Effect of rotavirus infection on lipid composition and glucose uptake in infant mouse intestine

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Objective: To study the effect of rotavirus infection on lipid composition and glucose uptake in small intestine of infant mice. Methods: Thirty six 7-day old balb/c mice were given 50 mL (100 ID₅₀) rotavirus suspension orally; 36 control animals received only normal saline. Body weight of animals was recorded. Six animals in each group were sacrificed on days 0, 1, 3, 5, 7 and 10 post-inoculation (pi). Intestines were removed, everted and homogenized in ice-cold saline. Cholesterol and phospholipid content, glucose uptake and alkaline phosphatase in the jejunum and ileum were determined. Results: Rotavirus infection led to a significant decrease in body weight with on days 3 and 5 pi as compared to controls, and an increase in cholesterol and phospholipid content of the intestine. Uptake of glucose also increased significantly. Alkaline phosphatase activity was significantly reduced on days 3 and 5 pi as compared to controls. Conclusion: This study provides characterization of the lipid composition and uptake of glucose in infant mice small intestinal segments during rotavirus infection. [Indian J Gastroenterol/2001;20:18-21]

Key words: Cholesterol, phospholipid

Rotavirus is an important cause of diarrhea and death in young children throughout the world. Diarrhea during rotavirus infection is caused by a combination of factors that include a reduction in absorptive surface area of the small intestine, and replacement of mature villous enterocytes by immature cells leading to reduction in brush border disaccharidases and malabsorption of carbohydrates. Recently, it has been demonstrated that a non-structural protein, NSP4, in rotavirus stimulates the Ca²⁺-dependent signal transduction pathway, altering the intestinal epithelial transport in mice.

Membrane integrity in the intestine depends mainly on cholesterol/phospholipid ratio. This ratio is known to be altered during malnutrition. We have previously shown that mild to moderate malnutrition occurs during rotavirus infection in infant mice, resulting in reduction of body weight.

In the present study, we estimated the cholesterol/phospholipid ratio and glucose uptake of the brush border membrane during rotavirus infection in infant mice.

Methods

Inbred balb/c mice were procured from our central animal house. They were confirmed negative for rotavirus antibodies by ELISA and were kept in autoclavable polypropylene cages (Tarsus, Cutlwa) for breeding. Infant mice (7 days old) were selected for the study. Murine rotavirus EB-strain (serotype 3) used in the study was kindly gifted by Dr H B Greenberg, California, USA, and was maintained throughout in an infective form by serial in vivo passaging in 7-day-old mice; ID₅₀ was determined according to the method of Reed and Muench.

Protocol

The study protocol was approved by the institute’s ethics committee.

The infant mice (n=72) were divided into two (control and rotavirus-inoculated) groups. Control animals were orally given 50 mL normal saline whereas rotavirus-inoculated animals were inoculated with 50 mL of rotavirus suspension containing 100 ID₅₀. Infants were separated from their dams beginning half an hour before and after inoculation. They were examined daily for diarrhea and their body weights were recorded.

Animals were sacrificed under light anesthesia on days 0, 1, 3, 5, 7 and 10 post-inoculation (pi). All animals were sacrificed mid-afternoon to avoid diurnal variations in enzyme activities. Entire small intestine from pylorus to ileocecal junction was taken out and divided into two parts: the proximal one-third portion, which was considered as jejunum, and the latter two-thirds ileum.

Small segments of small intestine from each of the two regions were excised and fixed in 10% formalin. Tissues were processed and embedded in paraffin wax; 4-5 μm thick sections were stained with hematoxylin and eosin.

Lipid composition

Lipids were extracted and washed as described by Bligh and Dyer and were redissolved in small volumes of chloroform. Cholesterol content was estimated by the method of Zlatkis et al using glacial acetic acid-ferric chloride reagent. Lipid phosphorus was determined by the method of Bartlett as modified by Marinetti. The protein content was estimated by Lowry’s method using bovine serum albumin (Sigma, USA) as standard.
Glucose uptake

*In vitro* uptake of radioactive (U-14C) D-glucose (Bhabha Atomic Research Center, Trombay) by small segments of the jejunum and ileum was measured by a technique which has been described previously.13,14

Alkaline phosphatase activity

Alkaline phosphatase activity was assayed according to the method of Bergmayer using p-nitrophenyl phosphate as substrate.15 One enzyme unit of alkaline phosphatase was equal to one micromole of substrate hydrolyzed per minute under standard assay conditions.

