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Review article:

ROTAVIRUS INFECTION: MOLECULAR CHANGES AND PATHOPHYSIOLOGY

Sankar Surendran*

Department of Pediatrics, The University of Texas Health Science center, Houston, TX

* Corresponding author: Sankar Surendran M.S., PhD., The University of Texas Health Science Center, Houston, TX 77030, Tel: 713 500 5740; e-mail: sankar_surendran@yahoo.com

ABSTRACT

Rotavirus infection causing gastroenteritis is one of the major health concerns throughout the world. Millions of children are affected by the disease. Studying molecular mechanism and pathophysiology of the disease is important to understand and interpret possible therapeutical targets. Studies suggest that rotavirus infection alters phosphorylation of p70S6K, mitogen activated kinase (MAPK/ERK) and myosin light chain; induced inflammatory agents such as prostaglandin E2 and nitric oxide levels; and enhanced corticosterone levels to damage villi enterocytes in the small intestine. These changes lead to malabsorption, abnormal motility and diarrhea. Although Rotarix and RotaTeq vaccines are available, proposals are emerged to produce new candidate vaccines.

Keywords: vacuolated villi, diarrhea, intestine, rotavirus infection, gastroenteritis

INTRODUCTION

Rotaviruses belong to the family Reoviridae, which are non-enveloped viruses with an 11-segment double-stranded RNA genome (Velazquez et al., 1996; Franco et al., 2006; Bass et al., 2007). The name rotavirus comes from the characteristic wheel-like appearance of the virus when viewed by electron microscopy (the name rotavirus is derived from the Latin *rota*, meaning "wheel"). Rotavirus infection is highly contagious. Group A viruses are the major cause of rotavirus diarrhea in the U.S. and groups B and C can cause gastroenteritis in adults. Rotaviruses cause approximately 111 million episodes of gastroenteritis per year including 25 million clinic visits, 2 million hospitalizations and numerous rotavirus-related deaths in children younger than 5 years of age worldwide (Parashar et al., 2003, 2006). Children between the ages of 6 and 24 months are at greatest risk for developing severe disease from rotavirus infection.

Rotavirus infection in the bowel is the most common cause of severe diarrhea and causes the death of about 600,000 children worldwide annually. Children acquire immunity to rotavirus after several infections with different strains of the virus. In the United States rotavirus related hospitalization is 4–5 %, and between 1 in 67 and 1 in 85 children will be hospitalized with rotavirus-mediated gastroenteritis by 5 years of age (Malek et al., 2006). This rate has not declined between 1993 and 2002 (Charles et al., 2006). In adults rotavirus infection effect is usually mild. Rotavirus is transmitted by the fecal-oral route, via contact with contaminated hands, surfaces and objects. Studying molecular and pathophysiological changes in diseases are useful to understand and interpret possible therapeutical targets (Surendran, 2005; Surendran and Kumaresan 2007; Surendran et al.,

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2007). Therefore the present study was aimed to understand molecular and pathophysiological changes during rotavirus infection.

GENOME, PROTEOME AND CLASSIFICATION OF ROTAVIRUS

The genome of rotavirus consists of 11 double helix molecules of RNA containing 18,555 base pairs. Each helix is a gene, numbered 1 to 11 by decreasing size. Each gene codes for one protein, except genes 9 and 11 code for two. The RNA is surrounded by a three-layered icosahedral protein capsid. Viral particles are up to 76.5 nm in diameter and are not enveloped.

The viral proteins VP1, VP2, VP3, VP4, VP6 and VP7 are *structural* proteins, can form the virus particle (virion). The *nonstructural* proteins, that are only produced in cells infected by rotavirus are NSP1, NSP2, NSP3, NSP4, NSP5 and NSP6.

VP1 is an RNA polymerase enzyme, located in the core of the virus particle. VP2 forms the core layer of the virion and binds the RNA genome. VP3 is an enzyme guanylyl transferase that catalyses the formation of the 5' cap in the post-transcriptional modification of mRNA. VP4 binds to molecules on the surface of cells called receptors and drives the entry of the virus into the cell. VP6 is highly antigenic and can be used to identify rotavirus species. VP7 is a glycoprotein that is involved in immunity to infection.

NSP3 is bound to viral mRNAs in infected cells and it is responsible for the shutdown of cellular protein synthesis. NSP4 is a viral enterotoxin to induce diarrhea. NSP5 is encoded by genome segment 11 of rotavirus A and in virus-infected cells NSP5 accumulates in the viroplasm.

