Available online at www.sciencedirect.com

ScienceDirect

www.nrjournal.com

Original Research

Bifidobacterium lactis 420 and fish oil enhance intestinal epithelial integrity in Caco-2 cells

Kati Mokkala [a,b,c], Kirsi Laitinen [a,c,*], Henna Röytiö [a,c]

[a] Institute of Biomedicine, 20014 University of Turku, Finland
[b] Department of Medical Microbiology and Immunology, 20014 University of Turku, Finland
[c] Functional Foods Forum, 20014 University of Turku, Finland

ARTICLE INFO

Article history:
Received 5 August 2015
Revised 17 November 2015
Accepted 24 November 2015

Keywords:
Caco-2
Intestinal permeability
Probiotic supernatant
Fish oil
In vitro
TEER

ABSTRACT

Increased intestinal permeability is a predisposing factor for low-grade inflammation–associated conditions, including obesity and type 2 diabetes. Dietary components may influence intestinal barrier integrity. We hypothesized that the dietary supplements *Bifidobacterium lactis* 420, *Lactobacillus rhamnosus* HN001, and fish oil have beneficial impacts on intestinal barrier integrity. In addition, we hypothesized that the coadministration of these components results in synergistic benefits to the integrity of the intestinal barrier. To study this, we investigated the impact of cell-free culture supernatant from dietary supplements *B lactis* 420 and *L rhamnosus* HN001, and fish oil, separately and in combination, on intestinal permeability in a CaCo-2 cell model. Administered separately, both *B lactis* 420 supernatant and fish oil significantly increased the integrity of the intestinal epithelial barrier, as determined by an increase in transepithelial electrical resistance (TEER), whereas *L rhamnosus* did not. The TEER increase with *B lactis* 420 was dose dependent. Interestingly, a combination of *B lactis* 420 supernatant and fish oil negated the increase in TEER of the single components. mRNA expression of tight junction proteins, measured by real-time quantitative polymerase chain reaction, was not altered, but the mRNA expression of myosin light chain kinase increased after fish oil treatment. To conclude, single dietary components, namely, *B lactis* 420 and fish oil, induced beneficial effects on intestinal barrier integrity in vitro, whereas a combination of 2 beneficial test compounds resulted in a null effect.

1. Introduction

In the gastrointestinal tract, epithelial cells form a barrier that enables the absorption of dietary nutrients but at the same time provides a defense against intestinal pathogens, allergens, and toxins. Maintaining the integrity of the intestinal barrier is essential for human health, and indeed, an increased intestinal permeability exists in several disorders such as obesity, obesity associated insulin resistance, type 1 diabetes, and type 2 diabetes [1–4]. Impairment of the barrier function may increase the risk for metabolic endotoxemia, a condition where excess amounts of gram-negative bacterial

Abbreviations: Caco-2, colon cancer cell line; CGM, cell growth medium; CFS, cell-free supernatant; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; IFN, interferon; JAM, junctional adhesion molecule; LPS, lipopolysaccharide; MLCK, myosin light chain kinase; TEER, transepithelial electrical resistance; TJ, tight junction; qPCR, quantitative polymerase chain reaction; TNF, tumor necrosis factor.

* *Corresponding author at:* 20014 University of Turku, Finland. Tel.: +358 23336063.
E-mail address: kirsi.Laitinen@utu.fi (K. Laitinen).

http://dx.doi.org/10.1016/j.nutres.2015.11.014
0271-5317/

endotoxin, lipopolysaccharide (LPS), passes through the intestinal wall into blood circulation, resulting in an increased risk for systemic low-grade inflammation [5, 6]. Compared with healthy subjects, higher levels of LPS were measured in a range of conditions, including obesity, diabetes, cardiovascular disease, and nonalcoholic steatohepatitis [5, 7–9], which suggest that intestinal permeability and metabolic endotoxemia may have a role in these lifestyle-associated diseases. Maintaining the integrity of the intestinal epithelial barrier is important to prevent both metabolic endotoxemia and systemic low-grade inflammation–associated conditions.

