New Therapies for Calf Diarrhea: Therapy and Prevention for the New Millennium

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Introduction

As we look to the turn of the century, many new theories and technologies are evolving to help us succeed in decreasing the onset of diarrheal disease or diminishing its impact on the neonatal bovine. This discussion will focus on many of these disease agents. The bovine practitioner must realize that definitive work on applied use of many of these factors (e.g. "field studies") is not yet under way. Existing products of which the authors are aware will be mentioned, but should not be interpreted as an endorsement. They are mentioned to provoke thought and provide information about potential future therapies that may be used to bring further success in therapy and prevention of diarrheal disease.

We would first like to discuss agents that alter the ability of enteropathogens to colonize the gut or adhere to the gut through interference of mucosal binding. The second part of this paper will discuss our greater comprehension of new elements of oral rehydration solution (ORS) therapy. We will describe our efforts to manipulate these solutions to enhance net absorption of water and increase the healing of the damaged intestinal villus.

Factors Influencing Gut Enteropathogen Colonization

Interference with mucosal binding

One of the great advances in prevention of neonatal diarrhea has been isolation of purified fimbrial antigens responsible for attachment to binding sites in the gut of the neonate. The primary bacterial antigen in the calf is K99+, a fimbrial antigen found in strains of enterotoxigenic *Escherichia coli* (ETEC). Oral administration of monoclonal antibodies directly to the newborn, or vaccination of the dam to produce antibody-rich colostrum have both been highly successful means of controlling what once was a frequently fatal and very common form of infectious diarrheal disease in the calf.

Because the K99+ binding sites in the gut disappear about 72 hours after the calf is born, transient immunoprophylaxis provided by specific antibodies can be successful in eliminating the threat of this disease. In addition to genetically engineered monoclonal antibodies, products derived from hyperimmunized horses or chickens also have been successful at controlling ETEC.[1] The analagous ETEC virulence factor in swine is the F88+ antigen. Recently, a pineapple juice derivative was used successfully to prevent F88+ strains of *E. coli* from adhering to gut mucosa and causing diarrhea in neonatal pigs. Studies to assess applicability of this therapy to K99+ sites have been proposed.[2]

Loss of coliform fimbrial adhesion may be the mechanism through which juice from the American cranberry protects countless women against urinary tract infections. An immediate inhibition of binding has been described, as well as long-term effects similar to those seen with sub-inhibitory concentrations of antibiotics.[3] Guava, pineapple, mango, grapefruit and blueberry juice have been found to directly inhibit type 1 fimbrial adhesin, but only cranberry and blueberry juice contained an adhesion-inhibiting agent directed against the P fimbriae expressed by uropathogenic *E. coli*.[4] Similar effects against bacterial flora in the intestinal tract have been postulated.[5]

Other studies have been utilized to study inhibitory effects of human milk on the binding ability of enteric pathogens or toxins, and have been summarized.[6] Those that apply most directly to the neonatal calf include efficacy against Rotavirus as well as enterpathogenic, enterohemorrhagic and enterotoxigenic strains of *E. coli* (ETEC). Monnosilated glycoproteins in human milk inhibit adherence of enterohemorrhagic *E. coli*. Fucosylated oligosaccharides inhibit enteropathogenic strains of coliforms. Non-immunoglobulin fractions of human milk and colostrum inhibit ETEC; glycoproteins and free oligosaccharides are thought to mediate these inhibitory effects. Human milk mucin can bind to Rotavirus and protect mice

against experimental infection. Monoclonal technologies ultimately may result in milk additives that could conceiveably protect the calf against specific enteropathogens.

Other factors that inhibit enteropathogens

Factors concentrated in colostrum have long been known to suppress the ability of the enteropathogen to colonize the neonatal gut. Colostrum is rich in lactoferrin, a substance that helps prevent coliform multiplication in the gut by binding iron in the diet. Since coliforms require iron to proliferate, the presence of lactoferrin in the gut helps to suppress coliform overgrowth. This has such significant potential for neonatal health in developing countries that transgenic studies have centered on splicing the lactoferrin-producing segment of the genome into the goat in order to produce milk significantly richer in this natural antimicrobial agent.

