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Paneth Cells Distribution of Small Intestine in Male Rabbit: A Light and Electron Microscopic Study

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Abstract: The Paneth cell lineage is one of four epithelial lineages derived from the small intestine's multipotent stem cell and differentiate during a downward migration to the crypt base. These cells are pyramidal secretory granulated epithelial cells in the base of the crypts of the small intestines. The effect of area on intestinal paneth cells was evaluated in 10 mature rabbits by light and electron microscope. Our results showed that further of the cells filled by secretary granules, in the positive reactions with phloxine- tartrazine. Histometric results showed except for ileum, crypt number and paneth cell number per crypt, increased from duodenum toward jejunum. The results obtained of transmission electron microscope of paneth cells indicated that the principal recognizable structure of the paneth cell was great secretory granules, Golgi complex and dense small granules.

Key words: Paneth cell · Rabbit · Small intestine

INTRODUCTION

The epithelium of the small intestine is a dynamic tissue which renews and propagates itself rapidly [1]. Cell proliferation is confined to the crypts of Lieberkühn where four cell types, the undifferentiated crypt cells and the differentiated Paneth, goblet and enterochromaffin cells, are found [2]. These cells migrate distally to the bottom of the crypt where they are adjacent to undifferentiated anion secreting cells expressing cystic fibrosis transmembrane conductance regulator unlike other epithelial lineages that derive from stem cells located in the crypts of Lieberkühn [3, 4]. The function of the Paneth cells has not yet been clearly defined. In recent studies [5-7] have been detected that Paneth cells contain of immunoglobulins, lysozyme, the bacteriolytic enzyme and the anti-microbial peptide termed cryptidins (defensin/corticostatin-like peptide) and are also responsible for the elimination of heavy metals and phagocytosis of bacteria [8].

Paneth cells are pyramidal secretory granulated epithelial cells in the base of the crypts of the small intestines found in the small intestine of most vertebrates [8]. Paneth cells are located at the base of small intestinal crypts of Lieberkuhn that synthesize and secrete granules containing a variety of peptides and proteins with known host defense functions. Unlike villus enterocytes, though, which have lifetimes of 2-3 days, Paneth cells are long-lived, with half-lives measured at 20 days in mice. In mammals, a predominant group of antimicrobial peptides are defensins [9, 10] and Paneth cells are the major source of *á*-defensins and distributed from the duodenum to the ileum [11, 12]. Inada *et al.* [13] in human stomach, in very rare cases, have been found that small numbers of Paneth-like cells, with prominent eosinophilic granules in their cytoplasm in such glands.

Paneth cells have an extensive endoplasmic reticulum, well-developed Golgi and large apically located granules. In addition to *á*- defensins, Paneth cells also produce other antimicrobial proteins including lysozyme and secretory phospholipase A2 [6]. Then this cells secrete these secretory granules into the crypt lumen in response to bacterial products (such as muramyl dipeptide, a component of bacterial peptidoglycan) [2, 14].

Studies on Paneth cells have been carried out on rather laboratory rodents (rat, mouse) kept under pathogen-free conditions. To date, the effects of area on the distribution of the Paneth cells in the different anatomical regions in rabbit small intestine have not been investigated satisfactorily. Thus present study to investigate effect of area on paneth cells in the small intestine.

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MATERIALS AND METHODS

Ten healthy adult rabbits aged 3 months old were selected from the rabbit breeding colony established under the genetic and quality controls on our institute. The animals were kept in under standard environmental conditions in stainless steel cages and off feed before sampling for 48 hours. After sacrificing of the rabbits, the whole small intestine in its junction to the pyloric part of stomach and the ileocecal junction was removed from the abdominal cavity. Samples were obtained from the duodenum (middle parts of descending and ascending parts), jejunum (proximal, middle and distal parts) and ileum (near its junctions with jejunum and cecum).

Tissue samples were fixed in 10 % Merck buffered formalin, dehydrated and embedded in paraffin and washed through a series of graded alcohols. Then were blocked in paraffin and 5-6 micrometer-thick sections were stained with Lendrum's phloxine-tartrazine. Stained sections were investigated with optical microscopy to obtain distribution and scattering of the Paneth cells in areas of interest.

For electron microscopic study, the sections of 1 mm^3 were taken and immediately were kept for 24 hours in glutaraldehyde-paraformaldehyde pre-fixing (pH 7.4). Rinsed three times with cacodylate sodium buffer 0.15 M, pH 7.4 at 10-minute intervals and post-fixed in a phosphate-buffered solution of 1% osmium tetroxide at 37°C for 2 hr. washed again in distilled water and dehydrated by passage through a series of ethanol with increasing concentrations (35%, 50%, 75%, 95% and 100%). Tissue blocks Prepared in small-plastic capsules by pure resin after to transparent of samples with propylene. semithin sections stained by toluidine blue and ready for ultrathin and were observed under transmission electron microscope.