Statistical analysis

Intergroup comparisons were done using Student’s *t* test. Values are given as mean (standard error) of 6 replicates.

Results

Rotavirus infection was self-limiting and lasted till day 7 pi. Challenged animals developed yellowish, watery diarrhea on day 1 pi with maximum severity on days 3 and 5 pi. Diarrhea resulted in severe dehydration which was reflected by a significant reduction in body weight compared to the controls on days 3 and 5 pi; thereafter the weights were similar.

Histology

As previously reported,5 histological alterations in the small intestine of rotavirus-inoculated animals included focal areas of vacuolar degeneration in the lining epithelium, mainly at the tip of villi, on day 3 pi; the ileal segment was more severely affected.

Lipid composition

The cholesterol levels in the jejunum of rotavirus-inoculated animals was significantly higher than in controls on days 3 and 5 pi (185.7 [5.9] vs 143.3 [15.5] and 197.3 [4.7] vs 138.1 [10.7] mg/mg protein; *p*<0.001); subsequently levels were similar in the two groups. Similarly, levels in the ileal segment were higher on days 3 pi during rotavirus infection (*p*<0.02) (Fig 1). Phospholipid content was also significantly higher in the jejunum on 3 days pi in rotavirus-inoculated animals as compared to controls (*p*<0.001); no difference was observed in the ileum (Fig 2). The cholesterol/phospholipid ratio in the jejunum was higher in rotavirus-inoculated animals as compared to controls on days 5 pi (1.3 [0.1] to 2.1 [0.1]; *p*<0.001). In the ileum, the ratio was significantly higher (*p*<0.001) on day 3 pi in rotavirus-inoculated animals (15.0 [0.2] vs 18.0 [0.1]). On all other days there was no significant difference between the two groups.

Glucose uptake

*Fig 1*: Effect of rotavirus on intestinal cholesterol in infant mice

D-glucose uptake in the jejunum was lower on days 3 pi (0.69 [0.03] vs 0.47 [0.02] cpm/min/g tissue; *p*<0.02) and 5 pi (0.58 [0.02] vs 0.42 [0.01] cpm/min/g tissue; *p*<0.01) in rotavirus-inoculated animals compared to controls; thereafter, the levels were similar.

In the ileum also, a significant decrease in D-glucose uptake was observed on 3 and 5 days pi (*p*<0.05) in rotavirus-infected animals compared to controls (Fig 3).
Effect of rotavirus on mouse intestine

Fig 4: Effect of rotavirus on intestinal alkaline phosphatase activity in infant mice

Alkaline phosphatase activity

Alkaline phosphatase activity in the rotavirus-inoculated group was significantly lower on days 3 and 5 pi (p<0.001) in the jejunal (194.7 [6.6] vs 153.7 [3.8] and 204.6 [4.1] vs 175.8 [2.8] milliunits/mg protein) and ileal (193.5 [9.3] vs 148.5 [5.5] and 162.0 [9.8] vs 139.5 [3.1] milliunits/mg protein) sections compared to the control group. The activity was similar thereafter (Fig 4).

Discussion

This report provides characterization of lipid composition and uptake of glucose in infant mouse small intestinal segments during rotavirus infection.

The suckling mouse model presents almost the same advantages as rat or rabbit models in terms of cost, diarrhea incidence and duration, and histological changes. Infant mouse model has been used previously to study the pathogenesis of rotavirus infection.5,16,17

Electron microscopic and fluorescent-antibody labelling studies indicate that the virus invades and replicates in enterocytes lining the upper two-thirds of intestinal villi, resulting in damage and increased extrusion of cells from the villus tip.2 This phenomenon provides an explanation for the reduction in activity of alkaline phosphatase following rotavirus infection.

The lower glucose uptake in the intestinal segments with rotavirus infection could be due to a reduction in the number of mature epithelial cells as a result of damage, which reached a peak on days 3 to 5 pi. On days 7 and 10 pi, glucose uptake levels were similar to the controls values, corresponding to the histological status of the intestine.

The lipid composition of the small intestine may be affected by several factors like malnutrition, starvation and other diseased states.18,19,20 An increase in cholesterol and phospholipid levels, as observed in the present study in the infected animals, leads to a decrease in lipid fluidity, as seen in malnutrition states.19 This decreased lipid fluidity could also cause decreased glucose uptake during rotavirus infection.

In conclusion, rotavirus infection in infant mice resulted in decreased uptake of glucose and increased cholesteryl/phospholipid ratio in the jejunal and ileum; these changes recovered by day 7 pi.

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