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Probiotic control of diarrhoeal disease

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Probiotics have been suggested to be of use in many diarrhoeal disorders, particularly in the prophylaxis and treatment of infectious diarrhoea. Several different preparations are available commercially and they are widely used but consistent scientific documentation of their efficacy is lacking. Although their putative mode of action is not known, non-pathogenic organisms may prevent or displace enteropathogens from colonising the gut. *In vitro* studies suggest that some probiotics may exert a direct inhibitory effect on pathogenic organisms. There is some clinical evidence suggesting a possible role for probiotics in the prophylaxis of infectious diarrhoea in some circumstances, but there is little evidence of a beneficial effect in the treatment of established diarrhoea, except in cases of relapsing *C. difficile* infection. There are no convincing data at present demonstrating efficacy of probiotics in non-infective diarrhoeal disorders. Although the use of probiotics in diarrhoeal diseases is conceptually appealing, their use for this indication is not clearly supported by the available scientific literature at present. Further research into the role of the human microflora in diarrhoeal diseases is needed to aid the selection of appropriate non-pathogenic bacteria for clinical studies. Well conducted controlled clinical trials are then needed in order to determine the place of probiotics in the prevention and treatment of diarrhoeal disorders.

Introduction

Probiotics, (which may be defined as live microbial supplements which beneficially affect the host by improving its microbial balance)[1] have long been suggested to have a role in the management of diarrhoeal diseases. Many different probiotic preparations are available commercially and are widely used. They have been suggested for use as prophylaxis in acute infectious diarrhoea, (such as for traveller's and to prevent antibiotic associated diarrhoea) and as therapy to hasten the resolution of established infective diarrhoea. If a benefit with probiotics could be demonstrated it would be a significant clinical advance as acute infectious diarrhoea is one of the most common illnesses in the world and causes up to 4 million deaths annually in pre-school aged children, mostly in developing countries. In developed countries, acute infective diarrhoea is also common and a frequent cause of presentation to primary care physicians.

The aetiology of acute infectious diarrhoea is diverse and includes viruses, bacteria and parasites so it is unlikely that any single therapeutic approac 1000 h will be effective in all cases. Furthermore, regardless of the aetiology, most episodes are acute and self limiting so it is difficult to show a beneficial effect of a treatment when the natural history of the illness is brief. The mainstay of current management for established infection is supportive treatment with replacement of fluid and electrolyte losses. Specific antimicrobial therapy is not indicated or useful in most instances, although antibiotics are appropriate and effective for reducing the duration and severity of diarrhoea in some bacterial infections, (for example, dysenteric shigellosis and systemic salmonellosis)[2]. However, antibiotics are commonly and often inapprop-riately prescribed as empirical treatment. Antibiotics are effective in the prevention of traveller's diarrhoea and may reduce the attack rate by up to 80-90%[3-5]. Despite this, antibiotic prophylaxis during travel is not generally recommended. The problems of increasing drug resistance, poor compliance, cost and side-effects (which include antibiotic-associated diarrhoea) generally outweigh the potential benefits of prophylaxis in most instances. It follows that if effective non-antibiotic therapy was available that could prevent or shorten the duration of episodes of acute infective diarrhoea, this would be an important advance in therapy. Trials have established that non-antibiotic prophylaxis for diarrhoea in travellers using bismuth in either liquid or tablet form is relatively effective although this mode of management has never been popular[6,7]. The liquid form requires ingestion of inconveniently large volumes while the tablet requires a five times daily dosing regimen

which is unlikely to have high compliance. A probiotic that provided effective prophylaxis for traveller's diarrhoea would have widespread application.