Due to the antigenic and genomic diversity, rotavirus has been classified into 7 groups (A, B, C, D, E, F and G) and 4 subgroups within group A. Although species A is the main cause of disease in human, Band C also infects human beings. All the 7 groups cause disease in animals. The group A was further classified using the glycoprotein VP7 defining G types, and the protease-sensitive protein VP4 defining P types. The P-type is indicated by a number for the P-serotype and by a number in square brackets for the corresponding P-genotype. G-serotypes are similarly numbered but the G-genotype number is the same as the G-serotype. Approximately 14 G types and 20 P types have been reported, of which approximately 10 G types and 11 P types are identified in humans. Rotaviruses of different G and P types co-circulate and the main types found are G1P1A[8], G2P1B[4], G3P1A[8], G4P1A[8]. Naturally circulating rotaviruses undergo point mutations which can be used to classify lineages and sub lineages within types.

GEOGRAPHICAL DISTRIBUTION OF ROTAVIRUS

Rotavirus infection has been reported throughout the world. Studies between 1986 and 2006 showed that more than 51 rotavirus genotypes were found in Brazil. Approximately 43 of genotype was that of P[8]G1, followed by P[8]G9 (22) and P[4]G2 (7) (Gurgel et al., 2008).

In Kenya, the genotype G1 was mainly observed up to the year 2002. Then G9 has emerged as the most predominant genotype and followed by a less frequent genotype G8 (Kiulia et al., 2008). Genotype P[8]G9 was mainly found throughout Latin America (Araujo et al., 2001). In the United States, the G9 genotype was detected in a 1995–1996 outbreak (Ramachandran et al., 1998). In Australia, the overall G9 detection rate increased up to 29 % in 2001 (Kirkwood et al., 2003). In Japan, G9 was mainly reported in 1998–1999 (Zhou et al., 2000).

In India, G9 strains were detected and were usually found in combination with the P[11] or P[6] genotypes at a detection rate of about 20 % (Das et al., 1994). While genotypes G1P8, G2P4, G3P8, and G4P8

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were also seen among Indian children (33 %), strains of P6 (G1P6, G2P6, G3P6, G4P6, and G9P6), which primarily infect asymptomatic newborns but are rare in children with diarrhea were common in India (43 %) (Ramachandran et al., 1996). The P[8]G9 was found in New Delhi in late 1998 (Jain et al., 2001).

In Europe G1–G4 and G9 were the most prevalent genotypes identified: Genotype G1 was identified in Spain, Sweden, and the United Kingdom; G9 in Italy, France, and Belgium; and genotype G4 in Germany. Only the G4 and G9 genotypes were identified in all areas (Banyai et al., 2004; Damme et al., 2007).

SYMPTOMS OF THE DISEASE

The symptoms of rotavirus infection usually arise within 48 hrs period. Mild fever, vomiting, watery diarrhea and abdominal pain are the symptoms of the disease. Watery diarrhea occurs several times a day. Rotavirus infection occasionally leads to severe dehydration in infants and children. Symptoms of dehydration include lethargy, dry, cool skin, absence of tears when crying, dry mouth, sunken eye and extreme thirst.

DIAGNOSIS OF THE DISEASE

Rotavirus is shed in high concentration in the stool (~10^{12} viruses/G) of children with gastroenteritis. Therefore measurement of rotavirus antigen in the stool has been used to identify rotavirus infected patients. Enzyme immunoassay (EIA) directed at an antigen common to all group A rotaviruses has been widely used to determine the virus antigen presence in the stool. Latex agglutination method is also used and this technique may be less sensitive than EIA. Electron microscopy and polyacrylamide gel electrophoresis are also used to determine the virus (Beards, 1988). Reverse transcription-polymerase chain reaction can detect all species and serotypes of rotavirus (Nishimura et al., 1993; Fischer and Gentsch, 2004). Enzyme immunoassay for rotavirus serum immunoglobulin G (IgG) and immunoglobulin A (IgA) antibodies are the serologic methods used to determine rotavirus infections (Zijlstra et al., 1999; Zhang et al., 2000; Fischer et al., 2005).

