D(-)-lactic acid-producing probiotics, D(-)-lactic acidosis and infants

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There is mounting evidence that ingestion of selected probiotics can modify disease morbidity for specific conditions affecting humans, and there is growing interest in the amelioration or prevention of disease with probiotics. Modulation in gene expression of the cellular elements of the intestinal mucosa and interbacterial interactions are leading theories as to the mechanism whereby probiotics can effect benefit for the host. Furthermore, gene-environmental interactions are considered to be important in the development of disease in those at genetic risk. With the intestinal tract harbouring large numbers of bacteria, alteration of the microbial environment with probiotic microbes is being considered as a controllable factor that may limit disease expression for those at genetic risk. This reasoning has led to interest in the administration of probiotics to infants. However, there are significant developmental changes occurring in many organ systems from the time of parturition and during the first months of life. Because there is little in the published scientific medical literature regarding the effects of long-term administration of probiotics to infants, potential problems must be considered; one such issue is that of administration of D(-)-lactate-producing probiotics. An appraisal of the current knowledge of this potential adverse effect is the subject of this communication.

Key Words: *D-lactic acidosis; Infants; Probiotics*

There are a number of potential benefits to the ingestion of probiotics that are particularly significant to infants and children. Perhaps the most documented therapeutic application of probiotics has been for infectious diarrhea, a condition that remains a significant concern due to morbidity, mortality and health care resource use. Moreover, bacterial resistance and problems related to the use of antibiotics have generated interest in new therapeutic modalities. Three reviews (1-3) have concluded that probiotic organisms are effective in the treatment of infectious diarrhea by reducing the duration and frequency of diarrhea. In addition, probiotics may have greater benefit when administered earlier in the course of illness (4,5) before significant disease is present (6), and may provide for beneficial long-term clinical impact on growth among those children in developing countries who are at risk from repeated infectious exposures (7). Whether long-term prophylactic probiotic administration can prevent infectious diarrhea remains to be proven (1,8,9); however, two recent meta-analyses (10,11) yielded evidence that probiotics may be useful in the prevention of antibiotic-associated diarrhea.

Probiotiques producteurs d'acide D-lactique, acidose D-lactique et nourrissons

De plus en plus de données tendent à confirmer que la prise de probiotiques sélectionnés peut modifier le cours de certaines maladies chez l'homme, et l'amélioration de la santé ou la prévention de maladies par les probiotiques suscitent un intérêt croissant. La modulation de l'expression génique de certains éléments cellulaires de la muqueuse intestinale et les réactions entre bactéries sont les principales théories visant à expliquer les mécanismes par lesquels les probiotiques peuvent produire un effet bénéfique chez l'hôte. De plus, les interactions gènes-environnement jouent, croit-on, un rôle important dans l'apparition de maladies chez les personnes qui ont une prédisposition génétique. Compte tenu du fait que l'intestin abrite un grand nombre de bactéries, la modification du milieu microbien par les probiotiques est perçue comme un moyen maîtrisable, qui peut limiter l'expression d'une maladie chez les personnes génétiquement prédisposées. Ce même raisonnement a conduit à l'administration de probiotiques chez les nourrissons. Toutefois, la période entre la naissance et les premiers mois de vie est témoin d'une multitude de changements importants dans de nombreux systèmes organiques. Comme il existe peu de données publiées dans la documentation médicale sur les effets de l'administration prolongée de probiotiques chez les nourrissons, il faut envisager l'existence possible de différents problèmes; parmi ceux-ci figure l'administration de probiotiques producteurs de D-lactate. Tel est le sujet du présent article : l'évaluation des connaissances actuelles sur l'effet potentiellement nocif de cette substance.

For infants and young children, another area in which small studies have reported benefit has been the use of probiotics for the prevention of dental caries (12) and atopic disease (13,14). Moreover, a preventive effect for atopic eczema beyond infancy is reported on four-year follow-up of those infants who had received perinatal probiotic administration (15). Along with the demonstration that the inoculation of germ-free mice with a commensal organism may upregulate expression of genes associated with gastrointestinal development and maturation (16), the influence of bacteria (and potentially artificially applied bacteria in the form of probiotics) on the health of children could be significant. In essence, by altering host-environment interactions, it is speculated that probiotic administration to the very young infant could alter time of onset or ultimate disease expression. However, clinicians should be aware that, at the current time, probiotic administration is a largely unregulated area of medical therapy without governmental end product quality control testing. There are concerns regarding the administration of probiotics to young infants, which include the unique condition of D-lactic acidosis.