Colostral antibody provides some protection against viral enteropathogens in neonatal calves when it is rich in anti-viral antibodies. It is added to milk or milk replacer at 1% of the total volume at each feeding for 7 or more days after birth.[7,8] Appropriate immunization of the cow during the final 40 days prior to gestation may result in production of colostrum with the potential to protect the calf when administered immediately after birth and daily.

Two commercial products containing anti-viral antibodies which can be used to supplement colostral antibody have been marketed in the US. One is derived from bovine colostrum and contains coronavirus-specific antibody as well as K99+ antibody (First Defense, ImmuCell, Portland, Maine). The second product is derived from egg yolks of hyperimmunized chickens (Pro-body Gel, Jorgenson Laboratories, Inc., Loveland, Colorado). A similar yolk-derived product from hyperimmunized chickens has been shown by the Japanese to be very effective in protecting the neonatal calf against rotavirus challenge.[9]

A new vaccine recently was tested at North Carolina State University College of Veterinary Medicine by Dr. Lance Perryman and others. This vaccine uses an epitope common to sporozoites and merozoites of *C. parvum*. Colostrum from hyperimmunized cows protected calves against oral challenge with 10^7 organisms when compared to calves fed colostrum from non-immunized cows.[10] Studies evaluating the protective effect of antibody in human exposure are expected.

An extract from *Guazuma ulmifolia* bark has been used by the Mixe Indians of Mexico for years to treat diarrheal disease. In vitro studies have shown if this extract is in contact with intestinal tissue prior to challenge with cholera toxin, Cl- hypersecretion by the tissue does not occur. The extract did not inhibit PGE$_2$-induced Cl- secretion, but stopped secretion when cholera toxin was pre-treated with the extract. The mode of action appears to be alteration of a specific subunit of the toxin.[11] Since cholera toxin is similar to the heat-stable toxin produced by ETEC, this could have some potential benefit in therapy for K99+ diarrhea. The active constituents appear to be polymeric proanthocyanidins. Catechins and theaflavins in black tea extract were thought to be responsible for reduced Cl- secretion when cholera toxin was treated with an extract of the tea.[12]

A milk replacer additive (Enterocin-C, Nutrizyme, Inc.) containing allicin fructooligosaccharides and gut-active bacteria recently was shown to have no beneficial response in calves challenged with 1.5 X 10^6 *C. parvum* oocysts. Decreasing the challenge by half still resulted in no statistical difference in fecal consistency scores or average daily gain between treated and control calves.[13]

New Factors in Oral Rehydration of Calves with Diarrhea

The goal of fluid and electrolyte therapy is to prevent or reverse metabolic acidosis, solute (Na+ and Cl-) depletion and decline in the extra cellular fluid compartment. For expediency, this discussion is limited to oral electrolyte fluid therapy.

Total Quantity of Liquid Intake

A few new factors should be kept in mind when the decision is made to begin oral rehydration therapy for neonatal diarrheal disease. In the past, we assumed that allowing the calf to ingest milk while suffering from diarrhea only worsened the diarrhea. Calves were held off feed for at least 48 hours while receiving only oral rehydration solutions (ORS). When calves are not fed milk in addition to ORS during diarrheal episodes they are likely to become hypoglycemic, lose weight and experience alterations in glucagon and insulin levels consistent with those that occur in starvation states.[14] Calves with severe diarrhea may require 7 to 8 L of fluid/day (milk plus ORS) to maintain hydration and meet maintenance fluid requirements. Electrolyte solutions do not meet the caloric needs of the calf, but several energy-dense electrolyte solutions exist that will help. Although these solutions are very hypertonic, they will help maintain blood glucose levels in the calf with diarrhea. No adverse effects have been attributed to bacterial overgrowth in the gut or increased severity of osmotic diarrhea in calves receiving these formulations.[15, 16] However, hypertonicity continues to be an issue with ORS in human products, and recommendations may change as to the efficacy of the more hypertonic solutions, especially in osmotic diarrhea.