MOLECULAR AND PATHOPHYSIOLOGICAL CHANGES

Rotavirus group A level decreases in the rat stomach by 72 hour post infection (hpi) and in the intestine between 4 and 9 days (Ciarlet et al., 2002; Crawford et al., 2006). 5 day old rat pups gavaged with rotavirus showed virus particles in the ileum even after 3 day period of infection. Approximately 30 % rats showed high level of virus presence in the ileum at 72 hpi. These studies suggest that either rotavirus does not severely infect all rats or virus level decreases with a progress of time period. Ileum villi length in the infected rats was decreased at 72 hpi.

Villus enterocytes are mature, non-proliferating cells covering the villi that regulate digestive and absorptive functions. The absorptive enterocytes are expressed on the apical surface and synthesize a number of disaccharidases and peptidases to carry out digestive functions. Absorption across the enterocyte barrier occurs both by passive diffusion of solutes along electrochemical or osmotic gradients and by active transport. Water transport occurs through transporters such as the sodium-glucose cotransporter 1 base (SGLT1) transport water along with solute (Loo et al., 2002). The crypt epithelium lines the crypts and is the progenitor of the villus enterocytes. Crypt cells do not have microvilli and absorptive functions of the enterocyte and actively secrete Cl^- ions into the intestinal lumen. In the normal animal, the combined activity of the enterocytes and crypt cells results in a constant bidirectional flux of electrolytes and water across the epithelium. We observed that rotavirus infects mainly enterocytes of the small intestine. Rotavirus group A and B infection causes vacuolation of the villi especially enterocytes in the severely infected rat ileum (Lane et al., 1993; Cow-

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ley et al., 1994; Reinhard et al., 1994). The vacuolation of enterocytes in the villi of rotavirus infected small intestine is shown in Figure 1. These studies suggest that rotavirus infects enterocytes of the small intestine and subsequently alters structure and function of the epithelium. Rotavirus group B infection in the neonatal rat reduced the villi height in both jejunum and ileum, and this height increased from 4 days on (Salim et al., 1995), suggesting auto-recovery of the rotavirus infected villi in the rat. Viral antigen and infectious virus frequently enter the circulation. Approximately 67 % of intestinal fluid and electrolyte secretion during rotavirus infection was due to the activation of enteric nervous system. Lundgren et al. (2000) suggest that rotavirus infection primarily impedes enterocytes development and subsequently affects the function of not only the epithelium but also the enteric nervous system. Neurological disorder observed in children with rotavirus infection was concomitant convulsions (Keidan et al., 1992), as rotavirus has ability to infect the central nervous system (Lynch et al., 2001).

P70S6Kinase (p70S6K) belongs to growth factor-regulated serine/threonine kinase family. In many cell types phosphorylated p70S6K (pp70S6K) regulates transit of cells from G1 to S phase of the cell cycle (Lane et al., 1993; de Groot et al., 1994; Chou and Blenis, 1995). Thus pp70S6K has the capacity to regulate gene expression. Recent studies have shown that pp70S6K can directly phosphorylate nuclear factor cAMP-responsive elements modulator (CREM) (de Groot et al., 1994). Activation of p70S6K can occur by activation of a mTOR-dependent pathway targeting Thr389 and an ERK1/2-dependent pathway targeting Thr421/Ser424 (Koltin et al., 1991; Ferrari et al., 1993; Fruman et al., 1995; Lehman et al., 2003; Wing et al., 2005). Mitogen-activated protein kinase (MAPK) has been shown to play a role in transducing extracellular signals into a cellular response. The $p^{42/44}$ MAPK is also known as extracellular signal-regulated kinase (ERK), which regulates cell growth and differentiation (Cowley et al., 1994; Hill and Treisman, 1995).

Phosphorylation of p70S6K (THR421/SER424) and mitogen activated protein kinase (MAPK/ERK) were found to be decreased in the rotavirus severely infected ileum. Since pp70S6K (Chou and Blenis, 1995; Surendran and Kondapaka, 2005; Surendran et al., 2005) and p42/44 MAPK (Cowley et al., 1994; Hill and Treisman, 1995) regulates cell growth, down regulation of these molecules and vacuolation of the ileum villi in the rotavirus infected rats suggest that these proteins likely to contribute to vacuolation of the villi seen in the infected rats. Destruction of villi enterocytes by the virus likely to affect structural and functional integrity of the epithelium and thus contribute to malabsorption.

