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The role of heterolactic lactobacilli in diarrhoea of short small bowel patients

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Abstract

Patients with a short small bowel (SSB) suffer continuously from abdominal pain, flatulence and diarrhoea. Their faeces contains a very characteristic flora, that normally consists of >70% (sometimes even up to 99%) of lactobacilli. In this study, we intended to prove that the mentioned inconveniences are mainly caused by fermentative activity of the massively present heterolactic lactobacilli. Bubbling SSB faeces was examined for bacterial composition and gas production. The effect of oral feeding was studied in a parenterally fed SSB girl and the effect of glucose loading on biochemical and microbiological parameters and on symptoms was studied in an SSB woman. Faecal flora of these SSB patients consisted of >85% lactobacilli. Of these, just *Lactobacillus fermentum* (10^{12} cfu/g wet faeces) produced CO_2 as the only gas in the bubbling faeces. The parenterally fed SSB girl suffered from flatulence, abdominal pain and diarrhoea after oral feeding. Soon after the glucose loading the abdomen of the woman became very distended and painful, and the patient became increasingly flatulent and sick due to massive intestinal CO_2 production. After enormous deflation and diarrhoea all symptoms had gone. The data presented here demonstrate that intestinal heterolactic lactobacilli, such as *L. fermentum,* give rise to abundant CO_2 production and concomitant flatulence, abdominal pain and diarrhoea.

Introduction

In patients with a short small bowel (SSB) >70% of the small bowel has been resected (1). Frequent causes of resection are (i) in newborns and young children, necrotizing enterocolitis and congenital anomalies, such as malrotation with volvulus and ensuing small bowel necrosis; (ii) in younger people, malrotation with volvulus, and recurrent resections due to Crohn's disease; (iii) in the elderly, vascular accidents of mesenteric circulation. Due to massive resection the capacity for uptake of carbohydrates and other nutrients is markedly reduced (2,3), and therefore SSB patients suffer from malabsorption on enteral feeding. Consequently, they develop a characteristic flora of mainly lactobacilli (2,4). By massive lactic acid production and the resultant low pH, these lactobacilli inhibit intestinal growth of other bacteria (2). In addition, this flora causes daily D-lactic acidaemia and aciduria (2,3,5), less frequent acute D-lactic acidosis (with hyperventilation) (6), and occasionally D-lactic acidosis-associated encephalopathy (1). Another important

inconvenience of SSB patients is that they continuously suffer from flatulence (defined as anal release of intestinal gas), abdominal pain and diarrhoea, i.e. symptoms that are closely related as they arise from intestinal gas production.

Most SSB patients suffer from both malabsorption and a rapid intestinal transit, which results in diarrhoea and additional malabsorption of nutrients. Only in sporadic cases intestinal stasis is seen instead of rapid transition. To reduce stool frequency our SSB patients are normally on an essentially lactose-free, but otherwise normal oral diet with carbohydrates as the main caloric source, with soymilk replacing dairy products and with micronutrient supplementation.

Faeces of SSB patients contains a very characteristic flora, that consists of >50% and sometimes even up to 100% of lactobacilli. The genus *Lactobacillus* (7) may be subdivided into three groups: (i) the (obligately) homolactic lactobacilli, (ii) the obligately heterolactic lactobacilli, and (iii) the facultatively heterolactic lactobacilli. Homolactic lactobacilli

(e.g. *Lactobacillus acidophilus*) produce only 2 mol lactate from fermentable monosaccharides (such as glucose, fructose or galactose) and no gas, whereas obligately heterolactic species (e.g. *Lactobacillus fermentum*) produce 1 mol lactate, 1 mol ethanol and 1 mol of the gaseous CO_2. The facultatively heterolactic lactobacilli (e.g. *Lactobacillus casei*) strongly resemble the homolactic lactobacilli as regards the fermentative metabolic pathway of the main fermentable monosaccharides, but still produce some CO_2. In other words: if lactobacilli can produce gas, it will always be CO_2.

