Pergamon

Food and Chemical Toxicology 36 (1998) 1085–1094

Food and Chemical Toxicology

Safety and Tolerance of *Lactobacillus reuteri* Supplementation to a Population Infected with the Human Immunodeficiency Virus

B. W. WOLF*, K. B. WHEELER, D. G. ATAYA and K. A. GARLEB

Department of Medical Nutrition Research and Development, Ross Products Division of Abbott Laboratories, 625 Cleveland Avenue, Columbus, OH 43215, USA

(Accepted 3 May 1998)

Abstract—Probiotic supplementation may provide health benefits, especially for individuals with an underlying disease state that makes them more susceptible to infections. The purpose of this experiment was to evaluate the safety and tolerance of *Lactobacillus reuteri* ingestion by subjects infected with the human immunodeficiency virus (HIV). Thirty-nine subjects consumed a freeze-dried preparation of *L. reuteri* or a placebo for 21 days in a double-masked, parallel design experiment. Serum chemistry, haematology, immune profile, urinalysis, physical examination, gastrointestinal tolerance and faecal microbiota data were collected. No clinically significant changes were noted in any of the safety parameters measured. Overall, tolerance was good in both groups. Consumption of *L. reuteri* tended to increase faecal levels of *L. reuteri* on days 7, 14 and 21 of treatment feeding ($P < 0.06$, $P < 0.11$ and $P = 0.05$, respectively). However, faecal levels of *L. reuteri* and total *Lactobacillus* species were lower than levels previously observed in healthy male adults. Overall, this study documents that *L. reuteri* may be fed to HIV-positive individuals at 1×10^{10} colony forming units/day without any clinically significant safety or tolerance problems.

Keywords: *Lactobacillus reuteri*; safety and tolerance; human immunodeficiency virus.

Abbreviations: AIDS = acquired immunodeficiency syndrome; CFU = colony forming units; GI = gastrointestinal; HDL = high-density lipoprotein; HIV = human immunodeficiency virus; spp = species.

INTRODUCTION

Historically, lactic acid bacteria (e.g. lactobacilli) have been recognized as beneficial, health promoting organisms (Douglas, 1911). Over the past few decades, scientific evidence has documented that intestinal microbiota provide protection against various diseases. For example, germfree animals are more susceptible to disease than are their conventional counterparts with a complete gut flora (Collins and Carter, 1978; Moberg and Sugiyama, 1979). Additional evidence supporting the protective effect of gut flora is the finding that animals, including humans, are more susceptible to infection by organisms such as *Clostridium difficile* after receiving antibiotics (Bartlett, 1994; Larson and Borriello, 1990).

One approach used to promote the beneficial microflora of the gastrointestinal (GI) tract is through the consumption of probiotics. A probiotic has been defined as a mono- or mixed-culture of live microorganisms that beneficially affect the host by improving the properties of the indigenous microbiota when consumed (Havenaar and Huis in't Veld, 1992). One microorganism of particular interest is *Lactobacillus reuteri*, a normal inhabitant of the GI tract of healthy humans and many animals (Axelsson *et al.*, 1989; Chung *et al.*, 1989). It has been discovered that *L. reuteri* can produce a broad spectrum antimicrobial agent, reuterin, that may contribute to the survival of *L. reuteri* cells within their gastrointestinal ecosystem.

Recently, Alak *et al.* (1996, 1997) have shown that *L. reuteri* may possess a prophylactic benefit for individuals susceptible to cryptosporidiosis. They demonstrated that *L. reuteri* prevented intestinal infectivity and reduced the faecal shedding of *Cryptosporidium parvum* oocysts in immunosuppressed mice challenged with *C. parvum*. Considering this, it may be beneficial for individuals who are at risk of cryptosporidiosis [e.g. immuno-

*Author for correspondence at: Dept 105670/RP3-2, Ross Products Division, 625 Cleveland Ave., Columbus, OH 43215-1724, USA.

0278-6915/98/$19.00
PII S0278-6915(98)00090-8

suppressed persons such those infected by the human immunodeficiency virus (HIV)] to consume supplemental *L. reuteri*.

As with all new probiotic ingredients, it is important that studies be conducted to identify (document) the positive effects of consuming the ingredient. Equally important, however, is the research that relates to the identification of any negative "side-effects" associated with the consumption of the food. In a double-masked, placebo-controlled study, Wolf *et al.* (1995) fed healthy male subjects 1×10^{11} colony forming units (CFU) supplemental *L. reuteri* per day for 21 days. They concluded that *L. reuteri* may be fed up to 1×10^{11} CFU per day without any clinically significant safety or tolerance problems. The current study proposed to test the safety and tolerance of supplemental *L. reuteri* in an HIV-positive (HIV+) population. The objectives of this study were (1) to assess safety and tolerance of supplemental *L. reuteri* consumption compared with placebo in HIV+ adult subjects (as measured by serum chemistry, haematology, immune deficiency and urinalysis profiles) and (2) to determine the effect of the consumption of *L. reuteri* on the level of total *Lactobacillus* species (spp) and *L. reuteri* in the faeces of this population.