RESULTS

Light Microscopic Result

Morphology: The under light microscope, the paneth cells mainly distinguished in the end of crypt with basophilic basal cytoplasm. The nucleus was found a round or oval shape that lies toward base of the cells. Furthermore the apical portion of their cytoplasm is filled with acidophilic secretory granules. They were distributed in the cytoplasm matrix with vary sizes but no was found the granules in the bottom of cells, between nucleus and cell base. The granule size enlarged in the part of the cell apex in the direction of crypts.

Table 1: Means and standard deviation of crypts number per mm of length

	Regions		
Groups	Duodenum	Jejunum	Ileum
Male	8.3±1.3	12.4±2.5	11.6±2.1

Table 2: The number of paneth cells per crypt

	Regions		
Groups	Duodenum	Jejunum	Ileum
Male	2.2±0.2	2.7±0.28	0.8±0.1

While further of the cells filled by secretary granules, in the positive reactions with phloxine- tartrazine, red granules of inside the lumen demonstrated that their cells contents to liberate into the crypt lumen.

Histometry: The crypts mean per mm length of the tree regions of small intestine, (duodenum, jejunum and ileum) indicated in the Table 1.

Results obtained from the present study showed that the mean of crypts number increased in duodenum and jejunum statistically but the reverse is true about ileum ($P>0.05$).

Table 2 show the number of paneth cells per crypt. Significant difference found among three regions, number of paneth cells decreasing toward ileum. This results show that majority of the cells density observed in jejunum.

Electron Microscopic Result

Ultrastructure of Paneth Cells: The paneth cell that located in the crypt end of intestine, were identified by their great secretory granules supranuclear zone. Nucleus has been placed near of cell base than apex. The cell are shaped pyramid to polygonal mid the base lie against the basal lamina of crypt. The cell membrane as a thin electron dense separated cell cytoplasm from basement membrane.

Regularly shaped nucleus enveloped by smooth membrane of nuclear, were recognized at the base of cell and near the cell membrane that showed polarization of cell. The principal recognizable structure of the paneth cell was great secretory granules, Golgi complexes and dense small granules. At the apical of cell, variable the homogeneous granules were seen voluminous and greatest of the electron viewpoint. The contents of the granules comprised a face of low electron contrast. This secretory granules glut the upper and apex of the cell.

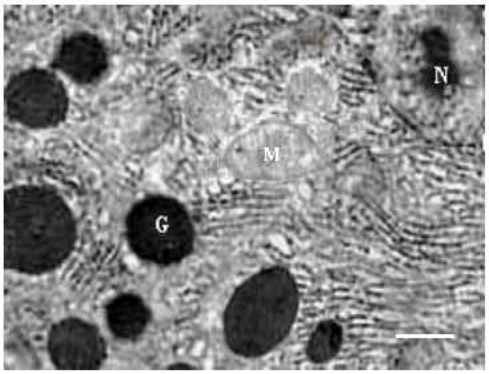

Fig. 1: A paneth cell with the nucleus (N) was found at the base of the cell; the rich in endoplasmic reticulum (ER); and the apical cytoplasmic granules (G) and mitochondria (M); (duodenum, bar= 1 μm).

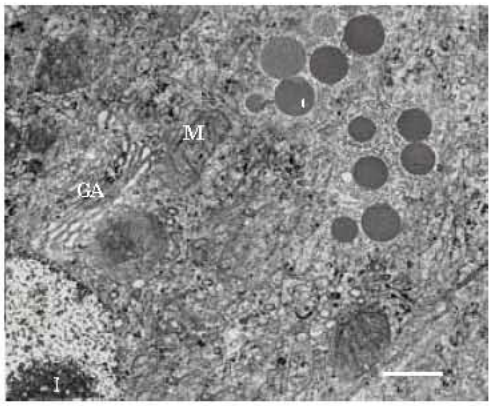

Fig 2: The nucleus (N); Golgi apparatus (GA); granules (G) and mitochondria (M); (jejunum, bar= 0.75 μm).

Apart of granules, small granules seen dens and few scattered into cytoplasm. The Golgi complex in the paneth cell was rich and lies between the cytoplasmic granules and the nucleus, fundamentally in the overhead nucleus region. The mitochondria are chiefly scattered throughout medio-basal zone and rarely found in the cytoplasm apex. They are limited by an outer double membrane and ones matrix is denser than cytoplasmic matrix. Cytoplasm was endoplasmic reticulum environs of nucleus (Figure 1, 2).