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METABOLIC ACIDOSIS

Acidosis in humans may result from two major causes. The first is inadequate pulmonary excretion of CO_2 (eg, respiratory acidosis) and the second is alterations in the balance between production and excretion of acid (eg, metabolic acidosis). In the latter, systemic acidosis may result from the increased accumulation of blood hydrogen ion concentration due to the inadequate excretion of hydrogen ions or excessive loss of bicarbonate in the urine or stools. Metabolic acidosis may also occur as a consequence of increased absorption of hydrogen ions from the intestinal tract. The two sources for increased organic acids in the gastrointestinal tract are increased intake from an exogenous source or increased endogenous production.

The clinical manifestations of metabolic acidosis are non-specific. One manifestation can be hyperventilation. Other clinical manifestations involve neurological signs and symptoms and, in the young, these may include vomiting, altered consciousness, poor feeding, inappropriate behaviour and crying, slurred speech, ataxia and coma (17). Chronic acidosis is associated with poor growth (18). Making a diagnosis of acidosis is difficult at any age because of the nonspecific nature of the signs and symptoms involved. In the very young, this is compounded by difficulties in eliciting signs and symptoms in young patients. Adding to the complexity of diagnosis is that normal physiological parameters change rapidly in the developing human infant. For instance, the normal respiratory rate for an infant in the first three months of age is 35 to 55 breaths/min whereas in the older child, 14 to 22 breaths/min is normal (19). Crying can be a normal phenomenon or a symptom of a problem in infants. The amount of time spent crying peaks at around six weeks of age with crying for up to 3 h/day; it then decreases to approximately 1 h/day by three months of age (20). Sleep time also changes; in the first month of life, the average infant will sleep approximately 16 h/day, compared with 13 h/day for a one-year-old (20). Increased or decreased amounts of sleeping may signify a medical problem, but the diagnosis of this is difficult. Thus, due to the difficulty in diagnosis of a condition with nonspecific signs and symptoms such as metabolic acidosis in the very young, diagnosis requires clinical perception and relevant laboratory analysis.

INFANT PHYSIOLOGY

The infant brain undergoes rapid enlargement in size during the first two years of life, accompanied by a period of rapid cognitive development (21). Metabolic acidosis can have a negative impact on developmental outcome in infants (22).

Nutrient digestion is not fully developed at birth. Each of the different digestive enzymes produced by the exocrine pancreas appears at a distinct time in gestation and their functional capacity increases postnatally (23). For instance, pancreatic amylase is scarcely detectable at birth and begins to rise after the first month, continuing to increase during the first two years. Other sources of amylase (breast milk, intestinal, salivary) also confer some benefit until the infant matures and the pancreas assumes a more dominant role. In contrast, the developmental pattern of disaccharidases reveals that full term infants are able to digest lactose and other disaccharides from birth (24).

Lowering blood pH, which occurs with systemic acidosis, leads to physiological responses to normalize pH in the host, including stimulation of the respiratory centre to increase the respiratory rate; the kidneys also increase excretion of hydrogen

ions. Compared with older children and adults, acid loading of infants results in larger falls in blood pH and total CO_2 , a smaller and less rapid fall in urinary pH, and much smaller increases in urinary titratable acid and ammonium. The immaturity of renal acidification accounts for the relatively large changes in blood pH resulting from minor changes in diet. During acidosis, older subjects can double or triple their baseline rates of acid secretion, whereas the infant is already operating at relatively higher rates of acid secretion and is limited in the ability to further increment this response (25). Clinically, the immaturity of renal acidification means greater metabolic acidosis risk to the infant than the older child for the same accumulation of hydrogen ions in the blood.

D(-)-LACTATE IN HUMANS

L(+)- and D(-)-lactate are optical isomers that differ only in the position of the alpha-hydroxy group. The predominant form of lactate normally found in the blood of humans and other vertebrates is L(+)-lactate, which is derived from pyruvate by the action of L-lactic dehydrogenase. Normally, small quantities of D(-)-lactate may be found in the blood (26) and excreted in the urine of humans (27). Humans can metabolize D(-)-lactate (28) at a rate 30% slower than L(+)-lactic acid (29), and renal clearance of D(-)-lactate is slower than its optical isomer (29-31). Very small amounts of D(-)-lactic acid can originate from its generation by the endogenous methylglyoxylase pathway (32). However, if blood D(-)-lactic acid levels were elevated, the source for this elevation would be as a result of absorption from the intestinal tract either following an ingestion of D(-)-lactate (33) or following increased production in the intestinal tract. In situ rat studies have demonstrated that, with the administration of D- and L-lactic acid to the jejunum, absorption of the L-isomer is lower than that of the D-isomer (34).