MATERIALS AND METHODS

Probiotic preparation

Lactobacillus reuteri (strain SD2112, formerly called strain MM2) was obtained from BioGaia Biologics, Inc. (Raleigh, NC, USA). *L. reuteri* was grown in proprietary selective media (Marschall Products, a division of Rhône-Poulenc, Madison, WI, USA) and then freeze-dried in the presence of a cryoprotectant (non-fat dry milk powder, maltodextrin and sucrose). Individual 5-g foil packets were manufactured with a level of approximately 5×10^9 CFU *L. reuteri*/packet. Sucrose was used as the carrier and packets were sealed under a nitrogen flush. The placebo was devoid of *L. reuteri*, but contained all other ingredients present in the packet consumed by the "*L. reuteri*" group. Packets were processed and coded (to maintain a double mask) by Anderson Packaging (Rockford, IL, USA). To ensure viability of the *L. reuteri*, packets were stored in a freezer (–4°C) until consumed.

Experimental design

This study protocol was approved by an institutional review board assembled by Great Lakes Institute for Health and Economic Research (Columbus, OH, USA). 51 HIV+ adults (male and female) were screened as potential subjects for this double-masked, placebo controlled experiment. Because these subjects were infected with HIV, it was anticipated that some may be receiving antiretroviral therapy. Thus, subjects were randomized within each block [i.e. antiretroviral therapy (only zidovudine, Burroughs Wellcome Co., Research Triangle Park, NC, USA) or no antiretroviral therapy] to one of the two treatment groups. The duration of the study was 35 days with the subjects consuming two packets of the supplement per day (approx. 12 hr apart) during the first 21 days followed by a 14-day washout period (day 22 through 35). Subjects were allowed to consume their normal diets; however, alcohol was not allowed.

Product consumption

During the first 21 days of the experiment, subjects consumed two packets/day resulting in a total daily intake of 10^{10} CFU *L. reuteri*. Subjects were directed to add packet contents to one of the approved beverages (no hot drinks, >37°C). The following beverages were determined to be adequate for product mixability and *L. reuteri* viability: tap water, milk, orange juice (from concentrate), apple juice, grape juice, cranberry juice, and regular or diet 7-Up (unpublished data).

Subject selection

Subjects were selected according to the following criteria: (1) had pathologically confirmed HIV infection as defined by the revised CDC classification scheme (Centers for Disease Control, 1992); however, must have had a CD4+ T-lymphocyte count greater than 400 (per μl blood); (2) was a male/non-pregnant female at least 6 wk postpartum and non-lactating between 18 and 60 yr of age; (3) was not more than 20% below ideal body weight; (4) had blood chemistries within a normal range or not considered clinically significant for HIV+ patients if outside the normal range; (5) had subjects' assurance that they were free from known metabolic or gastrointestinal diseases and had no known food allergies; (6) had subjects' assurance that they had not taken antibiotics for a period up to 3 months prior to the start of the study; (7) was not currently taking an antiretroviral drug, or if the subject had been prescribed an antiretroviral drug it must have been zidovudine only and the subject must have been on the medication for a minimum of 1 month prior to study initiation; (8) was not taking medications that would interfere with nutrient absorption, metabolism or excretion or compromise gut microbiota (e.g. laxatives, antidiarrhoeal agents, antibiotics, etc.); (9) had a negative stool culture for enteric pathogens; and (10) voluntarily signed an informed consent form.

Subject withdrawal

A subject was discharged or dropped from the study when any of the following occurred: (1) if, at any time during the study, antibiotic or antiretroviral therapies were instituted; (2) if the subject con-

sumed alcohol during the study; (3) self-determination or investigator judgement.

Subject screening

Prior to admittance into the study, all potential subjects were screened to determine whether they met the selection criteria. In general, the following analyses were conducted: serum chemistry, haematology and immunology profile, urinalysis, medical history, and stool culture (see below).

Physical examination

Physical examinations were conducted on days 1 (baseline), 21 and 35. The following physical examination parameters were taken: body weight, oral body temperature, pulse rate, respiratory rate, systolic and diastolic blood pressure, and a review of systems (only occurred at baseline). These parameters along with serum chemistries, haematology and immunology profiles and urinalysis were used to document the subject's "health" (other than HIV infection) prior to study initiation, after 21 days on the treatment, and after a 14-day washout (day 35). Washout refers to the time in which subjects are not consuming their dietary treatment. Physical examinations were conducted by a licensed physician.

Collection and analysis of biological samples

Fasting blood samples (minimum 10-hr fast) were drawn by venipuncture at screening and on days 21 and 35. Blood was drawn into EDTA tubes for haematology, acid citrate dextrose solution tubes for immune profile, and serum tubes for serum chemistries. In addition, blood was drawn into bottles containing blood culture broth for routine culture analysis on whole blood. The screening serum chemistry, haematology and immunology profile, and blood culture were used as baseline data. The parameters examined are listed in the Results section.

A random urine collection from each subject for routine analysis with culture was obtained at screening and on days 21 and 35. The screening urinalysis data was used as baseline data. The parameters examined are listed in the Results section. Expectorated sputum samples were to be collected on days 1 (baseline), 21 and 35. However, if a subject was unable to produce a purulent sputum sample, the analysis was not conducted for that subject at that time point. All routine clinical analyses were conducted by MetPath Laboratories (Columbus, OH, USA).

