Pathological Studies on Foals Experimentally Infected with Equine Rotavirus

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Pathological studies were performed on 2 neonatal foals with a febrile response by oral inoculation of equine rotavirus. Severe diarrhea was observed in Foal No. 2 necropsied 5 days after inoculation, but not in Foal No. 1 necropsied 2 days. Main gross lesions were mild edema in the small and large intestines. Histological changes in the small intestine consisted of shortening and fusion of villi, desquamation of the villous epithelial cells, denudation of the villous lamina propria, and infiltration of lymphocytes and an increase in number of histiocytes in the villous lamina propria. In the large intestine, the cecum and large colon showed epithelial degeneration and infiltration of lymphocytes and plasma cells in the submucosa. Scanning electron microscopy exhibited the reduction or lack of microvilli in some villous epithelial cells. A great deal of tissue fluid flowed from the villi to the intestinal lumen. From these results, foal diarrhea caused by equine rotavirus was pathologically classified as an acute catarrhal enteritis.

Key words. diarrhea, equine rotavirus, foal, pathology

Introduction

Rotavirus has been known as an important pathogen for gastroenteritis in neonatal animals of many species and children.1) Equine rotavirus (ERV) is also considered to be the most important, principal causative agent for foal diarrhea. ERV infection in foals seems to be widely spread in many countries,2-5) including Japan.6-8) Pathological changes in experimental rotavirus infection caused in calves,9,10) piglets,11,12) lambs,13) pups14) and foals5) have been almost confined to the small intestine. It seems, however, that there may be some morphological variations in species. Severe changes were observed in piglets, and mild to moderate ones described in calves and pups, but no pathological changes were described in lambs. In addition, there has been a little information about pathological findings in ERV infection in foals,5) as compared with the clinical descriptions.3,5)

This studies were undertaken to illustrate pathological changes in foals experimentally infected with ERV, as examined by light microscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM).
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Materials and Methods

Experimental materials and procedure. The animals, virus and method of inoculation employed in this study were the same as described in the other paper.15)

Pathological examination. After intravenous injection with 0.2 mg of suxamethonium chloride solution (Succin produced by the Yamanouchi Pharmaceutical Co. Ltd., Tokyo), per kg of body weight, 2 foals were exsanguinated by severing the carotid arteries and necropsied immediately. Histological samples were collected mainly from the gastrointestinal tract and its associated lymph nodes. They were fixed in 10% neutral buffered formalin solution, dehydrated through an ethanol series, embedded in paraffin, and cut into sections. The sections were stained with hematoxylin and eosin, periodic acid-Schiff, colloidal iron. Some frozen sections were prepared from formalin-fixed tissue blocks and stained with osmium tetroxide for lipid.

Electron microscopy. Samples were taken from the formalin-fixed duodenum, jejunum and ileum, and re-fixed in 2.5% glutaraldehyde solution at 4°C for 1 h. Then they were subjected to the staining method with tannic acid and osmium tetroxide to conduct electricity. The specimens were dried in a critical-point drying apparatus with a CO₂ gas phase, and examined by a Hitachi S-405 scanning electron microscope. For TEM, small tissue specimens from formalin-fixed intestinal samples were fixed in 2.5% glutaraldehyde solution in 0.1 mol/l phosphate buffer solution (pH 7.4) at room temperature for 2 h, postfixed in 1% osmium tetroxide, dehydrated in a series of ethanol, and embedded in epoxy resin (Poly/Bed 812 produced by Polysciences Inc., Warrington, U.S.A.). Ultrathin sections (60–80 nm thick) were prepared, stained with uranyl acetate and lead citrate, and examined by a Hitachi H-600 transmission electron microscope.

Results

Foal No. 1 fell into a stage of discomfort within postinoculation day (PID) 1. It had a rectal temperature of 39.8°C on
Histopathologically, prominent lesions were found in the villi of the duodenum, which were stunted with a clumped epithelial cell layer (Fig. 1). On the contrary, the villi of the jejunum and ileum were long and slender and covered with vacuolated epithelial cells (Fig. 2). Toward the lower part of the small intestine, the vacuolation increased in severity (Fig. 3). The vacuoles were proved to contain lipoid substance by specific staining with osmium tetroxide of frozen sections. Slight depletion or necrosis was noted in lymphatic follicles in the submucosa of the small intestine.