Infective diarrhoea is conceptually an ideal disease where treatment with a probiotic or nonpathogenic bacteria may be beneficial. In the prophylaxis of infection, the probiotic organism may occupy the ecological niche in the gut that a pathogen may otherwise find. Perhaps by altering the microenvironment it may inhibit a pathogen from successfully colonising and exerting its deleterious effects. Similarly, if such a non-pathogenic organism could displace an already established pathogen, recovery from infection may be hastened. How a probiotic may interfere with colonisation by a pathogen in the gut is unclear. It may involve secretion of substances toxic to the pathogen that are either directly inhibitory or alter the local chemical milieu[8]. It may compete with a pathogen for luminal nutrients that are rate limiting substrates or occupy adhesion receptors and inhibit attachment to the mucosa[9]. There may be indirect effects that result from enhancement of host responses such as activation of macrophages or stimulation of secretory antibody[10,11]. These possible mechanisms would be dependent on the ability of the probiotic to survive and colonise the gut. Human and human adapted organisms may be expected to be more successful at colonisation than non-human adapted isolates. The most commonly used probiotic organisms are the lactic acid bacteria, lactobacilli (*Lactobacillus acidophilus*), bifidobacteria (*Bifidobacterium bifidum*) and enterococci, (*Enterococcus faecium*) all of which may be found in the human intestine. Nonhuman derived organisms, such as those used in yoghurt (*L. bulgaricus, Streptococcus thermophilus*) have also been used. Even with human isolates, long term colonisation may not occur and continuous ingestion may be needed to affect an alteration in the host microflora. In human colonisation studies, a reduction in the faecal bacterial enzymes glucuronidase, nitroreductase and azoreductase was evident only as long as *L. acidophilus* was being ingested[12].

There are three main areas of scientific study examining the suitability and efficacy of probiotic agents in infectious diarrhoea. *In vitro* studies have examined theoretically desirable characteristics of probiotic agents in a variety of models. Secondly, there is experimental animal and veterinary data using probiotics in a number of circumstances. Lastly, there is a modest amount of human clinical data available where probiotics have been used for the prevention of a diarrhoeal disease but there are very few human therapeutic trials examining the benefit of probiotics given to treat established diarrhoeal illness.

In vitro studies

For a non-pathogenic organism to survive and exert an effect in the gut several characteristics are desirable. Organisms should have stability in acid, resistance to the toxic effect of host bile and proteases and have the ability to attach to human enterocytes. Organisms should also survive in the presence of faecal bacteria and show antagonism to human pathogens. Demonstration of some or all of these characteristics *in vitro* may aid selection of isolates for clinical studies but unfortunately may not predict survival *in vivo*, particularly with non-human derived isolates.

Activity of putative probiotics against gut pathogens has been demonstrated in a number of ways. In an elegant study, a Caco-2 cell cultured cell line was used as a model to demonstrate inhibitory effects by a *L. acidophilus* isolate against a variety of gut pathogens. Lactobacilli inhibited adhesion of enterotoxigenic *E. coli* and *Salmonella typhimurium* to the cell monolayer and inhibited cell invasion by several organisms including *Yersinia pseudotuberculosis*, *S. typhimurium* and entero-pathogenic *E. coli*[9]. In other work, human gut isolates of lactobacilli, bifidobacteria and enterococci have been shown to inhibit *C. botulinum*[13]. Similarly, a variety of intestinal bacteria have been shown to inhibit *C. difficile*[14]. A commercially available lactobacilli preparation has been shown to neutralise *E. coli* enterotoxin *in vitro*[15] and this has also been demonstrated in animal studies[16] although a clinical trial with this preparation failed to show a protective effect when tested in travellers[17].

Animal Studies

Ligated rabbit ileal loop preparations have been used to test the efficacy of commercially available lactobacilli preparations in reducing fluid secretion due to *E. coli* enterotoxin. A reduction in the loop fluid ratio compared to positive controls was demonstrated but it is not clear what role the other ingredients of the commercial product played in this effect[16]. Other studies have shown an inability of selected lactobacilli to inhibit the heat-labile and heat-stable enterotoxin effects of *E. coli* B7A[18].