The major part of intestinal gas leaves the body by flatulence, a common process that occurs in all people. The driving force of flatulence is generally ascribed to the intestinal production of H_2, and in many people also of CH_4, whereas the malodour must be ascribed to bacterial production of H_2S and some N-derived gases. Since the amount of bacteria other than lactobacilli is normally limited in SSB patients, neither production of bacterial methane nor of gaseous malodorous compounds that arise from bacterial amino acid fermentation ought to be expected. The smell is often slightly acidic due to massive intestinal production of lactic acid.

CO_2 is a fermentation product of the intestinal heterolactic lactobacilli. Its production depends on intestinal sugar concentrations and on the number of bacteria. Intestinal sugar concentrations may be high due to (i) a strongly decreased uptake by the resected gut wall (severe malabsorption) of depolymerized sugar molecules that have been produced by enzymatic depolymerization of polysaccharides, but also (ii) due to excessive consumption of low molecular sugars, especially glucose, fructose and galactose. As concentrations of $10^{11}-10^{12}$ living lactobacilli per gram SSB faeces are quite normal, whereas in healthy non-SSB persons they amount to 10^7-10^8 cfu/ml wet faeces (2), it is suggested that massive CO_2 production is quite normal with a high carbohydrate diet and may even explain flatulence, abdominal pain and diarrhoea.

Since lactobacilli are predominantly present (up to 100%) in SSB patients (2), we postulate that the intestinal inconveniences, the occurrence of diarrhoea included, are mainly determined by the fermentative activity of the present heterolactic lactobacilli on fermentable sugars, such as glucose, fructose, sucrose and lactose.

In this study, we tried to confirm our deduced postulate with clinical casuistic data. In particular, both the faecal percentage and the faecal concentrations of lactobacilli are essential. We focused on several diarrhoea-related observations in SSB patients and on data from a glucose-loading test.

Patients and methods

Patients

The general clinical data of the specific SSB patients, CH-2, CH-4 and AD-7, aspects of which have been studied in detail, are listed in Table I. The small bowel of the girl CH-2 had been resected on the second day after her birth (2,6). The presented observations and experimental data were obtained when she was 4–6 years of age. The length of the small bowel of the girl CH-4 was 60 cm immediately after the first resection (in 1995) and 45 cm soon after the second resection. Initially, feeding was wholly parenteral, but later it was replaced by enteral feeding consisting of oral nutrition and drip-feeding via a gastrostomy. Amounts were adapted as required. The presented observations and experimental data were obtained when she was 7 years old.

The small bowel of the adult female patient AD-7 was resected in 1991. Besides normal oral feeding she received additional parenteral nutrition. The mean defecation frequency of patient AD-7 was about 16 times per day, and sometimes this increased to 20 times per day in spite of the use of loperamide as antidiarrhoeal medication. Glucose loading tests were performed 5 years after resection.

Patients CH-2, CH-4 and AD-7 were typical SSB patients as described above; they often suffered from flatulence, abdominal pain and diarrhoea. Another common feature was the characteristic Gram-positive flora with mainly lactobacilli (2) and an excessively distended abdomen, especially after each meal. Like all SSB children with merely oral/enteral feeding, patients CH-2 and CH-4 both failed to grow.

This study was performed in accordance with the Helsinki Declaration of 1975, as revised in 1983,

Table I. Clinical data of the three female SSB patients studied in detail.

Patient	Year of birth	Resection	Cause of resection	Small intestine	Ileocaecal valve	Colon
CH-2	1984	1984	Volvulus	28 cm	Present	Present
CH-4	1990	1994	Ischaemic incident	45 cm	Present	Present
AD-7	1950	1991	Mesenteric thrombosis	50 cm	Absent	85%

and approved by the Ethics Committee of the Radboud University Nijmegen Medical Centre.

