Clinical Nutrition xxx (2015) 1–8

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Randomized control trials

Cow's milk and rice fermented with *Lactobacillus paracasei* CBA L74 prevent infectious diseases in children: A randomized controlled trial

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ARTICLE INFO

Article history:
Received 7 July 2015
Accepted 10 December 2015

Keywords:
Acute gastroenteritis
Upper respiratory tract infections
Probiotics
Innate immunity
Acquired immunity

SUMMARY

Background & aim: Fermented foods have been proposed for the prevention of infectious diseases. We evaluated the efficacy of fermented foods in reducing common infectious diseases (CIDs) in children attending daycare.

Methods: Prospective randomized, double-blind, placebo-controlled trial (registered under Clinical Trials.gov identifier NCT01909128) on healthy children (aged 12–48 months) consuming daily cow's milk (group A) or rice (group B) fermented with *Lactobacillus paracasei* CBA L74, or placebo (group C) for three months during the winter season. The main study outcome was the proportion of children who experienced at least one CID. All CIDs were diagnosed by family pediatricians. Fecal concentrations of innate (α- and β-defensins and cathelicidin LL-37) and acquired immunity biomarkers (secretory IgA) were also evaluated.

Results: 377 children (193 males, 51%) with a mean (SD) age of 32 (10) months completed the study: 137 in group A, 118 in group B and 122 in group C. Intention-to-treat analysis showed that the proportion of children who experienced at least one CID was lower in group A (51.8%) and B (65.9%) compared to group C (80.3%). Per-protocol analysis showed that the proportion of children presenting upper respiratory tract infections was lower in group A (48.2%) and group B (58.5%) compared with group C (70.5%). The proportion of children presenting acute gastroenteritis was also lower in group A (13.1%) and group B (19.5%) compared with group C (31.1%). A net increase of all fecal biomarkers of innate and acquired immunity was observed for groups A and B compared to group C. Moreover, there was a negative association between fecal biomarkers and the occurrence of CID.

Conclusion: Dietary supplementation with cow's milk or rice fermented with *L. paracasei* CBA L74 prevents CIDs in children attending daycare possibly by means of a stimulation of innate and acquired immunity.

1. Introduction

Daycare centers and schools are ideal places for the occurrence and transmission of common infectious diseases (CIDs) affecting respiratory and gastrointestinal tract in young children, often resulting in many missed days of both daycare and parental work [1]. Young children attending daycare centers and schools have a 1.5–3.0 times higher risk of respiratory and gastrointestinal tract

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http://dx.doi.org/10.1016/j.clnu.2015.12.004
0261-5614/

Abbreviations

CIDs	common infectious diseases
AGE	acute gastroenteritis
URTI	upper respiratory tract infections
FPs	family pediatricians
HNP	1-3: α-defensin
HBD-2	β-defensin
LL-37	cathelicidin
sIgA	secretory immunoglobulin

infections than children cared for at home or in small family care groups [1]. These subjects have been shown to have more outpatient doctor visits, emergency room visits, and increased usage of prescription medicines than children not in daycare [2]. Daycare-related infectious illnesses have been estimated to cost $1.8 billion per year in the United States [3].

The prevention of infections in daycare is therefore of major importance. An option is to use fermented foods with probiotics [4]. The fermentation process provides a gain in these food products in terms of benefits for health, which makes these products useful strategy against pediatric infections [4]. The efficacy of these fermented foods is believed to be strain-specific and dose-dependent [4]. For these reasons, it is of fundamental importance to test each product in clinical trials.

The aim of this double-blind, randomized, placebo-controlled trial was to test the preventive effect of fermented products with *Lactobacillus paracasei* CBA L74 (i.e., fermented milk and rice) against CIDs in children attending daycare or preschool. We also tested whether these fermented foods are able to stimulate innate (α- and β-defensins and cathelicidin LL-37) and acquired (secretory immunoglobulin A) immunity factors.

2. Patients and methods

2.1. Study design

A prospective randomized, double-blind, placebo-controlled trial was conducted from January to March 2012 in collaboration with family pediatricians (FPs), who care for children up to 14 years of age in the Italian Public Health System. The study protocol was illustrated to and discussed with FPs during two meetings.