A faecal sample taken during subject screening was evaluated for the following: (1) enteric pathogens including *Salmonella*, *Shigella*, *Yersinia* and *Campylobacter*; (2) *Clostridium difficile* toxin; and (3) ova or parasites to include *Cryptosporidium parvum*. Faecal samples for faecal fat analysis were collected on days 1 (baseline), 21 and 35. Qualitative analysis of faecal samples were analysed for total faecal fat and neutral fat (MetPath Laboratories). Faecal samples for gut microbiota analysis were collected from the first defaecation on days 1 (baseline), 7, 14, 21, 28 and 35. It was anticipated that some subjects supplemented with *L. reuteri* may have high faecal levels of *L. reuteri* after 2 wk of washout. Thus, additional faecal sampling was planned for day 49 (4 wk washout) if this occurred. Samples were collected by the subject and given to the investigator within 5 hr of collection. Samples were processed for microbiota enumeration by diluting a 2-g subsample in 8 ml 0.1% peptone water (Bacto®-Peptone, Difco Laboratories, Detroit, MI, USA). Samples were homogenized using a Stomacher lab-blender (Seward, London, UK). Three aliquots of 1.5 ml each were stored separately in microfuge vials. Samples were quickly frozen (in alcohol and dry ice) and stored at -70°C. All samples were analysed within 1 wk of collection. *Lactobacillus* spp and *L. reuteri* were enumerated using standard anaerobic microbiological techniques and techniques developed by BioGaia Biologics, Inc. as previously described (Wolf *et al*., 1995).

Subjective tolerance factors

Using a daily questionnaire, subjects were asked to report the severity of the following symptoms: nausea, diarrhoea, cramping, distention, flatulence, vomiting, constipation, burping and reflux using the following scale: 0 = absent, 1 = mild, 2 = moderate or 3 = severe. In addition, bowel function was monitored daily (number and consistency). Subjects used the following scale to rate faecal consistency: 1 = hard, dry; 2 = hard, formed; 3 = soft, formed; 4 = soft, unformed; 5 = watery.

Statistical methods

Prior to conducting this experiment, a power analysis was conducted utilizing the data generated in a similar study conducted by Wolf *et al*. (1995). Faecal *L. reuteri* concentration was used as the variable to calculate power. A conservative estimated difference of one standard deviation between groups [a change of two standard deviations was found by Wolf *et al*. (1995)] was used for the power calculation. It was determined that a sample size of 36 would give 80% power (significance level of 0.05) to detect differences between treatments. Additional subjects were enrolled in the anticipation of dropouts.

Because of the limited number of subjects on zidovudine, the preplanned analysis that called for the use of a randomized block was not conducted. Microbiota counts were transformed to $\log_{10}$ scale for statistical analysis. Those subjects who had counts below the method's detection threshold (1×10^3) were substituted with values representing the half-way point (5×10^2 or 2.70 $\log_{10}$). For all continuous level parameters, weekly change scores

from baseline were calculated. All continuous data were analysed with a two sample *t*-test or Wilcoxon rank sum test where appropriate. When significant differences were found between the groups at baseline, those parameters were analysed utilizing an analysis of covariance. Data which were categorical in nature were analysed with a Fisher's exact test separately for each day of collection. Daily documentation of gastrointestinal tolerance were summarized with frequencies and percentages.

RESULTS

51 HIV+ adults (male and female) were screened as potential subjects for this double-masked, placebo controlled experiment. 39 subjects (age range: 23–50 yr) met the eligibility criteria and followed study procedures. 35 of these subjects completed the 35-day experiment. Partial data were evaluable from the remaining four subjects prior to their dropping from the experiment (no dropouts were associated with intolerance to either treatment). One subject in the placebo group dropped out on day 20. Three subjects in the *L. reuteri* group dropped out on days 8, 13 and 22, respectively. The study population was primarily male (37 *v.* two females).

Physical examination parameters were similar for both treatments (Table 1). The only statistical difference noted was in the change from baseline for diastolic blood pressure at day 21 (*L. reuteri* > placebo).

Serum chemistry profiles are summarized in Table 2. No differences ($P > 0.05$) were found in any of the chemistries analysed. Haematology profiles may be found in Table 3. At baseline, subjects randomized to the placebo had a higher ($P < 0.05$) red blood cell distribution width than those randomized to *L. reuteri*. Obviously this was not related to treatment and adjusted means for the treatments at day 21 and day 35 were similar ($P > 0.05$) using covariate analysis. At day 35 there was a significant ($P < 0.05$) difference in the change from baseline for differential percent lymphocytes (placebo > *L. reuteri*).

Results for the immunology profiles are shown in Table 4. At day 35 there was a significant ($P < 0.05$) difference in the change from baseline (placebo > *L. reuteri*) for the absolute number of CD4+ T lymphocytes. Data generated from urinalysis are summarized in Table 5. Change from baseline for specific gravity at day 21 was higher ($P < 0.05$) for the *L. reuteri* group.

All safety cultures for bacteria in blood samples showed no growth after 7 days of incubation. Only a few urine samples showed bacterial growth. Those with growth were considered to not be significant by the performing lab. Low-level bacterial growth is common in urine samples due to contamination when samples are collected. No sputum was obtained since no subjects were able to provide a sample, suggesting that no respiratory infections occurred during the study.