Intestinal bacteria express either a D(-)- or an L(+)-lactate-specific dehydrogenase, or both (35,36). Additionally, some *Lactobacillus* species have DL-lactate racemase which catalyzes the conversion between D(-)- and L(+)-lactate (35). Thus, colonic D(-)-lactate may be formed either from pyruvate by bacterial D(-)-lactate dehydrogenase or from L(+)-lactate by racemization (37). In addition, relative expression levels of L(+)- and D(-)-lactate have been shown in vitro to be affected by bacterial growth conditions (eg, sodium acetate, pH) for some bacterial species (38,39). Carbohydrates such as hexoses (eg, glucose, galactose and fructose) that are ingested into the intestinal tract are fermented by bacterial glycolytic pathways to pyruvate and either L(+)- or D(-)-lactate. L(+)- and D(-)-lactate are intermediary products that other colonic bacteria can metabolize to short chain fatty acids (eg, acetate, butyrate and propionate), and are used for energy by mucosal cells of the colon (35); lactic acid in itself can affect microbial survival.

D(-)-LACTIC ACIDOSIS

D-lactic acidosis is an unusual condition manifested with non-specific signs and symptoms similar to other causes of metabolic acidosis. There have been no large cohort studies but there have been two reviews of the condition that have detailed many of the case reports in the literature before 1999 (28,37); additional reports in children have been subsequently published (40,41). None of the reports included infants younger than one year of age, although a few young children have been

diagnosed with this condition. Clinically, most of the patients have presented with nonfocal neurological signs and symptoms such as confusion, ataxia, dysarthric speech, aggressiveness, inappropriate behaviour, stupor and even coma. The younger the patient, the more often episodes of irritability and drowsiness are reported; occasionally, hyperventilation was noted (40-45).

The condition has been almost exclusively recognized in patients with significant reductions in the small intestinal absorptive capacity following intestinal bypass surgery or in short bowel syndrome (28,39-41), essentially leading to a greater amount of fermentable products reaching bacteria in the intestinal lumen. With the absorption of organic acids into the body and a lack of compensatory increase in renal clearance, symptoms may develop. Although different organic acids may be increased in episodes of short bowel syndrome acidosis, it is mainly the D(-)-lactic acid load in blood and tissues that affects the serum pH (46). The use of non-D(-)-lactic acid-producing organisms to recolonize the bowel of patients with bacteria that do not produce (or produce lesser amounts of) D(-)-lactic acid has been attempted with success (47,48).

Mesenteric thrombosis, volvulus and Crohn's disease are the common conditions requiring massive small intestinal resection, resulting in short bowel syndrome in these patients. Other conditions in which there are single case reports of patients who developed D-lactic acidosis included a patient with a feeding tube in the colon (49) and a patient with exocrine pancreatic insufficiency (50) but most patients with short bowel syndrome and these other conditions do not develop D-lactic acidosis (51). Limited in-depth assessments of the compositional changes of intestinal microbiota have been performed but, in one such study (52), microbial analysis in short bowel syndrome suggested that disturbances of the intestinal microflora (either in type or metabolic activity) may play a role in the development of D(-)-acidosis.

DIETARY INGESTION OF ACIDS

Organic acids of dietary origin do not usually pose an acid-base threat in older children and adults because the quantity ingested is not large enough to exceed the capacity of the host to remove them by metabolic means (53). Studies in the English medical literature have described the effects of feeding acidified formulas to otherwise healthy infants (54-56). Jacobs and Christian (54) studied weight gain and stool pH in healthy newborn babies who received either an acidified milk (lactic acid isomer not specified) formula or an evaporated cow's milk/maltose-dextrin formula that was a standard at the time of the study. The acidified formula had a caloric density that was 15% greater than the evaporated cow's milk formula. Babies receiving the higher caloric density acidified formula gained weight faster than the babies receiving the lower caloric evaporated cow's milk formula. Stool pH was lower and stool consistency was greater in babies receiving the acidified formula, compared with babies taking the nonacidified evaporated cow's milk formula. Clinical episodes of metabolic acidosis were not reported. Blood chemistry analysis was not performed. Brusner et al (55) prospectively studied healthy infants under 12 months of age at two different centres. Infants from one centre received an acidified formula containing 0.23 g of lactic acid (isomer not specified)/100 mL of formula. Infants from the other centre received a comparable but nonacidified formula. Nurses performed follow-up evaluations

during twice-weekly visits. Episodes of diarrhea were the primary point of evaluation. Babies receiving acidified formula had fewer episodes of diarrhea than babies receiving nonacidified formula. Clinical episodes of metabolic acidosis were not reported and blood chemistry analysis was not performed. Stolley and Droese (56) summarized the results of feeding healthy newborn infants (in their first three months) a formula containing 0.35 g to 0.5 g of lactic acid to 100 g of milk formula for 10-day periods. They reported that 30% of infants had clinical evidence of metabolic acidosis (paleness, vomiting, retarded weight gain). In follow-up studies, formula with 80% L(+)- and 20% D(-)-lactic acid isomers led to development of severe acidosis symptoms in one infant (56). With the addition of 0.35 g of lactic acid/100 g of formula, there was increased secretion of organic acids in urine, including the D(-) isomer of lactic acid.