In one report, administration of killed *L. acidophilus* extended the survival of suckling mice infected with enterotoxogenic *E. coli* although the results were not unequivocal[19]. In another study the feeding of *Strepto-coccus faecium* concurrently with *E. coli* ameliorated or prevented the induction of diarrhoea in gnotobiotic pigs[20]. As with *in vitro* studies, the applicability of animal studies to humans is variable, being dependent on host factors, the pathogen and the characteristics and preparation of the probiotic.

Human Studies

Although *in vitro* and animal experimental studies may provide supportive evidenc 1000 e suggesting a role for probiotics in the prevention or treatment of infective diarrhoea, human studies provide the only direct information as to the clinical efficacy of these agents. Studies can be divided into colonisation studies, prophylaxis trials and treatment trials. Lactobacilli sp. are among the most studied probiotic agents. A variety of isolates derived from both human and non-human sources have been used in many different formulations, including fermented milk liquid or powder and encapsulated purified organisms. As both the probiotics used and the clinical setting and quality of trials vary it is difficult to directly compare study results.

Diarrhoea prophylaxis trials

The usefulness of probiotics given as prophylaxis for infective diarrhoea has been studied in four different clinical situations: for prevention of antibiotic-associated diarrhoea, for travellers to high risk destinations, in children admitted to hospital and in volunteer challenge studies.

A well conducted double blind placebo controlled trial demonstrated that supplementation of formula with *B. bifidum* and *S. thermophiles* significantly reduced the incidence of acute diarrhoea and shedding of rotavirus in infants admitted to hospital. Infants aged 5-24 months were randomised to receive standard infant formula or the same formula supplemented with both organisms. Of subjects who received the control formula, 8/26 (31%) compared with 2/29 (7%) who received the supplemented formula developed diarrhoea. Furthermore 39% of subjects who received control formula compared with 10% of those who received the supplemented formula shed rotavirus at some stage during the study[21]. Confirmatory studies of these findings are awaited.

Results of trials to prevent antibiotic-associated diarrhoea have been conflicting. A commercial preparation of dried *L. acidophilus* and *L. bulgaricus* has been used in an effort to prevent ampicillin-associated diarrhoea in adult hospital inpatients. The probiotic was co-administered with ampicillin for the first five days of therapy. The incidence of ampicillin associated diarrhoea in the placebo treated group was 14% while no cases were found in the probiotic treated group. Although the numbers in this study were small the data did support a beneficial effect of the probiotic[22]. However in another study, using the same probiotic preparation as prophylaxis against amoxycillin-induced diarrhoea in paediatric patients, no obvious beneficial effect was found[23]. *Lactobacillus* GG in yoghurt has also been used for the prevention of antibiotic-associated diarrhoea. The efficacy of this preparation in preventing erythromycin-associated diarrhoea was studied in healthy volunteers. Subjects receiving the probiotic with erythromycin had less diarrhoea than those taking pasteurised yoghurt as a control, but the number of subjects was small and the data only semiquantitative[24].

The prevention of traveller's diarrhoea has been a popular target for probiotic trials. As diarrhoeal attack rates are so high in travellers to many parts of the world, an effective and convenient mode of prophylaxis pther than antibiotics is highly desirable. In 50 volunteer travellers to Mexico from the USA a commercial lactobacilli preparation was tested in a randomised double blind trial. The subjects received one week of prophylaxis or placebo but over a 4 week observation period, the prevalence of diarrhoea between the 2 groups was not different[17]. In a European study 820 Finnish travellers to southern Turkey were randomised to receive either *Lactobacillus* GG or placebo. The incidence of diarrhoea in the placebo group was 46.5% compared with 41.0% in the probiotic group. An overall protecti 1000 on of 11.8% was claimed, although analysis of the data reveals that this difference was not statistically significant[25]. In a more recent study, the efficacy of two encapsulated lactobacilli strains (*L. acidophilus* and *L. fermentum*) was studied in a randomised placebo controlled trial involving British soldiers deployed to Belize. Overall 282 subjects were randomised to receive one or other of the lactobacilli strains or placebo beginning the day before travel and continuing for three weeks after arrival. The diarrhoeal attack rate was 28% after 4 weeks. However, there were no significant differences in the incidence of diarrhoeal episodes between subjects in any of the three groups after three or four weeks indicating that these lactobacilli preparations were not protective in this geographic area[26].