Ratings of subjective GI tolerance factors are presented in Table 6. Data have been summarized as a percent of subject days in which a tolerance factor was rated absent, mild, moderate or severe. A high percentage of total study days for most subjective GI tolerance factors were reported as absent. The only factor noted with frequency was flatulence, but results were similar for both treatments. Also, there appeared to be a trend towards more complaints of mild nausea for subjects consuming *L. reuteri*.

Results of faecal analysis for the enumeration of *L. reuteri* and total *Lactobacillus* spp is shown in Table 7. Consumption of supplemental *L. reuteri* tended to increase faecal levels of *L. reuteri* from baseline on days 7, 14 and 21 ($P = 0.054$, $P = 0.104$ and $P = 0.050$, respectively). Some individuals consuming placebo had detectable levels of *L. reuteri*, confirming the indigenous nature of this

Table 1. Effect of supplemental *Lactobacillus reuteri* on physical examination parameters of adults infected with the human immunodeficiency virus

	Treatment								
	Placebo			*L. reuteri*			Statistical analysis[a]		
Day[b]	Baseline	21	35	Baseline	21	35	Baseline	Day 21	Day 35
Parameter[c]	(n = 21)	(n = 20)	(n = 20)	(n = 18)	(n = 15)	(n = 15)			
Temperature [oral (°C]*	36.6 ± 0.1[d]	36.6 ± 0.1	36.5 ± 0.1	36.6 ± 0.1	36.7 ± 0.1	36.5 ± 0.1	NS	NS	NS
Pulse rate (bpm)	74 ± 2	81 ± 2	79 ± 1	73 ± 2	79 ± 1	73 ± 2	NS	NS	NS
Respiratory rate (rpm)	20 ± 0.3	20 ± 0.2	20 ± 0.3	20 ± 0.5	20 ± 0.0	19 ± 0.5	NS	NS	NS
Systolic blood pressure (mm Hg)	116 ± 3	119 ± 2	120 ± 3	124 ± 3	126 ± 3	121 ± 4	NS	NS	NS
Diastolic blood pressure (mm Hg)	79 ± 2	75 ± 1	78 ± 2	79 ± 2	84 ± 2	76 ± 3	NS	< 0.01	NS
Body weight (kg)	77.5 ± 4.2	78.0 ± 4.3	77.7 ± 4.3	81.7 ± 3.5	81.4 ± 3.9	81.5 ± 3.9	NS	NS	NS

[a]Baseline = level of significance between treatments at baseline; day 21 and day 35 = level of significance for change from baseline between treatments at the respective time points; NS = not significant ($P > 0.05$).
[b]Subjects consumed their respective supplement from day 1 through day 21. Day 35 data represent a 2-wk washout.
[c]bpm, beats per minute; rpm, respirations per minute.
[d]Values are means ± SE.
*Placebo, day 21 n = 19.

Table 2. Effect of supplemental *Lactobacillus reuteri* on blood metabolites of adults infected with the human immunodeficiency virus

