

Epidemiology and Potential Methods for Prevention of Neonatal Intestinal Viral Infections

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Viral infections of the gastrointestinal tract remain a major problem during the neonatal period. In addition to causing acute diarrhea, rotaviruses and other enteric viruses may be involved in the pathogenesis of necrotizing enterocolitis and other neonatal enteric diseases. There are several potential methods for the prevention and treatment of gastrointestinal viral infections. Antiviral immunoglobulins might be used to inhibit intestinal viral replication. Since only small concentrations of serum immunoglobulins are present at mucosal surfaces, oral administration of immunoglobulins might be utilized to maximize antiviral efficacy. Alternatively, inhibitors of specific glycoproteins of virus-cell binding might be used to prevent the productive infection of intestinal epithelial cells. In addition, since many enteric viruses require proteolytic enzymes for protein cleavage, protease inhibitors may prove effective for inhibition of intestinal viral replication. At this time, these methods have proven useful for the inhibition of rotavirus infection in experimental animals. The successful application of these and other methods for the prevention of enteric infections in humans might substantially reduce the morbidity and mortality associated with enteric diseases in high-risk neonates.

Viral infections remain important causes of morbidity and mortality in low-birth-weight infants. While a number of organ systems can be infected by virus during the newborn period, the neonatal gastrointestinal tract is an important target organ for these pathogenic agents. Epidemiologic studies have demonstrated that the replication of viruses within the neonatal intestinal tract can lead to a number of clinical consequences, ranging in severity from self-limited watery diarrhea to more prolonged diarrhea and, in some cases, to necrotizing enterocolitis [1-4]. In some cases intestinal infection with viruses can be inapparent; however, infants with asymptomatic infections may serve as reservoirs of virus that can be transmitted to more susceptible infants [5]. Epidemiologic studies also indicate that nosocomial infection can be transmitted by medical and nursing staff who have asymptomatic infections or infections associated with minimal symptoms [6].

No approved chemotherapeutic agents have been shown to be effective for the treatment of enteric viral infections. Efforts thus have to be directed at the im-

provement of methods for the prevention of disease transmission in the neonatal environment and the development of new therapeutic regimens for the treatment of viral enteric disease when it occurs. Traditional methods of infection control such as hand washing and barrier protection (e.g., gloves and gowns) remain the mainstay of disease prophylaxis. It is important to recognize enteric diseases in infected individuals and to institute appropriate measures as soon as infection is recognized. The complicated nature of gastrointestinal disease in neonates can delay recognition of disease and the institution of such measures. This is especially true in infants of very low birth weight, in whom several mechanisms in addition to the intestinal replication of infectious agents can cause changes in stool consistency and stool number. Moreover, it can be difficult to distinguish cases of necrotizing enterocolitis in which a transmissible agent may be involved from those in which no agent can be implicated. Institution of diagnostic assays for the detection of viral enteric pathogens such as group A rotaviruses and enteric adenoviruses might facilitate the identification and isolation of infected infants and medical staff members capable of transmitting infection to susceptible neonates [2, 7]. However, it is unlikely that such testing will result in the identification of all infected infants and care givers, and the testing would not

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prevent the acquisition of infection from fomites or other environmental sources. In addition, viral agents that cannot easily be identified by available assay systems, such as non-group A rotaviruses, astroviruses, coronaviruses, or Norwalk-like viruses, may be involved in outbreaks of enteric infections in neonatal intensive care settings [8–10]. Thus, it is necessary to develop additional measures for the prevention of enteric disease in susceptible newborns.

In this review we will discuss possible therapeutic interventions for the prevention of neonatal enteric infections. We will focus on the prevention and treatment of rotavirus infection since more information is available for this agent than for other viruses that might cause enteric infections in neonates. However, measures that are developed for the prevention and treatment of rotavirus infections in neonates may be applicable to the control of other enteric viruses that share pathogenic mechanisms and modes of transmission.

Pathogenesis of Neonatal Rotavirus Infections

Rotaviruses are double-stranded RNA viruses of the family *Reoviridae* that have been recognized as the most common agents of severe viral gastroenteritis in infants and young children in virtually all areas of the world [11]. Antigenically diverse, the rotaviruses have been divided into at least six distinct serotypes on the basis of neutralization with monoclonal antibodies or polyclonal immunoglobulins from experimental animals that react primarily with the VP7 surface protein of the virus [12]. However, immunization with bovine serotypes of rotaviruses can provide some degree of protection against human strains that have been shown to be antigenically distinct *in vitro* by neutralization analyses [13]. Also, infection in the neonatal period can confer protection later in life from serious infection with a wide range of rotavirus strains [14]. Thus, some degree of immunologic cross-protection against symptomatic infection with heterologous serotypes probably occurs following infection. To some extent this cross-protection probably is mediated by shared epitopes located on the VP4 surface protein and/or on conserved regions of VP7 [15–17]. It is also possible that conserved epitopes on other proteins may provide some degree of cross-protection, perhaps by the mediation of T cell reactivity [18].