Factors that stimulate mucosal regeneration and repair

Information gathered by different research groups suggests manipulation of the mucosal healing process may be possible in the future. Some factors are particularly suited to stimulate migration of gut epithelial cells onto the denuded segment of the villus, and other factors stimulate local cell proliferation. Agents such as glutamine, arginine, as well as prostaglandins and certain growth factors have been shown to stimulate mucosal repair in in vitro studies. Fetal bovine serum and bovine serum concentrates may contain some of these factors that assist in rapid mucosal repair.

Glutamine is an amino acid that may play a role in diarrheal disease through two mechanisms: (1) Maintainance of mucosal integrity of the gut and (2) A direct effect on absorption of solute in the bowel. Glutamine is a limiting amino acid in mucosal regenerative processes. It can prevent radiation damage to the gut if administered prior to radiation therapy and is used in some oncology protocols. It spares the integrity of the mucosa in nutrient-deprivation states in many species, and for this reason it is an integral part of hyperalimentation therapy in humans and animals when it can directly contact the mucosa. It has been demonstrated that glutamine will maintain the integrity of the mucosa in nutrient deprivation situations in the neonatal calf.[17] When glutamine was combined with TGF alpha (a growth factor found in breast milk), it stimulated recovery from ischemia/reperfusion injury[18,19] and also in porcine Rotavirus enteritis.[20] Neither substance was effective alone.

Arginine is an amino acid that also has been shown to have the capacity to promote intestinal repair. It is more effective during restitution (the first stage of repair of the gut, during which columnar cells migrate to cover basement membrane) than is glutamine. To fully benefit from the positive effects of arginine, it may be necessary to have it present prior to the introduction of the enteropathogen.

Bovine serum concentrate is a defibrinated, spray-dried commercial product used as a pig and dog chow additive that is reported to enhance performance of early weaned pigs.[21,22] It is FDA-approved, inexpensive, and has been included as a dietary component of veal calf milk replacers in recent years. About 20 growth regulatory peptides and cytokines are estimated to be present in serum.[23] Investigations are ongoing in third world countries to evaluate BSC as a therapeutic agent for diarrhea in infants. Increased weight gain, improved energy absorption and reduced fecal output occured when BSC was administered orally to children with malnutrition and acute diarrhea.[24] Studies are in progress to assess whether BSC will modulate the severity of *C. parvum* diarrhea.

Strong Ion Solutions

It is now recommended that selection of ORS be made based on strong-ion difference of solutions. ORS solutions that alkalinize most effectively are those in which the difference between the strong ions in the solution exceeds that of plasma (about 45 mEq/L). In order to determine if a strong-ion solution is advisable, the serum concentration of Na+, K+, and Cl- and the degree of acidosis of the calf should be evaluated. If the sum of the cations (Na+ plus K+) minus the major anion (Cl-) is less than 40 mEq/L or the *anion gap* [(Na plus K) minus (Cl plus CO_2)] is greater than 16 mEq/L, the calf is acidotic. If the acidosis is severe, an electrolyte solution with a strong-ion difference greater than plasma (45 mEq/L) will be most alkalinizing. Examples include Lifeguard® (SmithKline Beecham), Lifeguard® HE (SmithKline Beecham), and Biolyte® (Upjohn). Scouring calves with normal serum electrolyte concentrations can be maintained on solutions with a small strong-ion difference like Resorb® (SmithKline Beecham).

Pathophysiology, glucose and glutamine

In primary hypersecretory diarrhea (as occurs in ETEC), cells on the tips of the villus are not damaged, although Cl- secretion from the crypt cells results in net Cl-, Na+ and water loss into the bowel lumen. Glucose-coupled Na+ absorption can occur in the mature enterocytes on the villus tip. This makes it possible for the human with cholera or the calf with K99+ ETEC to passively absorb Na+ (and therefore water and Cl-) efficiently enough to overcome the simultaneous Cl-hypersecretion from the crypt cells. This is the primary reason why 2% glucose (and glycine) are presently added to ORS. Glutamine is similarly capable of stimulating glutamine-coupled Na+ absorption in the mature enterocytes.