DISCUSSION

It is considered that paneth cells help maintain the gastrointestinal barrier in the crypt lumen and contain lysozyme, a bacteriolytic enzyme, as well as immunoglobulin. They contribute to the host's defense against pathogenic microorganisms and this coupled with findings of degenerating bacteria and protozoa within their lysosomal elements suggest that Paneth cells may regulate the microbiological flora in the gut.

Parallel section through the intestine mucous membrane shows the crypts (glands) of Lieberkühn. The diameter of these crypts is smaller than that of the villi. Apart from the surface cells, the epithelium of the crypts also contains typical goblet cells. Clusters of cells with apical granulation are found in the intestinal epithelium at the fundus of the crypts. They are also called oxyphilic granule cells in reference to their staining characteristics. Paneth cells are exocrine glands. Their granulation is caused by secretory granules, which contain the bacteriolytic enzyme lysozyme or several peptidases [13]. As reported earlier by Merzel and Glerean and Castro [10, 15].

The villi increase the surface area of the small intestine to many times what it would be if it were simply a tube with smooth walls. Paneth cells sentries inhabit tiny pits in the intestine called crypts and secrete antimicrobial peptides, sterilize the contents of the intestine. The principal defense molecules secreted by Paneth cells are alpha-defensins, also known as cryptdins. These peptides have hydrophobic and positively-charged domains that can interact with phospholipids in cell membranes. This structure allows defensins to insert into membranes, where they interact with one another to form pores that disrupt membrane function, leading to cell killing.

Deschner [6] showed that human Paneth cells were not only limited to the bases of the crypts but also to embrace the entire length of the crypts and the villi [6]. Whiles Bjerknes and Cheng and Garabedian *et al.* [4, 9] were demonstrated that the Paneth cells differentiate as such towards the base of the crypts and hence the finding of the young cells at the top and the matured cells at the bottom of the crypts. In present study, no Paneth cells were observed in the villi of the small intestines of the rabbit and this cells occupied fundus of crypts. On the other hand, it is in line with this that these cells are plenty in the neck and bottom regions of the intestinal crypts.

Elmes *et al.* [7] reported that the effect of tissue preparation on crypts per mm was carried out by techniques of estimating paneth cell populations using linear measurements are more subject to error than those using a defined number of crypts. However, this researcher represented in adults when surgical specimens from the jejunum and ileum were compared more crypts per mm were found in the jejunum than the ileum that probably anatomical variation rather than tissue

shrinkage. In our findings, jejunum had maximum value than duodenum but in the compare with ileum different mean not significant. This pickup perhaps is indicative the point that activity and absorption of food stuff increased in jejunum.

In our study, the granular size was seen great towards the base of the crypts and these granules release their contents into the lumen of the crypts of Lieberkühn this finding was reported by various investigators and also histochemical studies corroborate the protein nature of the Paneth cell granules [10, 15].

The effect of age and its correlation with measure of the apical secretary granules were reported [4, 9]. In our study, the granules size observed toward the base of the crypt to enlarge in the rabbit intestine crypt. To seem that process of granules change express age or maturity of cells mentioned. Observation the granules that release their contents into the lumen of the crypts reported by various researchers [18].

In this study, the Paneth cells to have not a similar distribution along the entire length of the small intestine, with the region of highest cell density being the jejunum. The duodenum was observed to have the lower number of cells than the jejunum. In the ileum the cell count was lower than to jejunum but different not significant. We suggest that density of paneth cells in jejunum than to other regions due to the Paneth cell proliferation observed is an attempt to prevent bacterial overgrowth and or may be related to the functional requests and longer processes of digestive in this region.

Apart of lack paneth cells in the cat, dog and pig, these cells detected in man, monkeys, rats, guinea pigs and ruminants and contains abundant numbers of these cells in the crypts [4, 9]. Paneth cells to be much denser in the duodenum and ileum in human and less dense in the jejunum, but suggested that at the terminal ileum the number of crypts and Paneth cells are decreased due to the increased lymphoid tissue [16]. In rat these cells to be show an increase in number from the duodenum towards the ileum [1]. Their findings are in complete disagreement with those presented in this paper.

In the guinea pig, the human and bat fine structure of the Paneth cell granules showed that are large and filled with morphologically homogenous material. In the hamster it includes different electron densities [19]. In our finding, similar to hamster, were observed electron dense granules. Specimens studied about these granules with the electron microscope gave values of 1.5 and 2 μ, respectively [11].

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