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Evaluation of clinical safety and tolerance of a *Lactobacillus reuteri* NCIMB 30242 supplement capsule: A randomized control trial

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ABSTRACT

A significant number of human clinical trials have reported no adverse effects associated with consumption of *Lactobacillus reuteri* (*L. reuteri*). In the present study, the clinical safety and toxicology of oral ingestion of supplement capsules containing *L. reuteri* NCIMB 30242 was investigated. A randomized group of 131 subjects received a dose of 2.9×10^9 CFU *L. reuteri* NCIMB 30242 capsules ($n = 67$) or placebo capsules ($n = 64$) twice daily for 9 weeks. Clinical chemistry and hematological parameters of safety were analyzed. The frequency, duration and intensity of adverse events (AE)s and clinical significance of safety parameters were recorded for both groups. No clinically significant differences between the probiotic capsule and placebo capsule treated groups were detected in either the blood clinical chemistry or hematology results. The frequency and intensity of AEs was similar in the two groups. These results demonstrate that administration of a twice daily dose of 2.9×10^9 CFU was safe and well tolerated in the population evaluated over 9 weeks.

1. Introduction

The concept of probiotics evolved from a theory that was proposed by Metchnikoff at the beginning of the 20th century, who suggested that certain microbes beneficially affected the host by improving its intestinal microbial balance (Metchnikoff, 1907). Today, probiotics are defined as live microorganisms, which when administered in adequate amounts confer a health benefit on the host (WHO and FAO, 2001). Growing scientific evidence exists for the beneficial effects of live bacteria on a variety of gastrointestinal, immunological and metabolic conditions (Marteau, 2002). The demonstration of the efficacy of probiotics offers vast opportunities to improve human health; however, before a probiotic can be used to benefit human health, several aspects, including safety, functional and technological characteristics have to be taken into consideration.

The most frequently used strains include members of the genus *Lactobacillus*. Their presence in human flora and long history of use in foods and dairy products without significant complications has led to the conclusion that they are safe for human consumption and, as such, many strains have obtained generally recognized as safe (GRAS) status or have been determined to be safe for their traditional food uses under the Qualified Presumption of Safety (QPS) assessment process (EFSA Scientific Committee, 2007). The species *Lactobacillus reuteri* was first isolated from human fecal and intestinal samples in the 1960s and later was found to be present in most mammals and birds and in many food sources (Lee et al., 2009; Molin et al., 1992; Naito et al., 1995; Taranto et al., 2003). A number of human clinical trials have reported no adverse effects associated with consumption of *L. reuteri* (Coccorullo et al., 2010; Connolly et al., 2005; Rosander et al., 2008; Weizman and Alsheikh, 2006; Wolf et al., 1998, 1995).

There is growing interest in the identification of specific probiotic strains that can be used as foods or supplements to improve health and prevent and treat diseases. In support of this, *L. reuteri* NCIMB 30242 was selected for its ability to lower cholesterol. The

Abbreviations: AE, adverse events; ALT, alanine transaminase; APA, alginate-poly-l-lysine; AlkP, alkaline phosphatase; AST, Aspartate Aminotransferase; bpm, beats per minute; BSH, bile salt hydrolase; BMI, body mass index; Ca^{2+}, calcium; Cl^-, chloride; CSV, clinically significant value; GGT, gamma-glutamyl transpeptidase; ICH–GCP, International Conference on Harmonization–Good Clinical Practice; LAB, lactic acid bacteria; *n*, number of subjects; ANOVA, one-way analysis of variance; PRO, patient-reported outcome; PO_4^{3-}, Phosphate; K^+, potassium; QC, quality control; QPS, Qualified Presumption of Safety; Na^+, sodium; RBCs, red blood cells; SOP, standard operating procedures; WBCs, white blood cells.

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presence of a bile salt hydrolase (BSH) enzyme enables this strain to deconjugate bile acids which is hypothesized to lead to a reduction of serum cholesterol (Jones et al., 2011). Extensive *in vitro* characterization using molecular and metabolic techniques has demonstrated that the genome of *L. reuteri* NCIMB 30242 does not encode functional virulence factors or transmissible antibiotic resistance elements (Branton et al., 2011). In addition, the strain does not produce potentially harmful by-products such as biogenic amines and D-lactate at levels that can produce adverse effects in sub-sets of the population (Branton et al., 2011). Based on these results, *L. reuteri* NCIMB 30242 was deemed safe for human consumption.

The aim of the present study was to evaluate the safety and tolerability of *L. reuteri* NCIMB 30242 delivered in capsule format in a randomized, double-blind, placebo-controlled study of closely-monitored human subjects. Included was sequential documentation of the clinical chemistry, hematology and fecal parameters of the treated subjects to determine whether the values obtained extended beyond normal limits or differed significantly from the findings in the placebo group. Additionally, the number, duration and intensity of AEs reported were closely monitored and the clinical significance of safety parameters was assessed.