Rotavirus infections in children 6 months to 2 years of age are strongly associated with the development

of symptoms characteristic of viral gastroenteritis, such as loose watery stools, fever, vomiting, and abdominal cramps [11]. However, the clinical consequences of rotavirus infection in neonates are more varied. Some investigators have reported that rotavirus infections in this age group are largely asymptomatic, while others have shown a high correlation between rotavirus infection and clinical symptoms [1, 5, 19–21]. Rotavirus infection has also been associated with the development of bloody diarrhea, pneumatosis intestinalis, and frank necrotizing enterocolitis in susceptible newborns [22–25].

Undoubtedly, some of the variation in response to infection is related to host factors such as gestational age, postnatal age, nutritional status, levels of maternal antibodies, and the maturity and integrity of the gastrointestinal tract. However, the exact roles of specific factors that confer protection against symptomatic infection have not been clearly delineated. Serum IgA, intestinal IgA, T cell-mediated defenses, and nonimmunologic factors such as interferons and intestinal mucins, for example, may play a role in providing protection. It is also possible that some of the discrepancies are related to the difficulty in evaluating the signs and symptoms of gastrointestinal infections in very young neonates [26–29]. In addition to these host-related factors, genomic regions of the virus that appear to correlate with low degrees of symptomatic infections in neonates have been identified, suggesting that the variable expression of disease may be related to the inherent pathogenicity of the virus [30].

The definition of the clinical spectrum of rotavirus infection in neonates has been further complicated by the nonspecific results generated by some first-generation, commercially available enzyme immunoassays for the detection of rotaviruses in tests of stools from neonates [31]. Fortunately, this problem appears to have been alleviated by the introduction of more specific immunoassay systems for rotavirus detection [32].

Prevention of Rotavirus Infections

In light of these factors, several strategies are available for the prevention of rotavirus infection. These approaches can be divided into immunologic interventions, which make use of specific antibodies to the virus, and nonimmunologic interventions. Among the immunologic interventions, active oral immunization with live attenuated organisms has been the

most widely investigated for the prevention of disease outside the neonatal period [13, 33]. However, it is unlikely that active immunization with live viruses will play an important role in protection of high-risk neonates until its safety and the ability of neonates to mount an effective immune response following oral immunization have been demonstrated. The possibility of transmission of rotavirus to other susceptible individuals in the hospital, such as children with congenital or acquired immunodeficiencies [34], must also be considered. While it is possible that maternal immunization may result in the transplacental passage of antibody to their offspring, the irregularity of transmission of antibodies before the last month of gestation would preclude the benefit of antibodies to the premature infants who may be most in need of protection.

For these reasons, the passive administration of antibodies with antiviral activities might be considered as a means of preventing viral infections in high-risk neonates. Since rotaviruses replicate primarily in the epithelial cells of the gastrointestinal tract, one approach to passive immunization would be the oral administration of immunoglobulin preparations. In fact, we have shown that orally administered human immunoglobulins derived from breast milk or serum can survive within the gastrointestinal tract and inhibit the intestinal replication of rotaviruses in humans and animals [32, 35]. We also have found that oral administration of immunoglobulins derived from cows or chickens immunized with rotaviruses can confer protection against infection in experimental animal model systems [36, 37]. Limited data for humans also suggest that the administration of such immunoglobulins can limit the intestinal replication of rotaviruses and other enteric pathogens [38]. In fact, the oral administration of a human serum-derived IgG-IgA mixture to neonates has recently been shown to prevent the development of necrotizing enterocolitis in infants who could not receive human milk [39]. However, the limited oral intake possible by very-low-birth-weight infants early in life would limit this approach. The use of orally administered immunoglobulins would be particularly problematic during outbreaks of necrotizing enterocolitis, since oral feedings are usually withheld in the presence of signs or symptoms suggestive of intestinal dysfunction. This restriction would make it difficult to employ oral immunoglobulins that have short biologic half-lives and thus require repeated administration.