2.2. Ethics

The study was approved by the Ethics Committee of the University of Naples "Federico II" and was registered in the Clinical Trials Protocol Registration System (ClinicalTrials.gov) with the identifier NCT01909128.

2.3. Study subjects

Consecutive healthy children (12–48 months of age) attending daycare or preschool at least five days a week, were invited to participate to the study. Anamnestic, demographic and clinical data, including vaccination status, were collected by the FPs and reported in a specific clinical chart. The exclusion criteria were: age ≤12 months or ≥48 months, concomitant chronic systemic diseases, congenital cardiac defects, gastrointestinal or urinary or respiratory tract surgery, active tuberculosis, autoimmune diseases, immunodeficiency, chronic inflammatory bowel diseases, cystic fibrosis, metabolic diseases, history of suspected or challenge-proved food allergy, lactose intolerance, malignancy, chronic pulmonary diseases, malformations of gastrointestinal or urinary or respiratory tract, severe malnutrition (z score for weight-for-height <3 standard deviation scores), and use of pre/pro/synbiotics, antibiotics or immune stimulating products in the 2 weeks before the enrollment.

2.4. Intervention

The investigators were blinded to the treatment at all times, i.e. allocation, intervention, laboratory analysis and statistical analysis. The study subjects were distributed into three groups (A, B and C) according to a computer-generated randomization list. The FPs assigned for each child the next available number on entry into the trial. The FPs, parents and children were not aware of the dietary treatment assigned. Subjects were supplemented daily for 3 months with a dietary product deriving from cow's milk (group A) or rice fermentation (group B) with *L. paracasei* CBA L74, or placebo (group C).

In Table 1 is reported the composition of the study dietary products. They were provided in powder by Heinz Italia SpA, Latina, Italy, an affiliate of H.J. Heinz Company, Pittsburgh, PA, USA. The fermented milk was prepared from skim milk fermented by *L. paracasei* CBA L74. The fermentation was started in the presence of 10^6 bacteria, reaching 5.9×10^9 colony-forming units/g after a 15-h incubation at 37 °C. After heating at 85 °C for 20 s in order to inactivate the live bacteria, the formula was spray-dried. Thus, the final fermented milk powder contained only bacterial bodies and fermentation products and no living microorganisms. The fermented rice product was obtained using the same procedure. The placebo consisted of maltodextrins with similar energy content of fermented milk and rice products. Study products were provided in tins containing 400 g of powder, and the packaging was similar. Study products were stored at room temperature and in a dry environment.

The FPs instructed parents about the daily amount of the assigned study product and the method of preparation. All subjects received 7 g/day of study products diluted in a maximum 150 ml of cow's milk or water. After dilution, the look and the taste were the same for all study products. Parents were encouraged to contact the FP if necessary and to maintain the habitual diet of the child, but to exclude prebiotics, probiotics, synbiotics and immune stimulating products during the 3-month study period.

During episodes of acute gastroenteritis (AGE), children were instructed to continue the assigned study product.

An independent clinical trial monitor, blinded to the treatment assignment, was involved in the research. Study monitoring included on-site visits and telephone communications with FPs, to ensure that the investigation was conducted according to the protocol. The clinical trial monitor collected clinical forms, ensured compliance with the clinical trial protocol, reviewed the clinical forms for completeness, clarity, and consistency, and communicated with the clinical research coordinators before the final analysis.

2.5. Study outcomes

The primary outcome of the trial was the proportion of children experiencing at least one episode of CID. The secondary outcomes

Table 1
Composition of the study dietary products.

Value for 100 g of product	Fermented milk	Fermented rice	Placebo
Energy, kcal	367	397	388
Proteins, g	24.0	7.8	0
Carbohydrates, g	66.4	88.9	97
Fats, g	0.6	1.1	0
Lactobacillus paracasei CBA L74, CFU[a]	5.9×10^{11}	5.9×10^{11}	–

[a] Killed bacteria.

Please cite this article in press as: Nocerino R, et al., Cow's milk and rice fermented with *Lactobacillus paracasei* CBA L74 prevent infectious diseases in children: A randomized controlled trial, Clinical Nutrition (2015), http://dx.doi.org/10.1016/j.clnu.2015.12.004