INFANTS AND PROBIOTICS

The fetal intestinal tract is sterile but, following birth, there are a number of factors that influence intestinal colonization including mode of parturition, diet, timing of birth and environmental factors (57-59). From a gradually increasing diversity and density of organisms over the first few days of life, the full term breastfed infant creates an environment favouring bifidobacteria (59). While various factors within breastmilk are thought to influence microbial colonization of the infant gastrointestinal tract, the practice of breastfeeding itself can be a source of lactic acid bacteria (60). Taken together with the potential benefits of ingested probiotics in infants, producers of infant formulas are now manufacturing infant formulas supplemented with probiotics in Europe and Asia, and are considering doing so for North America. This concept is not new (61) but there has been little study of the effects of deliberate administration of large numbers of microbes to infants for extended periods of time in the form of probiotic supplementation of infant formulas. Saavedra et al (62) prospectively followed 80 infants aged three to 24 months of age who consumed infant formula supplemented with *Bifidobacterium lactis* strain Bb 12 and *Streptococcus thermophilus* strain Th4 for an average of seven months. Compared with a control group receiving a similar infant formula without added probiotics, no differences were reported for growth or health care attention seeking. These two strains of probiotics used for the study are not D(-)-lactate producers (JM Saavedra, personal communication). Among other trials that have included infants and children, only small numbers of infants under one year of age and very few infants under six months of age were included. These studies used probiotics that were not D(-)-lactate-producing microbes (or it was unknown whether they were D(-)-lactate producers), involved trials of very short duration of probiotics administration, or were trials in which fluid status was closely monitored, making it less likely that acid-base problems would develop (1-6,8-15,57,60,61,63-71). Premature infants have been provided with probiotics microbes, including a combination of *Lactobacillus acidophilus* and *Bifidobacterium infantis* or *Lactobacillus rhamnosus* strain GG (63,64,67). *L. rhamnosus* strain GG is not a D(-)-lactate producer and information as to the strain or whether the *L. acidophilus* strain is a D(-)-lactate producer is not included in the report (67). No studies with the primary end point being the evaluation of D(-)-lactate levels in infants administered microbes producing this molecule have been reported.

SUMMARY

The potential for modifying disease presentation and expression with consumption of probiotics may be greater in infants than any other age group. However, infant organ systems undergo age-specific maturation of physiological processes in a complex interdependent developmental process between host and environment. The exposure of the intestines to microbes can affect the infant both directly and indirectly. Ensuring normal growth and development is of utmost importance if physicians are to advocate deliberate long term ingestion of high concentrations of metabolically active dietary products such as probiotics in vulnerable populations such as infants. Previously, ingestion of acidified formulas by infants has led to problems of acidosis. Anatomically susceptible patients, such as those with short bowel syndrome, have demonstrated that the production of D-lactic acid from intestinal organisms can be problematic, resulting in the development of D-lactic acidosis. For those without anatomic susceptibility to the development of D-lactic acidosis, there are no reports of otherwise healthy infants or children developing D-lactic acidosis. However, no randomized controlled clinical studies, cohort trials or case-controlled trials involving primary analysis of this issue have been undertaken to determine whether infants are at risk for development of D-lactic acidosis following administration of

D(-)-lactate-producing microbes based on physiological susceptibility. Specific studies to determine whether D-lactic acidosis only develops in anatomically susceptible patients or whether infants represent a physiologically susceptible population that can develop D-lactic acidosis following deliberate dietary ingestion of D(-)-lactate-producing probiotics are needed.

The determination of D-lactic acid blood levels is not widely available. Given the difficulty in clinical diagnosis of D-lactic acidosis in infants, it is recommended that caution be exercised in the administration of D-lactic acid-producing organisms to young infants until safety studies are performed. Furthermore, labelling of products available for human consumption with specifics as to whether a given probiotic strain has the capability of producing D-lactic acid would allow clinicians easy access to information when presented with products an infant has been ingesting should D-lactic acidosis be considered as a clinical possibility.

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