Diarrhoea in travellers and other clinical situations involves a variety of pathogens. In challenge studies the efficacy of a probiotic can be assessed against a single pathogen, however there are few such studies. In one well conducted double blind randomised study, a commercial preparation of dried *L. acidophilus* and *L. bulgaricus* was given to volunteers. Adult subjects were challenged with toxigenic *E. coli* strains in conjunction with either the probiotic or placebo. No significant difference in attack rate, duration, volume or severity of diarrhoea was noted between the two groups[27].

In summary there is no data that any of the probiotic preparations studied to date can significantly prevent or reduce the risk of traveller's diarrhoea. The data suggesting a benefit in the prevention of antibiotic-associated diarrhoea is

not convincing. There is some evidence that diarrhoeal illness in infants, particularly in a hospital setting may be reduced, but confirmatory data are needed.

Treatment trials

A difficulty in showing benefit with treatment trials in acute diarrhoea is that the natural history of the illness is usually short and self-limiting. There are few studies of probiotics used as primary treatment for established diarrhoeal illnesses. Most evidence for a beneficial effect for probiotics comes from studies of *C. difficile* associated pseudomembranous colitis. Faecal enemas from healthy adults have been shown to hasten recovery from this condition[28,29]. A clear benefit was evident in one study using *Lactobacillus* GG in patients with relapsing antibiotic associated pseudomembranous colitis[30]. Another approach in this infection is the use of non-pathogenic *C. difficile*[14]. Not much evidence is available in other infective diarrhoeal illnesses. An isolated report using *L. casei* suggested that this organism may hasten the recovery of children with acute diarrhoea. Children were randomised to receive *Lactobacillus* GG in a fermented milk product, or as a freeze dried powder or placebo (pasteurised yoghurt). The duration of diarrhoea after commencing the therapy was 1.4, 1.4, and 2.4 days respectively, with a positive weight trend maintained in each group[31].

Probiotics for non-infective diarrhoeal disorders

Probiotics have been suggested to be of use for a range of diarrhoeal disorders in which no enteric pathogen is recognised as causal. For example, various anecdotal claims have been made for a beneficial effect of probiotic use in conditions as diverse as diarrhoea-predominant irritable bowel syndrome, lactose intolerance and inflammatory bowel disease. Although manipulation of the gut microflora with probiotics as therapy in non-infective diarrhoeal conditions is an intriguing area which merits study, there is currently no con 1000 vincing scientific evidence documenting efficacy with this approach.

Summary and conclusions

Evaluation of the available scientific evidence is difficult for many reasons. There are relatively few studies in a wide range of conditions and these are disparate in design. Many clinical studies have involved relatively small numbers of subjects often without a double blind placebo controlled study design. Available studies also reflect a combination of human derived and non-human derived organisms of varying quality and viability used in different doses and delivery systems. For these reasons it is not surprising that the results are so variable. Unfortunately, the promotion of probiotic formulations has preceded scientific evidence establishing their efficacy. Improvements in the selection and preparation of organisms for study will aid research into their use in human illness. Probiotics to prevent and treat diarrhoeal illness is conceptually appealing and is already popular among some health workers and the public. However, there is only a modest and largely inconclusive body of scientific evidence suggesting any clinical benefit with the use of probiotics in diarrhoeal diseases and a consensus panel of experts has recently endorsed this view[32]. Further understanding of the role of the human microflora in diarrhoeal disease is needed as well as insights into the mechanisms whereby probiotics may have a beneficial effect. This may allow better selection of probiotic organisms. Well conducted controlled clinical trials may then establish the usefulness of probiotics in diarrhoeal disease.

Chinese abstract

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