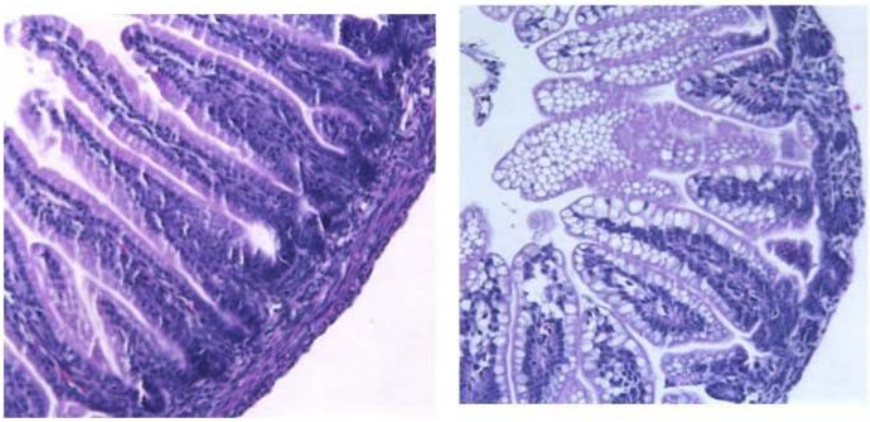

Figure 1: Immunohistochemical staining of rat small intestine at 72 hour post infection. Rat small intestine stained with hematoxylin and eosin showed severe vacuolation in the rotavirus infected villi enterocytes (magnification 20x).

Corticosterone is a glucocorticoid produced by the adrenal cortex, and is a precursor to aldosterone. Changes in the metabolism of glucocorticoids within peripheral lymphatic organs and in immune cells could modulate not only the suppression of cell activation by pro-inflammatory cytokines but also other immunomodulatory processes (Elenkov and Chrousos, 1999; McKay and Cidlowski, 1999). Corticosterone upregulation has been reported during inflammation in IBD patients and colitis (Hanauer, 2004; Ghia et al., 2007). In rats, high-dose of glucocorticoid acutely inhibits protein synthesis, decreases the phosphorylation of both 4E-BP1 and p70S6K (Shah et al., 2000). Rotavirus infected rat showing high level of plasma corticosterone suggests that corticosterone likely downregulates pp70S6K.

Elevated levels of prostaglandin E_2 production was observed in the rotavirus infected intestine (Zijlstra et al., 1999). Upregulation of prostaglandin E2 can induce cell death including epithelial cell death (Surendran, 2001). The viral protein NSP4 was found to have toxin-like activity (Zhang et al., 2000) and induced nitric oxide synthase (Borghan et al., 2007). Upregulation of nitric oxide synthase resulting elevated levels of nitric oxide leads to peroxynitrite production, to inhibit cell migration and cell growth (Surendran, 2008), and functional deficit in the severely infected intestine, as enteric neuron contact with smooth muscle is important for normal contraction and relaxation of the gastrointestine (Surendran, 2008). Meanwhile normal levels of nitric oxide contribute to cell migration and cell growth. Thus nitric oxide induces cell growth or cell death in a time and dose dependent manner.

Normal phosphorylation of myosin light chain (pMLC) is important for normal smooth muscle contractility (Patil et al., 2004). Decreased pMLC in the rotavirus infected rat ileum suggests that contraction of smooth muscle is likely suppressed in the rotavirus infected ileum.

TREATMENT OF THE DISEASE

Intake of fluid is important to avoid oral dehydration. In healthy subjects the disease lasts only a few days because of immune system. Antibiotics are administered intravenously. Electrolyte solution is administered into the vein of dehydrated patients. The rhesus rotavirus reassortant vaccine (Rotashield) was withdrawn from the market after the discovery of a rare association with intussusception. Vaccines to prevent rotavirus infection are available: the pentavalent bovine-human reassortant vaccine, Rotarix by GlaxoSmithKline and the monovalent human rotavirus vaccine, RotaTeq by Merck (Dennehy, 2008). Both are taken orally and contain disabled live virus. Both vaccines are safe and effective in western industrialized countries and in Latin America. Proposal is underway to develop new candidate vaccines with most safe, effective and economically affordable for the children of Third World nations (Ward et al., 2008).

CONCLUSION

Rotavirus severe infection decreased pp70S6K, pMAPK and pMAPK levels and increased prostaglandin E2 and nitric oxide toxicity to impede normal development of villi enterocytes of the small intestine and the consequent change in structure and function of the epithelium. These changes are likely to lead malabsorption and abnormal motility of the gastrointestine during rotavirus infection.

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