	Treatment								
	Placebo			*L. reuteri*			Statistical analysis[a]		
Day[b]	Baseline	21	35	Baseline	21	35	Baseline	Day 21	Day 35
Metabolites[c]	(n = 21)	(n = 20)	(n = 20)	(n = 18)	(n = 15)	(n = 14)			
Glucose (mmol/litre)	5.1 ± 0.3[d]	4.8 ± 0.3	4.5 ± 0.2	4.8 ± 0.1	4.7 ± 0.2	4.5 ± 0.2	NS	NS	NS
Calcium (mmol/litre)	2.25 ± 0.02	2.20 ± 0.02	2.23 ± 0.02	2.28 ± 0.02	2.25 ± 0.02	2.27 ± 0.02	NS	NS	NS
Phosphorus (mmol/litre)	1.02 ± 0.05	0.96 ± 0.04	0.90 ± 0.05	1.12 ± 0.04	0.98 ± 0.06	1.02 ± 0.06	NS	NS	NS
Ionized calcium (mmol/litre)	0.94 ± 0.01	0.92 ± 0.01	0.93 ± 0.01	0.94 ± 0.02	0.92 ± 0.01	0.92 ± 0.02	NS	NS	NS
Sodium (mmol/litre)	139 ± 0.4	138 ± 0.4	138 ± 0.5	139 ± 0.4	138 ± 0.3	138 ± 0.6	NS	NS	NS
Potassium (mmol/litre)	4.3 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.2 ± 0.1	4.1 ± 0.1	4.4 ± 0.1	NS	NS	NS
Chloride (mmol/litre)	104 ± 0.5	103 ± 0.5	104 ± 0.6	104 ± 0.6	103 ± 0.5	103 ± 0.7	NS	NS	NS
Magnesium (mmol/litre	0.84 ± 0.01	0.83 ± 0.01	0.85 ± 0.02	0.87 ± 0.01	0.89 ± 0.01	0.88 ± 0.01	NS	NS	NS
Iron (μmol/litre)*	16 ± 1	14 ± 1	19 ± 2	17 ± 1	15 ± 2	15 ± 2	NS	NS	NS
Triglycerides (mmol/litre)	1.54 ± 0.17	1.80 ± 0.28	1.89 ± 0.30	2.40 ± 0.51	2.72 ± 0.72	2.81 ± 0.78	NS	NS	NS
Cholesterol (mmol/litre)	3.97 ± 0.21	4.05 ± 0.22	4.06 ± 0.23	4.32 ± 0.25	4.20 ± 0.22	4.18 ± 0.25	NS	NS	NS
HDL (mmol/litre)†	0.91 ± 0.08	0.90 ± 0.09	0.93 ± 0.10	0.80 ± 0.04	0.89 ± 0.07	0.83 ± 0.07	NS	NS	NS
LDL (mmol/litre)‡	2.35 ± 0.18	2.49 ± 0.20	2.38 ± 0.18	2.53 ± 0.22	2.30 ± 0.18	2.42 ± 0.22	NS	NS	NS
Renal function									
BUN (mmil/litre urea)	4.6 ± 0.2	4.5 ± 0.2	5.1 ± 0.3	4.6 ± 0.3	4.6 ± 0.2	4.7 ± 0.4	NS	NS	NS
Creatinine (μmol/litre)	78 ± 3	75 ± 2	78 ± 3	77 ± 2	81 ± 3	80 ± 3	NS	NS	NS
Uric acid (μmol/litre)	380 ± 15	370 ± 13	393 ± 20	370 ± 23	361 ± 24	356 ± 25	NS	NS	NS
Hepatic function									
Albumin (g/litre)	44 ± 1	44 ± 1	45 ± 1	46 ± 1	46 ± 1	46 ± 1	NS	NS	NS
Total protein (g/litre)	77 ± 1	78 ± 2	78 ± 2	79 ± 2	80 ± 2	81 ± 2	NS	NS	NS
Total bilirubin (μmol/litre)§	11 ± 1	11 ± 1	13 ± 1	11 ± 1	14 ± 2	12 ± 2	NS	NS	NS
Direct bilirubin (μmol/litre)	2.4 ± 0.2	2.4 ± 0.2	2.7 ± 0.3	2.3 ± 0.2	2.6 ± 0.3	2.3 ± 0.2	NS	NS	NS
ALT (μkat/litre)	0.48 ± 0.06	0.57 ± 0.09	0.59 ± 0.10	0.53 ± 0.10	0.54 ± 0.10	0.58 ± 0.14	NS	NS	NS
AST (μkat/litre)	0.47 ± 0.04	0.54 ± 0.06	0.57 ± 0.08	0.44 ± 0.06	0.49 ± 0.07	0.49 ± 0.08	NS	NS	NS
GGT (μkat/litre)	0.56 ± 0.08	0.59 ± 0.11	0.56 ± 0.09	0.85 ± 0.21	0.72 ± 0.17	0.71 ± 0.15	NS	NS	NS
ALP (μkat/litre)	1.4 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	NS	NS	NS
LDH (μkat/litre)‖	2.88 ± 0.13	3.07 ± 0.12	3.24 ± 0.15	2.60 ± 0.12	2.77 ± 0.14	2.86 ± 0.14	NS	NS	NS

[a]Baseline = level of significance between treatments at baseline; day 21 and day 35 = level of significance for change from baseline between treatments at the respective timepoints; NS = not significant ($P > 0.05$).
[b]Subjects consumed their respective supplement from day 1 through day 21. Day 35 data represent a 2-wk washout.
[c]HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; BUN, blood urea nitrogen; ALT, alanine aminotransferase (serum glutamic–pyruvic transaminase); AST, aspartate aminotransferase (serum glutamic–oxaloacetic transminase); GGT, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase.
[d]Values are means ± SE.
*Placebo, day 21 and day 35 n = 19; *L. reuteri*, day 21 n = 14.
†*L. reuteri*, baseline n = 17.
‡Placebo, day 21 n = 18, day 35 n = 19; *L. reuteri*, baseline n= 15, day 21 and day 35 n = 12.
§Placebo, day 21 n= 19; *L. reuteri*, baseline n = 16, day 21 n = 14.
‖Placebo, day 21 n = 19

Table 3. Effect of supplemental *Lactobacillus reuteri* on clinical haematology of adults infected with the human immunodeficiency virus