On the other hand, parenteral immunoglobulins can be safely administered to human infants regardless of the integrity of their gastrointestinal tracts [40, 41]. Since most adult blood donors possess antibodies to rotaviruses in response to past infections, most lots of available immunoglobulin preparations can be expected to have antibodies to a wide range of human rotaviruses. The question can be raised about the value of parenterally administered antibody for the prevention of infection at a mucosal surface. While we cannot provide definitive answers with regard to the protective effect of parenteral antibody, we have found that most infants who received parenterally administered immunoglobulins that contained antibodies to rotavirus developed antibodies that could be measured in their fecal or intestinal samples. As depicted in table 1, 12 of 13 infants given a single 500-mg/kg dose of immunoglobulin intravenously achieved intestinal or fecal levels of antibodies to rotavirus of >20 ng/g of stool or intestinal contents and eight achieved levels of >100 ng/g. These antibodies were capable of binding to solid-phase rotavirus antigens and of being recognized by antibodies to human IgG in an enzyme immunoassay format. Detectable levels of antibodies to rotavirus were noted in the stools of the infants for up to 13 days following parenteral administration.

Table 1. Intestinal or fecal excretion of antibody to rotavirus by infants given a single 500-mg/kg dose of immunoglobulin intravenously.

Sample, patient no.	Birth weight (g)	Age (d)	Maximum concentration of antibody (ng of Ig/g)	Duration of detectable antibody (d)
Ileostomy				
1	930	34	>10,000	10
2	840	20	4,600	13
3	2,970	26	380	8
Stool				
4	720	45	>10,000	10
5	925	15	4,700	6
6	1,560	12	900	6
7	980	31	450	7
8	1,095	7	450	ND
9	690	59	73	ND
10	1,020	148	41	ND
11	1,005	19	40	ND
12	885	49	24	ND
13	1,485	29	<20	ND

NOTE. ND = not determined.

Since all these infants received immunoglobulin from the same lot, the variability noted in the levels of intestinal IgG antibody to rotavirus was undoubtedly related to differences in the permeability of the intestinal tract to serum proteins and to the survival of the IgG within the infant's intestine. The levels achieved in intestinal fluids, as measured in babies with surgical ileostomies, were generally greater than those measured in stools obtained per rectum, a finding which probably reflects breakdown of protein in the small bowel or colon. The younger and smaller babies appeared to have somewhat higher levels of intestinal or fecal antibodies, although the differences did not attain statistical significance.

These studies document that parenteral administration of IgG antibody can result in the prolonged excretion of antibody capable of binding specific antigens. Uncontrolled observations have indicated that the administration of immunoglobulins might be useful for the prevention of epidemics of necrotizing enterocolitis. However, the efficacy of parenterally administered immunoglobulins for the prevention of enteric infections and necrotizing enterocolitis should be the subject of controlled clinical trials.

Chemotherapy for Rotavirus Infections

In addition to these immunologic approaches to the prevention of rotavirus infection, there are several potential methods for the prevention of disease that do not require the administration of specific antiviral immunoglobulins. Such approaches might avoid the expense associated with the collection, testing, and storage of human or animal immunoglobulins and would allow for a greater degree of standardization than that achievable for complex mixtures of polyclonal immunoglobulins.

Although many potential methods are available for the chemotherapeutic inhibition of viral infections, most involve the inhibition of DNA or RNA synthesis with the use of nucleotide analogues. While the use of such drugs in the newborn period is not precluded, it would be preferable to use a therapeutic regimen that did not have the potential for interfering with host nucleic acid replication.

One promising approach to the chemotherapeutic prevention of enteric viral infections is based on the requirement by rotaviruses of proteolytic activation for efficient cellular penetration and viral replication [42, 43]. This activation, which results in the cleavage of one or more surface proteins, is

probably an adaptive mechanism ensuring efficient viral replication within the gastrointestinal tract. Recently, a number of therapeutic agents have been devised that specifically inhibit viral proteases [44, 45]. Furthermore, a number of naturally occurring protease inhibitors present in materials such as soybeans are suitable for human consumption [46].