2. Materials and methods

2.1. Study design

The objective of the study was to assess the tolerance, safety and impact on health parameters of otherwise healthy hypercholesterolemic human subjects to *L. reuteri* NCIMB 30242 capsules. The double-blind, placebo-controlled, multi-center study included 131 male and female subjects randomized into two treatment groups (*L. reuteri* capsule or placebo capsule). The study lasted a total of 13 weeks. The subjects were initially screened to determine whether they met the inclusion criteria prior to a 2-week wash-out period in which subjects were advised to follow general dietary recommendations throughout the entire study and a 2-week run-in period in which subjects consumed placebo capsules twice daily with the morning and evening meals. In total, subjects met with the investigational team at 3 time points over the 4-week screening period (initial screening visit, post-wash-out visit, post-run-in visit), after which inclusion was verified and subjects meeting all criteria were randomized. Post-randomization, subjects consumed either placebo or probiotic capsules twice daily with the morning and evening meals over a 9-week treatment period. Blood samples were taken at various time points from the participants for analyses. The study was performed by a Contract Research Organization, A-Pharma s.r.o. (Prague, CZ) according to the principles of the Declaration of Helsinki, and in compliance with International Conference on Harmonization – Good Clinical Practice (ICH-GCP). All procedures involving human subjects were approved by the Ethics Committee for Multi-Centric Clinical Trials of the University Hospital Motol, Czech Republic. The study protocol was carefully explained to all subjects before written informed consent was obtained from all subjects. Regular monitoring visits to the investigational sites were made prior to, during and post-treatment phase by A-Pharma s.r.o. monitors to ensure that the study was conducted in compliance with the protocol, the ICH-GCP guidelines and the applicable regulations for the clinical trial and that the rights, safety and well-being of the study subjects were protected at all times. The study was registered in the public registry ClinicalTrials.gov (NCT ID: NCT01341613).

2.2. Subject inclusion

Strict study entry criteria were assessed prior to enrollment into the study. Otherwise healthy hypercholesterolemic adult men and women were recruited from 6 centers in Prague, Czech Republic. Recruitment was performed by investigating physicians through patient record review or discussion of potential enrolment with patients. In certain centers, recruitment was further enhanced by local newspaper advertisement. Inclusion criteria for randomization were otherwise healthy males and females between the ages of 20 and 75 years old (inclusive); LDL-C >3.4 mmol/l with <15% variation between screening visits; triglycerides <4.0 mmol/l; body mass index (BMI) of 22–32 kg/m^2; accept to follow dietary recommendations; statin dose must be stable for at least 3 months; signed informed consent; subjects were permitted to take stable doses of thyroid hormone and anti-hypertensive agents; female subjects: effective contraceptive methods used. Dietary recommendations were advised, via the National Cholesterol Education Profile – Adult Treatment Panel III (NCEP ATP III) guidelines (NCEP Expert Panel, 2002), as part of the usual standard of care for hypercholesterolemic adult men and women. Exclusion criteria for randomization were use of cholesterol lowering prescription drugs other than statin monotherapy within the last 6 months; use of other cholesterol lowering non-prescription supplements within the last 3 months; history of chronic use of alcohol; use of systemic antibodies, corticosteroids, androgens, or phenytoin; having experienced any cardiovascular event in the last 6 months; diabetic; currently involved in a clinical trial or in an exclusion period following participation in another clinical trial; history of angina, congestive heart failure, inflammatory bowel disease, pancreatitis, gastrointestinal, renal, pulmonary, hepatic or biliary disease, or cancer; chronic user of probiotics or fiber laxative, or stimulant laxatives; history of eating disorders; exercise greater than 15 miles/wk or 4000 kcal/wk; pregnancy, breast feeding, or intent to get pregnant.

2.3. Examination of physical condition and vital signs

Subjects met with the investigational team at 7 different time points: Visit V0 (Week-4), V1 (Week-2), V2-1 (Week 0–1 day), V2-2 (Week 0, randomization and treatment baseline), V3 (Week 3, treatment midpoint), V4 (Week 6, treatment midpoint), and V5 (Week 9, treatment endpoint). The documented demographic characteristics of the participants included medical and surgical histories, alcohol and tobacco use and methods of contraception (for females of childbearing age). Abbreviated physical examinations were conducted at visits V2 (Week 0) and V5 (Week 9). Parameters recorded included the subjects' weight, BMI, vital signs (heart rate, systolic blood pressure, and diastolic blood pressure) as well as any detectable abnormalities of the skin, eyes, ears, nose and throat, heart, lungs, abdomen, musculoskeletal system, lymph nodes and nervous system. The subjects' dietary compliance and use of alcohol were also regularly assessed by investigating physicians at each visit and recorded on the CRF throughout the study period.

2.4. Preparation of test material

Lactobacillus reuteri NCIMB 30242 (Cardioviva™) was propagated in a 5000L fermenter, concentrated and lyophilized in compliance with standard operating procedures (SOP) and quality control (QC) procedures at Probiotical S.p.A (Novara, Italy). Microbiological analyses and bacterial culture purity were confirmed immediately after production, as well as at study baseline and throughout the study. Strain identity was confirmed using Amplified Fragment Length Polymorphism (AFLP) analysis; a technique involving whole genome DNA fingerprinting using selective amplification of restriction fragments (Vos et al., 1995). Generation of the AFLP profile and analysis was conducted by Belgium Co-ordinated Collections of Micro-organisms (BCCM/LMG, Belgium). Placebo and treatment capsules were produced at Probiotical S.p.A