QUANTIFICATION OF β-GALACTOSIDASE TO MEASURE INTESTINAL DAMAGE IN ROTAVIRUS INFECTED CALVES

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Summary

Quantitative β-galactosidase estimation in the intestinal mucosal cells of calves with diarrhoea under experimental conditions due to rotavirus were undertaken. A quantitative decrease of 40-70% in β-galactosidase activity was observed in proximal and middle segments of the small intestine of the infected calves, more so in the middle segments. The decrease in the distal part of the intestine, however, was lesser (5 to 30%). The decrease in the activity was more marked on the day 2 to 6 post infection indicating the degree of the damage of the villi of the small intestine.
(**Key Words**: Viral Enteritis, β-Galactosidase, Calves Enteritis, Intestinal Damage, Quantification)

Introduction

β-galactosidase (lactase) is essential for the hydrolysis of lactose, main source of carbohydrate in the diet of newborn calves. The β-galactosidase is confined to the mucosal epithelium of small intestine (Miller and Crane, 1961) and hydrolysis of lactose is considered to be an intracellular process (Bywater and Penhale, 1969). Earlier it was observed that the activity of β-galactosidase in small intestine decreased during diarrhoea due to rotavirus (Halpin and Caple, 1976; Tzipori et al., 1983; Pospischil et al., 1986; Stiglmair-Herb et al., 1986), the β-galactosidase activity having been determined qualitatively in the intestinal mucosa. The present communication deals with the quantitative measurement of β-galactosidase activity in the mucosal cells of small intestine of diarrhoeic calves experimentally infected with rotavirus to asses the degree of intestinal damage and the maturity of the cells.

Materials and Methods

A total of 15 Sahiwal × Jersy crossbred newly born specific pathogen free calves maintained in isolation immediately after birth under strict hygienic control were used for the study. The calves were divided into 5 groups of 3 each with one calf as uninfected control and 2 infected with 5×10^6 $TCID_{50}$ bovine rotavirus in MA 104 cells. All the calves, before infection were screened for the presence of rotavirus antigen in faeces by enzyme linked immunosorbent assay (Grauballe et al., 1981). The calves of group I to V were sacrificed on day 2, 4, 6, 8 and 10 post infection respectively, using high dose of intraval sodium as anaesthesia. Immediately after death of calves, 5 cm of the intestinal pieces were collected from proximal, mid and distal parts of small intestine. The intestinal pieces were thereafter cut open with scissor longitudinally and kept on a sterile filter paper with mucosal surface facing up. With the help of glass slides, the exposed mucosal cells were then scrapped from each part of the intestinal segments the proximal, middle and distal parts and 100 mg of these cells were taken in 2 ml of 0.2 M phosphate buffer saline (pH 7.0). The cell suspensions were then sonicated for 2 min, the suspension centrifuged at 10,000 xg for 20 min and the supernatant thus obtained tested for the activity of β-galactosidase using O-nitrophenyl β-D-galactosidase (ONPG) as subtrate as per the method of Saito et al. (1989). The activity of β-galactosidase was calculated as micromoles per ml. Two such samples were tested from each site and the mean of both reading was considered for expressing the value of β-galactosidase activity.

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Results

The β-galactosidase activity was found to decrease 40-70% in proximal and middle segments of small intestine of all the infected calves. However, its decrease was less (5-30%) in distal part of the small intestine. The average lactase activity in middle and proximal segments was 2.9 ± 0.69 and 3.65 ± 0.85 μ moles/ml in infected calves on day 2 whereas on day 4, it became 3.25 ± 0.75 and 4.25 ± 0.65 μ moles/ml (figure 1). On day 6 PI, the values of the lactase activity in middle and proximal segments were 3.05 ± 0.55 and 3.75 ± 0.55 in infected and 7.0 and 7.5 μ moles/ml in control calves (figure 1).

The changes in lactase activity were more pronounced on days 2 and 4 post infection. However, from day 8, its activity slightly decreased in all the segments of small intestine. The histopathological study of the intestinal tissue indicated damage including necrosis and desquamation of villus epithelium, shortened villi, congestion and mononuclear cell infiltration in submucosa. The pathological lesions remained confined to small intestine and mesenteric lymph nodes, the lesions being more pronounced upto 6 day post infection. Thereafter, the regenerative changes were evident.

