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Effect of Bacterial Infection and Administration of a Probiotic on Faecal Short-Chain Fatty Acids

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Faecal short-chain fatty acids (SCFAs) were determined in children with shigellosis (n=22) or salmonellosis (n=11) prior to treatment and 5 d and 10 d after treatment with an antibacterial drug (TMP-SMX or Polymyxin, 5 d), or Lactobacillus GG (10^10 - 10^11 CFU/d, 10 d), or both had been started. At admission the SCFA concentrations were very low. Acetic, propionic and iso-valeric acid were significantly higher in shigellosis than in salmonellosis. The SCFA concentrations increased significantly during treatment, reaching those of adults by the 5th day and exceeding them by the 10th day, and showed no difference between the diseases after the 1st day. Administration of Lactobacillus GG resulted in increased concentration of propionic acid by the 5th day of treatment and difference in iso-caproic acid in the 10th day samples: it was not found in any child who had received Lactobacillus GG but was present in half of the samples from the group treated solely with antibacterial drug. Iso-caproic acid is not found in healthy adults and may be indicative of Clostridium difficile. The disturbances in microbial ecology of the gut in enteric infections may have different characteristics depending on the aetiological agent. Treatment with Lactobacillus GG promotes recovery of the ecosystem as reflected by the faecal SCFAs.

KEY WORDS: SCFAs; diarrhoea; salmonellosis; shigellosis

INTRODUCTION

Acute enteric infections cause major disturbances in the microbial ecology of the gut. The administration of probiotic strains of lactobacilli has proven to be efficient in the treatment of acute diarrhoea of various aetiology, most likely by promoting re-establishment of normal microbial populations.14,24 Short-chain fatty acids (SCFAs) are the main end-products of anaerobic microbial metabolism of carbohydrates and proteins in the human colon. Alterations in the faecal concentrations and distribution of SCFAs may reflect disturbances in the intestinal microecology, for example those caused by antibiotics17 or malabsorption of some carbohydrates,1,25 as well as some dietary influences.2,30 SCFA concentrations are low in acute diarrhoea,24 but data on the effect of different aetiologic agents on SCFA profiles are scanty.

Lactobacillus GG can survive and temporarily colonise the human gastrointestinal tract.9 Administration of Lactobacillus GG influences the metabolic activity of the normal resident microbiota as shown by alterations in several enzyme activities.9,15 The effect of Lactobacillus GG on faecal SCFAs has been studied only in pre-term infants and no reaction was observed.31 The aim of this study was to investigate the alterations in microbial ecology of the gut induced by acute enteric infection (salmonellosis or shigellosis), and the effect of treatment, including Lactobacillus GG, on the microecology. Modified intestinal microecology was assessed by analysing the changes in faecal SCFA concentrations.

SUBJECTS AND METHODS

Subjects

Thirty-three children with mean age of 5.3 yrs (range 1-13 yrs), admitted to the Children's Clinic of the University of Tartu, Estonia, from
January 1992 to October 1993 due to acute diarrhoea, were included in the study. The clinical criteria for acceptance in the study were: history of diarrhoea from 1 to 3 d, two or more loose stools per day and/or haemorrhagic colitis, fever of 38°C or above and/or dehydration of the second stage. The laboratory criteria included verified diagnosis of salmonellosis (n=11, all *Salmonella enteritidis*) or shigellosis (n=22, all *Shigella sonnei*). An informed verbal consent was obtained from the parents of each patient.

Design of the study

At admission, anamnesis and clinical symptoms such as the duration of diarrhoea prior to hospitalisation, number of loose stools per day, character of stool, nausea, vomiting, stage of dehydration, severity and form of the disease, fever and pulse rate etc. were recorded and routine laboratory tests performed. If the patient was considered eligible for the study according to the clinical criteria he or she was randomly assigned to one of the three treatment groups: antibacterial drug (group ABD), *Lactobacillus GG* (group GG) or both (group ABD+GG).

Symptomatic treatment (i.v. or oral rehydration) was applied according to the need in all groups. Prior to initiating the therapy, stool samples were obtained for isolation of enteropathogenic bacteria and for faecal SCFAs. In the cases where *Shigella* spp. or *Salmonella* spp. were isolated, the child was finally included in the study and the faecal samplings, both for bacteriology and SCFAs, were repeated on days 5 and 10 after hospitalisation. As the randomisation to treatment groups took place before the laboratory criteria for acceptance became assessible the final groups were of unequal size. The SCFA samples were frozen and kept until analysis at -20°C. The effect of treatment on clinical symptoms, stool frequency and character, were followed daily in a diary.