SEM revealed destructive changes in the villi of the duodenum; that is, short, rough-surfaced and often clustered cells at the tips (Fig. 4). The villi of the jejunum (Fig. 5) and ileum were long and slender, like the normal ones. TEM presented viral proliferation in the absorptive epithelial cells of the jejunum. These affected

Fig. 4. Duodenum of Foal No. 1
Villi are markedly stunted with many irregular grooves giving their surface a rough appearance. Scanning electron microscopy (SEM), × 50.

Fig. 5. Jejunum of Foal No. 1
A long slender villus seems to have a normal appearance. SEM, × 140.

Fig. 6. An epithelial cell in jejunum of Foal No. 1
Endoplasmic reticulum (arrow heads) is dilated. It is apparently differentiated from lipoid (arrow). Transmission electron microscopy (TEM), × 6000.

Fig. 7. Enlargement of the rough endoplasmic reticulum shown in Fig. 6
An enveloped viral particle appears by budding process (arrow) from electron-dense area. TEM, × 30000.
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Table 1. Histopathological lesion, observation of virus by TEM and detection of specific immunofluorescent antigen (IFA) in foals inoculated with ERV

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Foal No. 1</th>
<th>Foal No. 2</th>
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<tbody>
<tr>
<td></td>
<td>Histopathological lesion</td>
<td>Virus particles</td>
</tr>
<tr>
<td>Stomach</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Duodenum</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Jejunum</td>
<td>– *</td>
<td>+</td>
</tr>
<tr>
<td>Ileum</td>
<td>– *</td>
<td>–</td>
</tr>
<tr>
<td>Cecum</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Large colon</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Small colon</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mesenteric lymph nodes</td>
<td>ND</td>
<td>–</td>
</tr>
</tbody>
</table>

Remarks. Histopathological lesion, virus particles by TEM and specific IFA: positive (+) and negative (–). ND: Not done. *: Vacuolation of villous epithelial cells was observed. **: Specific IFA was detected in lymph follicles of submucosa as well as epithelial cells.

Fig. 8. Duodenum of Foal No. 2 killed 5 days after inoculation
Desquamation is seen in epithelial cell layers. The villous lamina propria is denuded at its tip. HE, ×40.

Fig. 9. Jejunum of Foal No. 2
Villous edema is shown in the space under the villous epithelial cell layer. HE, ×40.

Fig. 10. Ileum of Foal No. 2
Villi are very short. HE, ×40.

cells had electron-dense granular viroplasm and dilated rough endoplasmic reticulum (Fig. 6), containing viral particles about 75 nm in diameter (Fig. 7). In Table 1, comparison was made among histopathological findings, observation of viral particles in the villous epithelial cells by TEM, and detection of specific immunofluorescent antigen which was described in the other paper.15)

Foal No. 2 started acute diarrhea with a mild febrile response about PID 3. Then it was depressed and anorectic through PID 5, when it was necropsied. Grossly, the distended cecum and colon contained a large amount of whitish brown, odorless fluid, but the small intestine contained little yellow fluid. The mucous membrane
was edematous in a portion of the intestinal tract extending from the duodenum to the large colon. No gross lesions were noted in any other organs.

Histopathologically, desquamation of villous epithelial cells was often observed in the duodenum (Fig. 8). Villi were edematous, short and frequently fused in the jejunum (Fig. 9) and ileum (Fig. 10). The space under the villous epithelial cell layer was filled with tissue fluid which was not stained with periodic acid-Schiff, colloidal iron, or osmium tetroxide. Many mitotic pictures were noted in the crypt epithelial cells. Vacuolated epithelial cells in the small intestine were fewer in Foal No. 2 than in Foal No. 1. Furthermore, histiocyte proliferation and lymphocyte infiltration in the villous lamina propria were remarkable in the small intestine (Fig. 11). The epithelial cells of the cecum and large colon degenerated and desquamated in places (Fig. 12). Slight infiltration of lymphocytes and plasma cells was noted in the submucosa of the cecum and large colon (Fig. 13).

SEM showed an apparent morphological difference in villi between 2 foals. In Foal No. 2, the villi of the duodenum were fairly tall (Fig. 14) and those of the
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jejenum (Fig. 15) and ileum (Fig. 16) short with fusion. The surfaces of the villi were smoother in this foal than in Foal No. 1. Highpower magnification revealed the reduction or lack of microvilli of the epithelial cell in the distal part of the villi in the jejenum and ileum (Figs. 17 and 18). In addition, mucoids were springing out through the intercellular junctions (Fig. 18). Findings of the affected epithelial cells under TEM were similar to those in Foal. No. 1. In the present observation, no viral particles were found out in the absorptive epithelial cells, except those of the small intestine (Table 1).