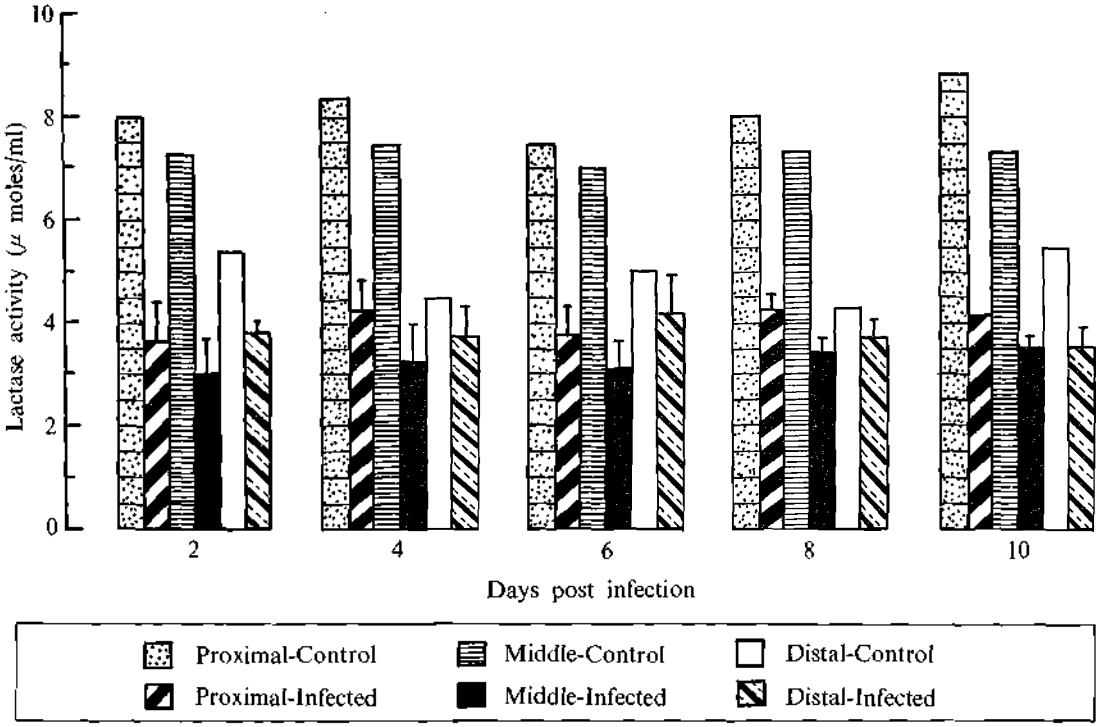

Figure 1. Showing the activity of β-Galactosidase in mucosal cells of small intestine.

Discussion

In calves infected with rotavirus and showing clinical symptoms of diarrhoea a quantitative decrease in β-galactosidase in small intestine was evident. The decrease was more pronounced in the proximal and middle segments of small intestine, particularly on day 2 to 4 post infection. It is considered that the activity of β-galactosidase is maximum in the mature cells at the top of villus and is localised in microvillus, brush border of the cells (Halpin and Caple, 1976). Normally for replacement of epithelium in the small intestine, cells divide in the crypts and migrate along the villi to be sloughed into lumen. This process is considered to take approximately 3 days (Moon and Joel, 1975). Immature cells in the crypts have a sparse microvillus border with negligible digestive enzyme activity. In rotavirus induced diarrhoea, the villi of small intestine become shortened

with progressive replacement of normal columar cells by squamous or cuboidal cells which lack in microvillus border (Woode and Bridger, 1975; Hall, 1988).

It has been shown that rotavirus causes damage to the epithelium in the top two third of the villi, leading to desquamation and as the loss of epithelium accelerates to that of cell production in crypts, immature cells are more evident to be present at the site (Mebus, 1990). If the β-galactosidase in the gut is inadequate due to immature cells, to cope with the lactose in the diet, the undigested lactose is fermented in colon by microflora leading to fermented diarrhoea (Bywater and Penhale, 1969) which further aggrevate the already existing condition. Mebus et al. (1971) earlier reported that the rotavirus primarily affect the villus epithelial cells causing necrosis and desquamation, resulting into decreased villus length. The upper, middle and lower small intestine were observed to be mainly effected. Whereas 4 to 6 hr after onset of the diarrhoea, the columnar cells were lost and were replaced by cuboidal or squamous type of cells (Mebus et al., 1973). Logan et al. (1979) were of the view that rotavirus induced pathological lesions predominantly in proximal part of small intestine.

The quantitative measurement of β-galactosidase thus could be successfully used to indicate the extent of damage of the gut mucosa as well as an index of the maturity of the gut enterocytes and for assessing the degree of infection in the animals.

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