Bacteriological analysis

The faecal samples (>10 g) were collected in a plastic container, transported to the laboratory as soon as possible, immediately placed into an anaerobic chamber, and then processed for microscopy and qualitative and quantitative bacteriological inventory. Thus, bacteriological analysis was started within 1 h after production of the faeces. Before study, the watery, faecal samples were homogenized by vigorous shaking. Microscopy was performed after Gram staining. Bacteriological analysis of faecal samples was performed as described previously (2).

The qualitative and quantitative anaerobic inventory were combined. For qualitative anaerobic inventory regarding all known intestinal bacterial species in the faecal samples a broad spectrum of selective and non-selective media (essentially according to the *Wadsworth Anaerobic Bacteriology Manual*) (8) was used. These media were: (non-selective) Brucella blood agar (anaerobes), β-phenylethylalcohol blood agar (most anaerobic bacteria; inhibition of enterics and certain other non-anaerobic Gram-negative bacilli), Bacteroides bile aesculin agar (*Bacteroides fragilis* group), Fusobacterium egg yolk agar (fusobacteria), kanamycin-vancomycin laked blood agar (anaerobic Gram-negative bacilli and *Veillonella* spp.), Veillonella neomycin agar (*Veillonella* and other Gram-negative cocci), neomycin egg yolk agar (clostridia), cycloserine-cefoxitin mannitol agar (*Clostridium difficile*), kanamycin aesculin agar (enterococci), Lactobacillus Selective (LS)-agar (some lactobacilli), De Man-Rogosa-Sharpe (MRS)-agar (lactobacteria) and Tomato Juice (TJ)-agar (lactobacilli). Since *Lactobacillus fermentum* grew on MRS- and TJ-agar, but not on LS-agar, MRS- and TJ-agar were essential for inventory.

Qualitative aerobic inventory was performed on (non-selective) blood agar, mannitol salt agar (pathogenic staphylococci), Salmonella Shigella agar (*Salmonella* and *Shigella*), Skirrow's Campylobacter isolation agar (*Campylobacter* spp.) and CIN agar (for *Yersinia* spp.). In a later stage, as we had recognized the characteristic SSB flora, we reduced the inventory programme for analyses of the SSB faeces.

For quantitative anaerobic inventory faecal suspensions were diluted with 10-fold dilution steps and all these dilutions were plated on the media mentioned above. The range was thus chosen such that 10^{14} cfu/g wet faeces could be detected on the plates. All manipulations for the inventory were carried out in an anaerobic chamber. A specific

count represents the highest count of identical colonies observed on the various media mentioned above. Thus, specific counts were determined from the highest dilutions at which colonies had grown. From plates with 5–30 separate colonies visually identical ones (up to 10) were isolated, cultured and compared.

For total counts of non-acid-tolerant bacteria, faecal samples were diluted and plated on both aerobic blood agar and anaerobic Brucella blood agar. For total counts of the acid-tolerant lactobacilli the same faecal samples were diluted and cultured anaerobically on MRS-agar (Oxoid CM-361) and on TJ-agar (Oxoid CM-113) (2). Faeces were always screened for intestinal pathogens, especially salmonellae, shigellae, campylobacters and yersiniae.

The following systems were used for identification: API-20E, API-20A, API-Staph and API-Strep (API System SA, Montalieu Vercieu, France). The standard procedure was that isolates from LS-, MRS- and TJ-agar were always screened for L- and D-lactic acid production and for gas production (see below). For lactobacilli from patients CH-2 and CH-4, identification was carried out at the RIVM (Dutch Institute of Public Health and Environmental Hygiene; Bilthoven, The Netherlands); lactobacilli from patient AD-7 were not identified at the species level.

The capacity of faeces and of pure isolates to produce gas was tested in a test tube equipped with a reversed Durham test tube in filter-sterilized 5% glucose solution.

Quality control of specific growth media (see above) preceded the qualitative and quantitative inventory. For quality control of the quantitative aspect we introduced faecal samples from antibiotic-free healthy persons. These samples were analysed in the same way regarding the counts of lactobacilli (normally 10^7–10^8 cfu/g wet faeces) and bacteroides bacteria (normally 10^{10}–10^{11} cfu/g wet faeces).