Discussion

In the diarrheal foal, pathological changes were mainly found in the small intestine by light and electron microscopy. They consisted of shortening with fusion of villi, desquamation of villous epithelial cells, infiltration of lymphocytes and an increase in number of histiocytes in the villous lamina propria, and vacuolation of epithelial cells in the small intestine. Although opinions are divided about the vacuolation of these epithelial cells, it was regarded as a physiological change of pinocytosis in neonates, because the vacuoles produced were proved to contain lipoid by light microscope and differentiated from dilated endoplasmic reticulum by TEM. All these findings were similar to those obtained from calves, pigs, and pups.

Although no one has ever described the lesions of the cecum or colon in any animal infected with rotavirus, the authors observed the degeneration of mucosal epithelial cells, and the infiltration of lymphocytes and plasma cells in the lamina propria. There may be a small difference in the distribution of intestinal lesions in rotavirus infection between foals and some other species of animals. At the
same time, since the cecum and colon play an important part in the digestion of food and the absorption of water in horses\textsuperscript{16}), those histological changes suggested functional disorders of such organs.

McAdaragh et al.\textsuperscript{11}) investigated villous morphological changes in piglets by SEM. They reported that villi were covered with round epithelial cells in the early stage of infection and that they were denuded of these cells at the tips later and fused with one another. By referring to their description, Foal No. 1 must have been in the early stage of infection and Foal No. 2 in the convalescent stage. In the latter stage, microvilli were reduced in number or disappeared from the distal portions of the jejunal and ileal villous epithelia. Reduction or lack of microvilli induced a decline in absorption of electrolytes and other nutritious elements, resulting in an increase in amount of intestinal fluid. Furthermore, springing out of mucoids through the intercellular junction indicates the flow of tissue fluid from the villi to the intestinal lumen. These findings show that the accelerated transportation of tissue fluid is a main cause of diarrhea.

Judging from studies on epithelial cell migration and turnover in the small intestine of sheep and calves, the replacement of the villous epithelium might occur in a few days.\textsuperscript{17}) If it is also the case with the organ of horses, the villi would be covered with regenerated epithelial cells in Foal No. 2. On the other hand, a study by the immunofluorescent antibody technique showed a viral replication in these new cells.\textsuperscript{15}) Viral replication in regenerated cells is also deduced from the results of isolation of virus which could be re-
covered for such a long time as 3 weeks in calf rotavirus infection. It has been said, however, that new regenerated cells are resistant to re-infection and lacking in receptors for the virus. These seem to contradict the above-mentioned immunopathological and virological results. Therefore, further studies will be necessary to solve this problem. Concerning this problem, it was speculated in this study that receptors for the virus might be on the microvilli. If this speculation is true, it will be mentioned that ERV can infect epithelial cells with microvilli, but cannot come into contact with epithelial cells without receptors.

Indeed, ERV is the most important, primal pathogen of acute diarrhea in foals, but many foals in the field may contract diarrhea by mixed or secondary infection with plural pathogens. It was ascertained, as mentioned in the other paper that no other microbiological agents were concerned with this experimental infection. From the results mentioned above, it was confirmed that ERV caused catarrhal enteritis in foals for itself.

Acknowledgments

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Literature Cited


要 約

馬ロタウイルス実験感染子馬の病理学的研究：和田隆一*・今川 浩*・塚田正信*・福永昌夫*・熊 桦野堂 慎*（*日本中央競馬会競馬総合研究所橋本支所）——馬ロタウイルスの経口接種によって発熱を呈した2頭の新生子馬を病理学的に調べた。下痢、接種後5日目に剖検された2号馬に顕著であったが、同2日目に剖検された1号馬には認められなかった。主な肉眼所見は小腸および大腸の粘膜の浮腫であった。小腸の病理組織学的変化は絨毛の短小化と融合、絨毛上皮細胞の剥脱による固有層の様化および絨毛固有層におけるリンパ球の浸潤と組織球の増殖であった。大腸では、盲腸と大結腸において粘膜上皮細胞の変性と固有層へのリンパ球やブラッサマ細胞の浸潤が認められた。走査型電子顕微鏡による観察で空腸と回腸の一部の絨毛上皮細胞の微細毛が減少ないし欠損していることが明らかにされた。さらに、これら上皮細胞の間隔から粘液粒子が漏出しており、大量の組織液が腸管腔へ移動していることが示唆された。以上の成績から、馬ロタウイルスの感染による子馬の下痢症は病理学的には急性カタル性腸炎に分類されるものであった。