	Treatment								
	Placebo			*L. reuteri*			Statistical analysis[a]		
Day	Baseline	21	35	Baseline	21	35	Baseline	Day 21	Day 35
Component[b]	(n = 21)	(n = 20)	(n = 20)	(n = 18)	(n = 15)	(n = 15)			
RBC ($\times 10^{12}$/litre)	4.6 ± 0.2[c]	4.6 ± 0.2	4.6 ± 0.2	4.7 ± 0.2	4.7 ± 0.2	4.7 ± 0.2	NS	NS	NS
WBC ($\times 10^{9}$/litre)	5.4 ± 0.4	5.5 ± 0.4	5.7 ± 0.4	5.2 ± 0.4	5.4 ± 0.4	5.2 ± 0.4	NS	NS	NS
Differential white blood cell count									
Neutrophils	0.51 ± 0.03	0.51 ± 0.04	0.48 ± 0.02	0.50 ± 0.02	0.52 ± 0.03	0.50 ± 0.03	NS	NS	NS
Lymphocytes	0.37 ± 0.03	0.37 ± 0.03	0.39 ± 0.02	0.36 ± 0.02	0.34 ± 0.03	0.36 ± 0.02	NS	NS	0.04
Monocytes	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	NS	NS	NS
Eosinophils	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.01	0.04 ± 0.00	0.03 ± 0.01	NS	NS	NS
Basophils	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	NS	NS	NS
Platelets ($\times 10^{9}$/litre)*	198 ± 14	208 ± 18	210 ± 13	225 ± 19	214 ± 17	230 ± 14	NS	NS	NS
Haemoglobin (g/litre)	147 ± 2	147 ± 3	148 ± 3	147 ± 3	146 ± 3	147 ± 3	NS	NS	NS
Haematocrit	0.43 ± 0.01	0.43 ± 0.01	0.43 ± 0.01	0.42 ± 0.01	0.42 ± 0.01	0.43 ± 0.01	NS	NS	NS
Red blood cell indexes									
MCV (fl)	95 ± 3	95 ± 3	95 ± 3	91 ± 3	92 ± 3	93 ± 3	NS	NS	NS
MCH (pg)	33 ± 1	33 ± 1	33 ± 1	32 ± 1	32 ± 1	32 ± 1	NS	NS	NS
MCHC	0.35 ± 0.00	0.34 ± 0.00	0.34 ± 0.00	0.35 ± 0.00	0.34 ± 0.00	0.34 ± 0.00	NS	NS	NS
RDCW	0.14 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	0.04	NS	NS
MPV (fl)†	8.4 ± 0.5	8.9 ± 0.2	8.3 ± 0.5	8.3 ± 0.5	9.2 ± 0.2	8.2 ± 0.7	NS	NS	NS

[a]Baseline = level of significance between treatments at baseline; day 21 and day 35 = level of significance for change from baseline between treatments at the respective time points; NS = not significant ($P > 0.05$).
[b]RBC, red blood cell; WBC, white blood cell; neutrophil (polymorphonucler leucocyte); MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin, MCHC, mean corpuscular haemoglobin concentration; RCDW, red blood cell distribution width; MPV, mean platelet volume.
[c]Values are means ± SE.
*Placebo, day 35 n = 19.
†*L. reuteri*, day 21 and 35 n = 14.

Table 4. Effect of supplemental *Lactobacillus reuteri* on the immunology profiles of adults infected with the human immunodeficiency virus

	Treatment								
	Placebo			*L. reuteri*			Statistical analysis[a]		
Day	Baseline	21	35	Baseline	21	35	Baseline	Day 21	Day 35
Parameter[b]	(n = 21)	(n = 20)	(n = 20)	(n = 18)	(n = 15)	(n = 15)			
CD3 (10^6/litre)	1583 ± 135[c]	1654 ± 148	1789 ± 158	1425 ± 104	1416 ± 169	1458 ± 149 p	NS	NS	NS
CD4 (10^6/litre)	441 ± 31	467 ± 34	484 ± 34	498 ± 39	461 ± 46	433 ± 33	NS	NS	0.05
CD8 (10^6/litre)	1034 ± 113	1094 ± 126	1193 ± 142	853 ± 90	896 ± 150	948 ± 140	NS	NS	NS
CD4:CD8 ratio	0.52 ± 0.08	0.54 ± 0.09	0.53 ± 0.10	0.70 ± 0.10	0.71 ± 0.14	0.63 ± 0.13	NS	NS	NS

[a]Baseline = level of significance between treatments at baseline; day 21 and day 35 = level of significance for change from baseline between treatments at the respective time points; NS = not significant ($P > 0.05$).
[b]CD3, CD3+ T lymphocyte count; CD4, CD4+ T lymphocyte count (helper/inducer); CD8, CD8+ T lymphocyte count (suppressor/cytotoxic); CD4:CD8 ratio, CD4 + to –CD8+ cell ratio.
[c]Values are means ± SE.

Table 5. Effect of supplemental *Lactobacillus reuteri* on urinalysis parameters of adults infected with the human immunodeficiency virus

	Treatment								
	Placebo			*L. reuteri*			Statistical analysis[a]		
Day	Baseline	21	35	Baseline	21	35	Baseline	Day 21	Day 35
Parameter	(n = 21)	(n = 20)	(n = 20)	(n = 18)	(n = 15)	(n = 15)			
pH	5.5 ± 0.2[b]	5.5 ± 0.2	5.6 ± 0.2	5.6 ± 0.3	5.4 ± 0.3	5.7 ± 0.3	NS	NS	NS
Specific gravity	1.02 ± 0.00	1.02 ± 0.00	1.02 ± 0.00	1.02 ± 0.00	1.02 ± 0.00	1.02 ± 0.00	NS	0.03	NS

Qualitative analysis of the following urinalysis parameters were not different between treatments: colour, bilirubin, ketones, glucose, character, nitrite, urobilinogen, leucocyte esterase, protein, blood, epithelial cells per low power field, crystals-amorphized, bacteria, mucosal threads, white blood cells per high power field, red blood cells per high power field.
[a]Baseline = level of significance between treatments at baseline; day 21 and day 35 = level of significance for change from baseline between treatments at the respective time points; NS = not significant ($P > 0.05$).
[b]Values are means ± SE.