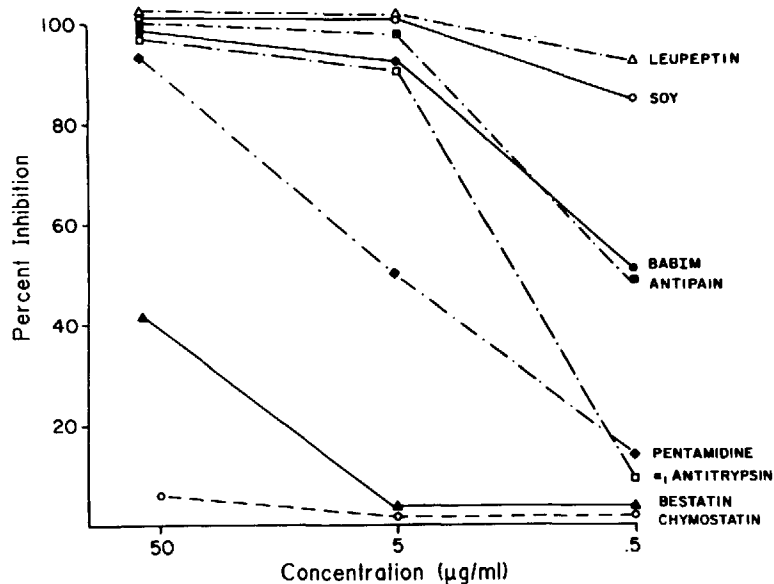
We have investigated the possible efficacy of protease inhibitors for the prevention of experimental rotavirus infections. As shown in figure 1, a wide range of inhibitors are capable of preventing the replication of rotaviruses in cell culture systems [47]. This inhibition, which was noted for a large number of rotavirus strains, occurred when the virus was cultivated in either the presence or the absence of exogenously added proteases. We also have investigated the efficacy of protease inhibitors for the prevention of infection in experimentally infected mice and have found several compounds that prevent intestinal replication in experimentally infected animals and disease transmission in a closed environment. One of the most effective low-molecular-weight inhibitors is the diamidine compound bis(5-amidino-2-benzimidazdyl)methane (BABIM). This compound has been shown to have inhibitory activity for other pathogenic viruses that require proteolytic cleavage for productive infection, such as respiratory syncytial virus [48, 49]. BABIM is highly specific for trypsin and thus might not interfere with proteases necessary for normal nutrition, coagulation, and other metabolic functions.

Many enteric viruses in addition to rotaviruses might be expected to require proteolytic cleavage for efficient replication and thus be susceptible to inhibition by protease antagonists. In fact, preliminary studies in our laboratory indicate that BABIM and related compounds can also inhibit the replication of enteric strains of adenoviruses.

Needless to say, a number of questions must be addressed before protease inhibitors can be considered as potential candidates for use in the prevention of viral gastroenteritis, including those related to safety and pharmacokinetics and to the potential impact of protease inhibitors on food digestion, nutrition, and other metabolic functions requiring enzymatic activation. However, if these questions can be satisfactorily answered, protease inhibitors have the potential for use in the prevention and treatment of a wide range of enteric infections.

Another possible method for the prevention of rotavirus infection has been suggested by data indi-

Figure 1. Effects of various protease inhibitors on the in vitro replication of SA-11 rotavirus in MA-104 cells. Each point represents the degree of inhibition of SA-11 virus plaque formation induced by addition of different concentrations of the protease inhibitors to cultures as compared with plaque formation of controls cultivated in the absence of inhibitor. Reprinted with permission from the *Journal of Clinical Investigation* [47].



cating that a number of glycosylated macromolecules can limit the replication of viruses in mammalian cells. We have found, for example, that mucins and other sialic acid-containing glycoproteins can inhibit the replication of a wide range of rotaviruses in cell culture systems. Furthermore, the feeding of bovine salivary mucins to laboratory animals can prevent both intestinal infection and the development of clinical disease following experimental infection [50]. While the mechanism by which these glycoproteins inhibit virus is not known with certainty, it is likely that they compete with viral binding sites on susceptible cells, thus impeding attachment of virus and preventing the initiation of the infectious process. It is of note that several of the effective sialic acid-containing glycoproteins are found in food sources and that animal mucins have been added to infant formulas used in Japan and Pakistan without apparent untoward effects. It is thus possible that the use of such glycoproteins might be a safe and efficient means of preventing rotavirus and other enteric infections. Additional studies relating to the chemical structure, pharmacokinetics, and mechanisms of action of the active glycoproteins need to be performed before the full potential of this approach can be assessed.

Conclusion

In this review, we have addressed several possible ap-

proaches to the prevention of viral gastrointestinal infections in the newborn period. In light of the complex nature of the neonatal gastrointestinal tract as well as of the pathogens that can infect neonates, it is unlikely that a single modality will prove effective for the prevention of infections under all clinical circumstances. On the other hand, it is possible that each of the approaches will prove useful under defined clinical and epidemiologic circumstances. It is hoped that the application of these or additional modalities, in combination with disease identification and traditional infection control techniques, will result in a decrease in the rate of serious gastrointestinal infections in low-birth-weight neonates and in the mortality and serious morbidity associated with such infections.

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