weaning in the present study supports these results. This is because, through the presence of the mucous gel layer, the effective surface area of the diffusion barrier in the intestine is the cylindrical surface area at the tips of villi and the minimum cylindrical surface area overlying tips of villi would explain the results. But, in rats before weaning, the condition of unstirred layer is likely to be different from rats after weaning; it should be examined in younger populations in the future.

Passive permeability of the small intestine has been summarised by Cooper[19]. If no specific carrier-mechanism exists, the principal factors determining whether a molecule will permeate the small intestine are lipid solubility and molecular size. If the molecule is not soluble in lipid, the critical factor is molecular size expressed by weight, radius or, most importantly, volume. Nimmerfall and Rosenthaler [20] point out the importance of mucin, which is secreted by goblet cells, as the decisive luminal barrier to the passage of a compound through the gut wall. In their study, the absorption of compounds of different chemical structure was directly proportional to their diffusion through isolated goblet cell mucin and inversely proportional to their retention in mucin and, with some reservation, also to molecular weight. In regard to molecular weight, the feature of passive permeability of the small intestine can be explained by the presence of a mucus gel layer covering the intestinal epithelium as found in the present study. We have also shown that total parenteral nutrition decreases luminal mucous gel and increases permeability of the small intestine[4]. So diseases related to increased permeability of macro-

molecules seem to be attributed to abnormality in the mucous gel layer covering the intestinal epithelium. The significance of increased permeability of macromolecules is almost completely unknown. However, it is conceivable that a more permeable small intestine would allow absorption of antigens, toxins, carcinogens and other compounds which are normally excluded. Their absorption could have etiological and pathogenetic implications for many intestinal and non-intestinal disorders, for example, atopic eczema, 'food' allergy, necrotising enterocolitis, other immunological and atopic disorders, malignant disease, Crohn's disease, celiac disease and the extra-intestinal manifestations of intestinal disorders. In celiac disease, increased intestinal permeability could be important in the etiology of intestinal damage, the associated malignant diseases, and the atopic and immunological disorders, including dermatitis herpetiformis, which are common among celiac patients.

The surface pH of intestinal epithelium is usually maintained between 5.5 and 6.0 (microclimate pH)[21], although the pH of the lumen is maintained at almost 7.0. The existence of the mucous gel layer[22] and brush-border Na+/H+ exchange[1] are thought to be important for the maintenance of microclimate pH. The gradient of H^+ is utilized in the transport of dipeptides that are the products of digestion of proteins through the apical membrane of the intestinal epithelial cell. The low surface pH seems to prevent bacteria from overgrowing. The development of the mucous gel layer after weaning can protect intestinal epithelium against exposure to many kinds of bacteria in food.

Since Davis[23] demonstrated that freezing could preserve luminal structures in gastrointestinal specimens, including the fragile microenvironment, freezing has been used in the method to evaluate the mucous gel layer covering the intestinal mucosa[24-26]. Celloidin stabilization of the mucous gel in cryostat sections was developed for the preservation of the preepithelial mucous gel of the colon for histochemical examination by Szentkuti and Eggers[27] and has advantages over other methods: there is no need for mucus-specific antiserum for immunostabilization[26], and shrinkage of cells in the mucosa is minimized compared with water-substituted and formaldehyde vapor-fixed sections[24]. This method enabled us to observe the distribution of mucous gel on air-dried cryostat sections across segments of the rat small intestine.

We observed morphological differences in the intraluminal mucous gel layer of the small intestine in rats of different postnatal ages. This was a simple descriptive study without a functional corollary. But developmentally-oriented morphologic study of the mucous gel layer of the small intestine is a necessary prelude to the study of the mucous gel layer's contribution to intestinal absorption and to host defense. The functional contribution of the mucous gel layer to absorption and intestinal barrier functions remains to be determined.

Acknowledgments

This work was supported by a Grant-in-aid for Scientific Research (05671490) from the Ministry of Education and Cultural Affairs and by a grant for paediatric research (C-3501) from the Ministry of Health and Welfare.

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Asia Pacific Journal of Clinical Nutrition (1997) Volume 6, Number 2: 111-115

大鼠成長過程中小腸黏液凝膠層分佈的改變

摘要

作者選用大鼠爲對象，觀察年齡在３日，１、２、４和６週小腸管腔内黏液的凝膠層，以研究大鼠出生後凝膠層的成長。他們把小腸連同管腔内容物用乾燥低溫切片，並用火棉固定，以保存管腔内黏液的凝膠層，用希夫氏高碘酸 (PAS) 染色。作者觀察了大鼠幾個年齡的小腸絨毛間黏液凝膠的形態學，發現出生２週後改變最爲明顯。本文指出，覆蓋大鼠小腸上皮帶黏性的黏液凝膠層，在大鼠斷奶後經歷迅速的成長。

凍結切片上の粘液をセロイジンを用いて安定化させる方法で、生後３日および２、４、６週齢のラットの小腸管腔内の粘液の分布形態の観察を行った。生後および１週では、villi間隙が粘液で満たされるには至らないが、生後２週目以降空腸、回腸ともにvilli間隙に粘液の存在が認められ、生後４週以降は、この粘villi間隙が満たされた。

ラットにおける粘液層の発達は、離乳と前後して認められ、消化吸収機能や粘膜防御機構等のやはり離乳と前後して起こると考えられる急激な変化との関詳細な検討が必要と考えられた。

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