Table 6. Effect of supplemental *Lactobacillus reuteri* on the frequency of subjective gastrointestinal tolerance factors in adults infected with the human immunodeficiency virus

		Percent of subject days for entire study, tolerance factor rating			
Parameter	Treatment	Absent	Mild	Moderate	Severe
Vomiting	Placebo	99.58	0.28	0.14	0
	L. reuteri	97.47	1.99	0.54	0
Flatulence	Placebo	62.89	23.67	12.89	0.56
	L. reuteri	67.45	19.53	9.4	3.62
Burping	Placebo	82.49	10.36	6.86	0.28
	L. reuteri	84.27	10.49	1.99	3.25
Reflux	Placebo	95.66	3.78	0.42	0.14
	L. reuteri	94.21	4.88	0.9	0
Nausea	Placebo	93.14	5.46	1.26	0.14
	L. reuteri	81.56	14.1	4.16	0.18
Cramping	Placebo	88.66	8.4	2.8	0.14
	L. reuteri	87.52	9.04	2.71	0.72
Diarrhoea	Placebo	94.26	3.22	1.96	0.56
	L. reuteri	91.14	4.52	3.07	1.27
Constipation	Placebo	97.06	1.82	1.12	0
	L. reuteri	93.31	3.62	1.27	1.81
Distention	Placebo	91.32	3.92	4.62	0.14
	L. reuteri	86.08	7.96	2.71	3.25

organism. Changes in faecal levels of total *Lactobacillus* spp were similar ($P > 0.11$) across all time points. Bowel function data (number and consistency) are summarized for each week in Table 8. Bowel movements per day and daily faecal consistency were similar ($P > 0.05$) between treatments.

Table 7. Effect of supplemental *Lactobacillus reuteri* on faecal levels of *L. reuteri* and total *Lactobacillus* species in adults infected with the human immunodeficiency virus

Treatment		*L. reuteri* ($\log_{10}$ CFU/g wet faeces)						
		Baseline	Day 7	Day 14	Day 21	Day 28	Day 35	Day 49
Placebo	Mean ± SE	2.70 ± 0.00	2.75 ± 0.04	2.71 ± 0.01	2.79 ± 0.06	2.79 ± 0.06	2.91 ± 0.16	2.70
	(n)	(21)	(21)	(21)	(20)	(20)	(20)	(1)
L. reuteri	Mean ± SE	2.70 ± 0.00	3.34 ± 0.24	2.86 ± 0.10	3.20 ± 0.19	2.87 ± 0.18	2.70 ± 0.00	2.70 ± 0.00
	(n)	(18)	(18)	(16)	(15)	(15)	(15)	(2)
	[a]*P*	not analysed	0.05	0.1	0.05	NS	NS	not analysed
		Total *Lactobacillus* species ($\log_{10}$ CFU/g wet faeces)						
Placebo	Mean ± SE	4.61 ± 0.26	4.86 ± 0.27	4.20 ± 0.26	4.54 ± 0.29	4.52 ± 0.32	4.92 ± 0.29	5.52
	(n)	(21)	(21)	(21)	(20)	(20)	(20)	(1)
L. reuteri	Mean ± SE	4.19 ± 0.30	4.03 ± 0.25	3.98 ± 0.28	4.36 ± 0.28	3.84 ± 0.28	3.67 ± 0.23	2.70 ± 0.00
	(n)	(18)	(18)	(16)	(15)	(15)	(15)	(2)
	P	NS	NS	NS	NS	NS	NS	not analysed

CFU, colony forming units; subjects who had counts below the method's detection threshold (3 $\log_{10}$ CFU/g wet faeces) were substituted with values representing the half-way point (2.70 $\log_{10}$ CFU/g wet faeces).
[a]Other than baseline, *P*-values represent the level of significance for change from baseline between treatments; NS = not significant ($P > 0.11$).

DISCUSSION

The use of probiotics for specific disease states may be warranted. Animal experimentation has shown positive benefits of *L. reuteri* in a murine acquired immunodeficiency syndrome (AIDS) model of cryptosporidiosis (Alak *et al.*, 1996, 1997). Prior to clinical experimentation in the AIDS population, the safety and tolerance of humans to *L. reuteri* must be demonstrated. Previously, Wolf *et al.* (1995) found no safety or tolerance concerns in healthy male adults consuming 1×10^{11} CFU *L. reuteri* per day. The current experiment was conducted to evaluate the safety and tolerance of supplemental *L. reuteri* in HIV+ adults.

It had been anticipated that many subjects would be concurrently on antiretroviral therapy. Thus, subjects were randomized to treatments in blocks according to antiretroviral use. Because zidovudine is the most commonly prescribed antiretroviral drug, subject enrolment was limited to individuals on zidovudine only (i.e. if they were currently on antiretroviral therapy). However, most volunteers for this experiment were not currently on antiretroviral treatment. Thus, a randomized block analysis was not completed since it would not be statistically meaningful.

Few statistical differences were found for the serum chemistry, haematology, immune deficiency and urinalysis data collected. All changes were not considered clinically significant nor related to treatment. It was noted that the total cholesterol levels of these individuals is low. However, the level of high-density lipoprotein (HDL) cholesterol was also very low (below the lab's normal values) making the cholesterol/HDL ratio unfavourable. This phenomenon has been noted before in HIV+ individuals (Shor-Posner *et al.*, 1993).