Treatment

ABD (antibacterial drug) group: trimethoprim-sulpha-methoxazole (TMP-SMX; Polfa, Poland) in the dose of 36 mg/kg per day for 5 d (six children) or Polymyxin (Kiev-medpreparat, Ukraine) in the dose of 100,000 U/kg per day for 5 d (three children). GG group: *Lactobacillus GG* (ATCC 53103) in the dose of 10^10^ CFU in three portions per day for 10 d (eight children). *Lactobacillus GG* has been identified as *L. rhamnosus* (strain GG) by genetic methods. ABD+GG group: doses as above, TMP-SMX+GG in 15 children, Polymyxin+GG in one child.

One child in the ABD+GG group developed allergic skin reaction and vomiting by the second day of treatment and the treatment was discontinued. Her SCFA data from the second and third sampling were excluded from statistical analysis.

Determination of SCFAs in faeces

The samples were thawed at room temperature and the aliquots (0.4-0.6 g) homogenised in 2 ml of distilled water containing 3 mmol/l of 2-ethylbutyric acid as internal standard and 0.5 ml of H₂SO₄ (0.5 mmol/l). The homogenate was vacuum-distilled and analysed for SCFAs as described by Zijlstra *et al*. with modifications by Høverstad *et al.*. Chromatograms were recorded, peak areas determined and concentrations (mmol/kg wet weight) calculated with the Shimadzu Data Processor Chromatopac C-R3A. Samples for SCFAs were not available from all subjects at the three sampling times: a few were missed during collection and some lost due to improper storage or too small sample volume taken.

Statistics

The Mann–Whitney U test for unpaired observations was used to compare the faecal SCFA concentrations of different sampling times, treatment groups and diagnosis. Fisher’s exact test was used to test the significance of qualitative differences in SCFA spectra (presence or absence of iso-caproic acid).

RESULTS

On the first day of hospitalisation the faecal concentrations of SCFAs were very low in children with diarrhoea (Table 1). Acetic acid was the only one found in all samples and accounted for 77 per cent of total SCFAs (range 15-0-100 per cent). Iso-butyric, n-valeric and n-caproic were detected in less than half the samples, and iso-caproic was found only in one sample.

The faecal SCFAs also exhibited a difference between the two diagnosis groups on the first day of hospitalisation (Table 1). Most acids, other than butyric (medians 1.3 and 0.7 mmol/kg), tended to be lower in salmonellosis than shigellosis, and the difference was significant for acetic (5.9 and 27.1),
propionic (1·2 and 5·3) and iso-valeric acid (0 and 0·4 mmol/kg, \( P<0·05 \)). The age of the patients, severity of the disease, number of stools per day and the degree of dehydration did not differ between the two diagnosis groups. The duration of disease before the hospitalisation had been longer in the salmonellosis group (mean 2·3 and 1·6 d, respectively, \( P<0·05 \)). Vomiting and blood in faeces were more common findings in shigellosis than in salmonellosis (\( P<0·05 \)), but there were no significant correlations between these symptoms and faecal SCFAs.

By the 5th day the concentrations of SCFAs increased markedly and did not differ significantly between the two patient groups. The total SCFA concentration and that of acetic, propionic and butyric acid were significantly higher compared to the first day (Table 1), and the median values of total and acetic were close to those found in adults. Acetic acid was still the only one present in all samples, accounting for 73 per cent of total SCFAs (range 51–97 per cent).

The antibacterial treatment had no significant effect on SCFAs on 5th day compared with the group where antibiotics were not used (GG). Administration of \textit{Lactobacillus GG} resulted in significantly higher faecal concentrations of propionic acid compared to the group where antibacterial drug was administered solely (Figure 1). Iso-caproic acid was found in two samples, both from the ABD group.

The 10th day samples contained the highest concentrations of SCFAs (Table 1). Compared with the values of 5th day the total concentration and that of acetic, propionic and butyric had significantly increased, and all these major acids were found in all samples. Acetic acid accounted for 65·9 per cent of total SCFA concentration (range 37–93 per cent). The SCFAs exhibited one significant difference between the treatment groups: iso-caproic acid was not found in any of the samples from the groups receiving \textit{Lactobacillus GG} (ABD+GG, 12 samples, GG, eight samples) but was measurable in four of eight samples in the ABD group (\( P<0·005 \), Fisher's exact test). Two of the four were salmonellosis cases treated with Polymyxin and two were shigellosis cases treated with TMP-SMX.
of respective substrates during the acute illness as well as changes in intestinal microbial community. The latter may be a direct effect of the invading pathogen (antagonism, competition for receptors etc) or indirect reaction as 'washout' due to the altered intestinal motility. Marked reduction of anaerobes and shift to comparatively more aerobic faecal bacteria has been reported during acute diarrhoea\textsuperscript{1,10} that may give rise to low SCFAs.