In other portions of the villus, electroneutral (commonly referred to as neutral) NaCl absorption also is occurring. This absorptive mechanism is driven by glutamine and remains intact even though the tips of the villus may be damaged, disrupting the glucose-coupled Na+ absorption. Most of the diarrheal diseases with which the calf must deal are *not* simple hypersecretory events; they are osmotic diarrheas resulting from damage to the mature enterocytes, with subsequent malabsorption/maldigestion. Crypt-cell Cl-hypersecretion also plays a role in many osmotic diarrheas. The most widespread agent in the US that causes osmotic diarrhea is cryptosporidiosis. *C. parvum* causes severe villus atrophy, osmotic diarrhea, and hypersecretion of Cl- (and thus Na+ and water) from the crypt cells. Effective pharmacologic preventative therapy is unavailable, and mucosal regeneration and repair takes from 10 to 14 days following infection. Rotavirus and

coronavirus also damage the tips of the villus, resulting in an osmostic diarrheal process.

Factors improving the absorptive capacity of the villus

A study by Brooks, et al showed inclusion of GLN in an oral electrolyte solution administered to K99+ infected calves resulted in water and electroyte absorption beyond that provided by glucose-based solutions alone.[25] This would be expected, since both the glutamine- and glucose-coupled Na+ absorption will still occur at the villus tips in ETEC and the glutamine-driven neutral Na+ absorption still will be occurring in the less-mature enterocytes on the edge of the villus. The combined works of Argenzio, Rhoads and others support the conclusion that glutamine stimulates H_2O/electrolyte absorption beyond that of glucose, probably through the mechanisms of Na+-coupled and neutral-Na+ transport previously mentioned.[26,27,28, 29]

The villus atrophy that occurs secondary to *C. parvum* infection can be expected to impair glucose- or glutamine- coupled Na+ absorption, but may leave areas of the villus capable of glutamine-stimulated neutral Na+ absorption. Although the neutral NaCl absorptive mechanisms remain functional in osmotic diarrhea, this mechanism is directly inhibited by the prostaglandin (PG) release that occurs locally with destruction of the tips of the villus. At the same time, Cl- secretion is enhanced in *C. parvum* infection by PG release.

In the disease processes where mature enterocytes are lost, inhibition of PG through administration of nonsteroidal anti-inflammatory drugs (NSAIDs) actually increases the severity of villus damage due to the cytoprotective effects of PGE_2. Thus, NSAIDs may be contraindicated in *C. parvum* (and probably viral) enteritis because they may enhance collapse of the villus. Studies by Argenzio have shown that Cl-hypersecretion can, however, be controlled through the use of such inhibiting agents as somatostatin. This drug has a direct inhibitor effect on the enteric nervous system of the gut, and stops hypersecretion of Cl- without resulting in villus collapse.

It is our hypothesis that somatostatin must be utilized to control the enteric nervous system in order to modulate the inhibitory effects of PG on the glutamine-stimulated neutral NaCl absorption. Somatostatin also should suppress Cl- hypersecretion in the crypts. These 2 factors would decrease production of diarrheal fluid and result in increased absorption of Na+, Cl- and water. Naylor, et al found that glutamine added to ORS resulted in more severe expression of diarrhea in calves with viral diarrhea.[30] This also supports our hypothesis, since they did not administer somatostatin to their calves. Therefore, the PG inhibition of glutamine-driven neutral Na+ absorption never occurred. Since the glutamine was not allowed to express its beneficial effect, it simply

acted as another osmotically active agent in the gut, contributing to severity of diarrhea. In support of this hypothesis, we have found that *C. parvum*-infected calves given a glutamine-based ORS, combined with systemic somatostatin therapy, experienced lower mean daily fecal outputs and significantly higher urine outputs than calves receiving a popular high-glucose, high-sodium, potassium-rich strong-ion ORS.

Summary

Many varied approaches are evolving that will help us more effectively prevent and treat diarrheal disease in the future. Some factors will act directly against the entropathogenic agent. Other therapeutic agents will enhance healing of the damaged intestine, increase the ability of the patient to absorb NaCl and water, or will curtail secretion of Cl-, Na+ and water. We hope this has helped prepare you for some of the changes you may expect to see in the future. We also hope we have provided a basic understanding of why some of these new findings should help us develop a more efficient means of therapy and prevention of neonatal diarrhea.

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