At day 35 there was a significant difference for the change from baseline (placebo > *L. reuteri*) for the absolute number of CD4+ T lymphocytes. This change was noted due to a decrease over time for the *L. reuteri* group versus an increase over time for the placebo group. Because this trend continued after 2 wk of washout it is not interpreted to be treatment related; however, additional studies should be conducted to corroborate this finding. It is interesting to note that CD4+ T lymphocyte

Table 8. Effect of supplemental *Lactobacillus reuteri* on bowel function in adults infected with the human immunodeficiency virus

Treatment		Average daily number of bowel movements				
		Week 1	Week 2	Week 3	Week 4	Week 5
Placebo	Mean ± SE (n)	1.59 ± 0.14 (21)	1.70 ± 0.16 (21)	1.81 ± 0.17 (20)	1.74 ± 0.18 (20)	1.69 ± 0.18 (20)
L. reuteri	Mean ± SE (n)	1.69 ± 0.17 (17)	1.83 ± 0.17 (17)	1.79 ± 0.18 (15)	1.73 ± 0.20 (15)	1.66 ± 0.17 (15)
	[a]*P*	NS	NS	NS	NS	NS
		Average daily faecal consistency score[b]				
Placebo	Mean ± SE (n)	2.77 ± 0.13 (21)	2.77 ± 0.11 (21)	2.87 ± 0.16 (20)	2.74 ± 0.12 (20)	2.72 ± 0.15 (20)
L. reuteri	Mean ± SE (n)	2.65 ± 0.16 (17)	2.64 ± 0.15 (17)	2.53 ± 0.19 (15)	2.56 ± 0.19 (15)	2.48 ± 0.18 (15)
	P	NS	NS	NS	NS	NS

Qualitative analysis for total faecal fat and faecal neutral fat were not different between treatments.
[a]*P*-values represent the level of significance for each week between treatments; NS = not significant ($P > 0.05$).
[b]Faecal consistency score: 1 = hard, dry; 2 = hard, formed; 3 = soft, formed; 4 = soft, unformed; 5 = watery.

counts were below the lab's normal range for both treatments. This is not surprising, and is probably an artefact of this patient population's HIV infection. Following a similar trend, CD8+ T lymphocyte counts were above the lab's normal range, resulting in a lower CD4+ to −CD8+ cell ratio for both treatment groups.

Faecal levels of lactobacilli were much lower for this HIV+ population (approx. 2×10^4) compared with a previous study (Wolf *et al*., 1995) in healthy male adults (approx. 1×10^7). Likewise, faecal levels of *L. reuteri* were low, even in subjects supplemented with 1×10^{10} CFU per day. These data could suggest that the enumeration methodology was poor in this experiment. However, samples from healthy male adults that were processed by the same laboratory found at least a 2 log higher lactobacilli count (data not shown), similar to the results of the previously mentioned study (Wolf *et al*., 1995). This would suggest that a low faecal lactobacilli count is characteristic of this population. It could be concluded that the HIV+ population has a low level of intestinal lactobacilli. However, the faecal analysis conducted in this study may be misleading. A majority of the subjects in this experiment were homosexual males. It is possible that the private lifestyle of this population would reduce the viability of microorganisms in the lower bowel (e.g. use of colonic irrigation is popular for this population). Therefore, microbiota counts in the proximal colon and ileum may be "normal" but cannot be determined via faecal microbial enumeration. It may also be concluded that *L. reuteri* do not survive passage through the GI tract of the HIV+ population. However, Welage *et al*. (1995) have shown that the environment of the stomach is less acidic in the AIDS patient, making the major obstacle of bacterial passage through the GI tract less challenging. One final explanation would be that the altered immune function of this population may modulate the indigenous microbiota composition. There appears to be a complex relationship between the indigenous microbiota and the mucosal immune system that is not completely understood (Gaskins, 1997; van der Waaij, 1989).

Two subjects had detectable *L. reuteri* on day 35, and were followed along with two subjects without *L. reuteri* at day 35. Only three subjects provided a day 49 sample, all showing no *L. reuteri*. After removal of the mask at the end of the study, it was determined that the two subjects who had *L. reuteri* at day 35 were on placebo, thus the *L. reuteri* found in these subjects was probably of ubiquitous nature (i.e. natural colonization). It is worth mentioning that even though no differences were noted in bowel function, the investigator correctly identified the active treatment after several weeks of processing faecal samples. The investigator noted that individuals consuming treatment A (*L. reuteri*) had less faecal odour and improved faecal consistency. Even though there was a 50% chance of guessing correctly, the investigator was correct with this subjective opinion. Again, the study was not unmasked until after the experiment concluded.

Overall, these data suggest that *L. reuteri* may be fed at a level of 1×10^{10} CFU per day to HIV+ subjects without any safety concerns. This experiment documents that *L. reuteri* fed at a level of 1×10^{10} CFU per day is safe and well tolerated in an HIV+ population. Faecal analysis proved to be a poor method for evaluating the survival of *L. reuteri* through the GI tract in this predominantly male population since faecal levels of total *Lactobacillus* spp were very low.

Acknowledgements—The authors wish to thank Dr Steven Delaveris, Georgeann Goheen and Bonnie Abbruzzese for their assistance in the co-ordination of this study.

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