The pathogenesis of infectious diarrhoea differs depending on the aetiological agent and still remains unclear even for some 'classical' pathogens such as salmonella.\textsuperscript{21} Little is known about the effect of different aetiological agents on intestinal microbial ecology in the course of acute infectious diarrhoea. Infection with \textit{Clostridium difficile} and rotavirus have been shown to produce some specific faecal SCFA patterns\textsuperscript{3,4} but no data are available about other enteropathogens.

In this study we observed some differences in the faecal SCFAs with respect to the aetiological agent. Concentrations of acetic, propionic and iso-valeric acid were significantly higher in shigellosis than in salmonellosis. This difference in the concentration may be a reflection of difference in severity of the diarrhoea. Although the number of stools did not show any difference between the diagnosis groups the volume of stools (not measured in this study) is known to be smaller in dysentery. Therefore, water loss could be greater in salmonellosis than shigellosis and SCFAs more diluted or washed out. Even lower SCFA concentrations than in this study have been reported in the acute watery diarrhoea of cholera.\textsuperscript{23} The fact that butyric acid did not follow the difference seen in other acids between the two patient groups, but showed rather an opposite trend, indicates that other related different characteristics of disturbances in the composition or metabolic activity of the resident microbiota are also possible.

Regardless of the type of treatment, the faecal SCFA concentrations showed significant increase by the 5th day of hospitalisation and the increase continued as seen in the 10th day samples. Surprisingly, no data are available about SCFA concentrations in children after 2 years of age. At 2 years of age the concentrations are higher, than in adults, and relative concentrations also partly differ. For example the proportion of acetic acid is higher and that of the minor acids lower.\textsuperscript{16} Although the SCFA patterns showed a tendency of becoming more 'adult like' during the treatment (the molar ratio of acetic acid decreasing and more
different acids present) some minor acids that are always present in health by the age of 9 months of age (iso-butyric and iso-valeric acid) were still missing in some of the 10th day samples, suggesting that the intestinal ecosystem had not yet fully recovered.

Lactic acid bacteria have been used in traditionally fermented food systems for hundreds of years and have a long list of suggested health benefits varying from treating diarrhoea to tumour suppression. Still, the opinions on their role in promoting health in humans are diverse (for review see reference 27) and in some areas the evidence poor. In this study we found a significant effect of administration of *Lactobacillus GG* upon the intestinal microecology in infective diarrhoea as shown by faecal SCFAs. The concentration of propionic acid was higher in children receiving *Lactobacillus GG* (solely or combined with antibiotic drug) at the 5th day after admission. Lactobacilli do not produce short chain fatty acids with more than two carbon atoms (at least in vitro), so this elevated propionic acid concentration is most likely not the direct effect of the administered probiotic bacteria. The major product of fermentation in *Lactobacillaceae* is lactic acid, which, in turn, is a substrate for many other bacteria in the gut that ferment it to SCFAs and other products. As for example, several representatives of *Bacteroidaceae*, one of the predominant genus of anaerobes in the gut, ferment lactate to propionic acid. The mode of action of probiotics during diarrhoea and recovery from that is far from clear. One of the possible mechanisms may be the resultant feeding of the suppressed resident bacteria with fermentation products such as lactic acid.

In the samples taken 10 days after admission to the hospital the effect of *Lactobacillus GG* on SCFAs seen on the 5th day was no longer evident. It may be reasonable to assume, that in the course of re-establishment of the normal microbiota the chances for the administered probiotic bacteria to adhere and to exhibit metabolic activity become limited. Hence, the faecal SCFAs still revealed one significant difference related to treatment with the probiotic: iso-caproic acid was not found in any of the children receiving *Lactobacillus GG* while it was present in half of samples from the group having been treated solely with an antibacterial drug. Iso-caproic acid is not normally found in faeces of healthy adults and only in single cases in children at 2 years of age, and may be indicative of *C. difficile*. Although the latter is recovered from the faeces of a high percentage of antibiotic-treated patients without diarrhoea it is considered to be the causative agent of pseudomembranous colitis, the most severe complication of antibiotic treatment. The exact mechanisms of changes in intestinal microecology during infective diarrhoea are not clear, but the main feature, decrease of the resident microbiota, coincides with that in antibiotic-induced alterations.

It is likely that the combination of infection and anti-bacterial treatment may even increase the risk of micro-ecological complications. *Lactobacillus GG* has been stated to be effective in treatment of relapsing *C. difficile* colitis. The results of this study suggest that administration of *Lactobacillus GG* as a part of treatment of infectious diarrhoea may promote recovery of a favourable ecosystem, as reflected in faecal SCFAs.

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