Biochemical analysis

For quantification of L-lactate in the blood and urine an enzymatic assay with L-LDH was performed, and for quantification of D-lactate the bacterial enzyme D-LDH replaced the enzyme L-LDH (3). Quantitative analysis of organic acids in blood and urine was performed by capillary gas chromatography-mass spectrometry (9). Urine was also studied by nuclear magnetic resonance spectroscopy (NMR) according to Wevers et al. (10).

CO_2 production was tested in the reversed Durham test tube. By addition of NaOH pellets (after growth) the only gas accumulated under the reversed Durham test tube that is fully absorbed by the

growth medium is CO_2 (chemical conversion to non-gaseous soluble bicarbonate).

Results

General diarrhoea-related features of SSB patients

During the many years we have studied SSB patients (with over 250 faecal samples), some very characteristic diarrhoea-related features have been seen (2). All these patients received dextrin-maltose-based food as their main carbohydrate source. Much more frequently than other patients and healthy persons, most SSB patients suffer from audible intestinal bubbling, a visibly distended abdomen, flatulence, painful abdominal cramps and diarrhoea. All these symptoms arise soon after a meal and decrease after flatulence and diarrhoea. This means that they are related to food consumption. In nearly all SSB patients apple juice and dairy products are diarrhoeagenic and therefore they have to be avoided. The frequency of bowel movements in most SSB patients varies markedly (up to 20 times per day), but normally ranges between 4 and 10 times per day. Thus far, the faecal composition in all our SSB patients (with oral nutrition and without oral antibiotic treatment) was very typical and completely different from all other faeces we have studied in more than 15 years. The fresh SSB faeces samples contained (2) as resident flora only the lactic acid producers, *L. acidophilus* and *L. fermentum* (each with $10^{10}-10^{12}$ cfu/g wet faeces), and *Escherichia coli* (10^8-10^{10} cfu/g wet faeces). Staphylococci, enterococci, enterobacteria, e.g. *Citrobacter*, *Klebsiella* and *Proteus* spp., bifidobacteria, eubacteria, propionibacteria, clostridia, peptostreptococci, *Bacteroides* spp. and the yeast *Candida albicans* have been seen sporadically (normally $<10^8$ cfu/g wet faeces, but sometimes even up to 10^{11} cfu/g wet faeces), but must be regarded as transient flora, that had fully disappeared after a few days. Whereas the homolactic *L. acidophilus* and the heterolactic *L. fermentum* constitute the major part of the lactobacillus flora, other lactobacilli such as *Lactobacillus jensenii* and *Lactobacillus minutus* have sometimes been observed (counts up to 10^9 and 10^{11} cfu/g wet faeces, respectively). The faeces always contained considerable amounts of lactic acid. The lowest faecal pH we have measured was 3.9 and this was mainly ascribed to the D-lactic acid that was massively present in the faecal SSB sample (2).

Proving the postulate

As mentioned in the introduction we postulate that the intestinal inconveniences, including the occurrence of diarrhoea, are mainly determined by the fermentative activity of the present heterolactic lactobacilli on fermentable sugars, such as glucose, fructose, sucrose and lactose. The faecal composition mentioned above absolutely deviates from the normal faecal composition. The first most intriguing result was that the total faecal concentrations that were counted at a nearly neutral pH (between 6 and 7), were normally 100–10 000-fold lower than the lactobacillus concentrations that were counted at a lower pH (between 4 and 5). This demonstrates the microbial supremacy of the lactobacilli present in the SSB intestines. Both the high serum D-lactate values and the enormous urinary D-lactate excretion also demonstrate the intestinal physiological supremacy and the consequent effect on patient's life (D-lactic acidosis and D-lactic acid-associated encephalopathy). Thus, it was theoretically deduced that intestinal inconveniences must be ascribed to heterolactic lactobacilli-mediated CO_2 production during intestinal sugar fermentation. Against this background we have studied two available sets of particular coherent data that may provide more insight into the role of heterolactic lactobacilli-mediated gas production in SSB and after that the effects of glucose-loading in one patient. In fact, these data are additional casuistic data to prove our postulate in several steps. All data are representative for our observations, but we have chosen data that stem from patients with high faecal concentrations of lactobacilli (85–90%).

CO_2 production by heterolactic L. fermentum

In the bubbling diarrhoea-like faeces of patient CH-2 the resident heterolactic *L. fermentum* ($10^{11}-10^{12}$ cfu/g wet faeces) was predominant. Normally numbers were 10–100-fold higher than those of the resident homolactic *L. acidophilus* and than those of the transient intestinal bacteria (2). The capacity of both a bubbling faeces with 90% Gram-positive rods (identified as lactobacilli; 10^{12} cfu/g wet faeces) as well as 10% Gram-negative rods, and of the isolated *L. fermentum* to produce CO_2 was demonstrated with glucose as sugar in the Durham test tube system (see Patients and methods section). Therefore, the bubbling of the diarrhoea was associated with fermentative CO_2 production by *L. fermentum*.

Intestinal inconveniences due to lactobacilli-mediated gas production

For a long time patient CH-4 suffered from increasing stool frequency, a distended abdomen and intense abdominal pain. At the age of 7 years her body weight fell from 19.5 kg to 17.5 kg within 3 months. From the moment she was admitted, she received both parenteral and oral feeding. Her faecal

flora then contained 85% Gram-positive rods (identified as *L. acidophilus* together with *L. fermentum*; 10^{12} cfu/g wet faeces), 10% Gram-negative rods and 5% yeasts. During *in vitro* incubation of her faeces with glucose only CO_2 was produced. After 4 days of beneficial parenteral feeding in combination with some oral feeding the composition of the faecal flora had not changed, and she still had severe abdominal pains and a distended abdomen. In the next 3 days of only parenteral feeding, abdominal pain and diarrhoea disappeared completely in a short time and the abdomen was no longer distended.

In vivo lactobacilli-mediated gas production

In patient AD-7 the diarrhoeagenic effect of glucose in SSB was studied twice (with an interval of 4 months) for 3 hours after loading with 50 g glucose dissolved in 200 ml water. Her faecal flora consisted of 90% Gram-positive rods, 5% Gram-positive cocci, 5% Gram-negative rods, <1% Gram-negative cocci and sporadic yeasts. The Gram-positive rods appeared to be a mixture of homolactic and heterolactic (CO_2-producing) lactobacilli. The total glucose intake lasted 5 minutes. Immediately after the intake intestinal fermentation started and could be heard due to bubbling. During the test an enormous amount of intestinal gas was produced. The patient felt distended, first in the upper part of the intestines, then in the lower part; and the whole bowel became visibly distended. After 15 minutes nausea started, and after 20 minutes abdominal pain and cramps started; after 30 minutes she became flatulent, and slowly she felt the urge to defecate. After 45 minutes she felt very miserable, and had to lie down. After 75 minutes she suffered from severe diarrhoea for about 15 minutes. After 90 minutes all these symptoms had gone. During the whole experiment H_2 was continuously ≤ 3 ppm in the breath test. Serum glucose increased from 4.5 mM to a maximum of 8.0 mM, D-lactate from 150 μM to a maximum of 400 μM, and L-lactate from 1000 μM to a maximum of 1600 μM. In addition, the presence of lactate was confirmed by NMR and only a slight increase of serum acetate was observed, but not of other organic compounds. Serum pH was continuously 7.33. Urinary excretions of total lactic acid just before and after the experiment were low: 30 mmol/mol creatinine. The gas produced after incubation of a small inoculum of faeces in a glucose solution was confirmed to be CO_2. Together, the microbiological and biochemical data described above indicate that after the glucose loading intestinally a massive instantaneous bacterial fermentation with concomitant gaseous CO_2 production had

occurred, that caused a very short transit time, and that finally resulted in severe diarrhoea. Thus far, the data from patients CH-2 and CH-4 and many previous data (2) point to *L. fermentum* as primary producer, but other lactobacilli are not excluded.

Discussion

It was expected that in SSB patients not receiving antimicrobial therapy and neutralization of gastric acid, and with a normal gastric acid production, the major faecal gas produced by the present intestinal flora with extremely high percentages of intestinal lactobacilli should be CO_2. The data sets collected for patients CH-2 and CH-4 agreed with this expectation. The fact that faecal gas consists only of CO_2 gas has not been reported before.

Antimicrobial therapy, neutralization of gastric acid, and therapeutically reduced gastric acid production may favour uncontrolled intestinal overgrowth of various fermentative microorganisms (2). The final effect is unpredictable. As yeasts produce nearly 2 mol of CO_2 per mol glucose, they may even increase flatulence, pain and diarrhoea. Heterofermentative microorganisms such as *Escherichia coli* produce both CO_2 and the nearly insoluble H_2. If H_2 is produced in SSB patients, it should be produced only by *E. coli* and/or some transient facultative and/or strict anaerobes, and not by either lactobacilli or yeasts.

For a closer understanding of the coherence between flatulence, abdominal pain and diarrhoea, the intestinal flora of patient AD-7 was manipulated by glucose loading. The data from this experiment confirmed that after glucose loading (i) the breath test for H_2 production was negligible, and (ii) upon incubation of the faeces with glucose the only gas produced was CO_2.

If in the loading experiment only 5 g (= 10% of the load) of the glucose (MW = 180) was fermented by heterolactic lactobacilli, then 600 ml of CO_2 gas should be produced (the molar gas volume is 22.4 L). With increasing CO_2 production both the intra-intestinal gas volume and gas pressure will increase, and be seen as abdominal distension and be felt as severe abdominal pain. Deflation will diminish the intra-luminal pressure.

Four main closely related factors together are causative for the massive CO_2 production: i.e. the patient's diet, malabsorption as a consequence of massive resection, heterolactic bacterial activity and the resultant low intestinal pH (2,6). During intestinal heterolactic bacterial fermentation in acidic watery solution gaseous CO_2 will arise massively because of its limited solubility in acidic solution. Consequently, the intestinal lumen will be filled

with gas and distended. The diarrhoeagenic character of apple juice (containing glucose and fructose) and of dairy produce (containing lactose) in these patients is thought to be based on the suitability of these sugars for intestinal lactobacilli-mediated fermentation.

Polymeric carbohydrates in more solid food are thought to be intestinally mixed with fermentative SSB flora, and just fermented after depolymerization by human amylase. Just as in fermenting dough, the volume of the intestinal fermenting food mass may increase greatly due to the gaseous CO_2 inside. Due to the limited space this spongy gas-filled food will cause an increasing intra-intestinal pressure on the lumen wall that also contributes to abdominal distension. As this gas pressure passes a threshold, it is thought to create a neurophysiological stimulus that forces lumen muscles to contract and the anal sphincter to relax and expel the intestinal contents (11) before all the normal digestive processes (dehydration included) have been performed; the result is seen as diarrhoea. The rather frequent SSB-associated and CO_2-mediated diarrhoea implicates a rather high defecation frequency. As this frequency is reduced, bacterial metabolites, e.g. D-lactate, will accumulate intestinally and contribute to acidosis and/or encephalopathy.

While the uptake of vitamins, minerals and trace elements (as calcium, iron and zinc) is supposed to be low in SSB patients due to strongly reduced absorptive capacity of the resected small bowel, diarrhoea additionally reduces the uptake because of the short contact time between food and bowel wall due to the rapid passage time.

It is concluded that in SSB patients a considerable proportion of dietary carbohydrates remains in the bowel for too long. Abundant heterolactic lactobacilli, such as *L. fermentum*, will ferment depolymerized carbohydrates leading to massive gaseous CO_2 production, and thus will be often responsible for frequent flatulence, abdominal pain and diarrhoea.

Acknowledgements

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