The integrity of the intestinal barrier is maintained by intercellular junction complexes, which consist of tight junctions (TJs), adherent junctions, desmosomes, and gap junctions [10]. Tight junctions, the most apical complex, are responsible for regulating the paracellular transport of ions, solutes, and water and are composed of multiple proteins, such as claudins [11], occludin [12], and junctional adhesion molecules (JAMs) [13]. Cytosolic zonula occludin proteins interact with the cytosolic domain of TJ proteins, which form an anchor between these transmembrane proteins and the cellular actin cytoskeleton [14].

Human intestinal Caco2 cells, obtained from colon adenocarcinoma, are widely used as a model for the intestinal epithelial barrier because, in cell culture, Caco-2 cells form a monolayer and TJ complexes characteristic of enterocytes [15]. Transepithelial electrical resistance (TEER) is a measure of the integrity of the intestinal barrier and is a method used to follow intestinal epithelial integrity in vitro.

Dietary components may influence the integrity of the intestinal barrier. Consumption of a high-fat diet is linked to increased intestinal permeability, which results in an increased LPS transfer into the circulation [5]. However, certain fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), prevent inflammation-induced disruption of TJ structure [16, 17]. Certain probiotics, live microorganisms that when administered in adequate amounts confer a health benefit to the host [18], may induce beneficial changes to the integrity of the intestinal barrier by altering TJ protein expression and localization [19].

We hypothesized that the dietary supplements *Bifidobacterium lactis* 420, *Lactobacillus rhamnosus* HN001, and fish oil improve the integrity of the intestinal barrier. In addition, we hypothesized that the combination of these dietary components would result in synergistic benefits to the integrity of the intestinal barrier. Thus, we investigated the impact of *B lactis* 420, *L rhamnosus* HN001, and fish oil, separately and in combination, on the integrity of the intestinal barrier using CaCo-2 cells as an experimental model and TEER as a measure of the integrity of the intestinal barrier. In addition, the potential mechanism of effect on intestinal epithelial integrity was studied by quantitative polymerase chain reaction (qPCR) for mRNA expression of TJ proteins and myosin light chain kinase (MLCK), a critical regulator of epithelial paracellular permeability.

2. Methods and materials

2.1. Epithelial cell culture

Human intestinal Caco-2 cells (ATCC HTB-39, LGC Standards, Manassas, VA, USA) were thawed and cultured according to ATCC instructions and Natoli et al (2012) [20]. Cell growth medium (CGM) was Dulbecco modified Eagle medium (Gibco, Paisley, UK; #21969-035) supplemented with 4 mmol/L L-glutamine (Gibco, #25030), penicillin 100 U/L and streptomycin 100 μg/L (Gibco, #15140-122), 1% nonessential amino acids (Gibco, #11140-050), and 20% heat-inactivated fetal bovine serum (Gibco, #10270-106). Passage numbers 25 and 28 were used for differentiating the cells for experiments.

2.2. Preparation of bacterial culture and fish oil capsules

Bifidobacterium animalis ssp *lactis* 420 (DSM 22089; Dupont, Niebuell, Germany) and *Lactobacillus rhamnosus* HN001 (ATCC SD5675; Dupont, Niebuell, Germany) were grown in de Man, Rogosa and Sharpe (MRS) broth (Oxoid, Hampshire, UK; #CM0359B) until colony-forming units reached about 10^8/mL, which corresponded to an OD600 of 0.86 for *L rhamnosus* HN001 and 0.75 for *B lactis* 420. The bacterial supernatant was used in experiments to examine the potential effect of compounds secreted by bacteria on the Caco-2 cells. As in a previous study [21], bacterial growth medium was centrifuged to collect cell-free supernatant (CFS). The CFS was filtered through a 0.2-μm sterile filter and used in cell culture experiments. As previously published [22], fish oil capsules containing omega 3-fatty acids in fatty acid ethyl ester form (Croda Europe Ltd, Leek, England) were used in this study. The contents of the fish oil capsules were suspended in 1 mL of 99.5% ethanol and diluted to CGM to correspond to a 1-mmol/L DHA/0.12-mmol/L EPA concentration, which was then added to the cells (ethanol concentration in final dilution < 0.05%). The fish oil capsules consisted of 9.4% EPA and 79.1% DHA as ethyl esters, with a total omega 3-fatty acids content of 88.5%. As a control for the effects of MRS broth, 10% MRS broth in CGM was used. To be able to compare the fish oil–treated cells with those treated with CFS, 10% MRS broth was added to the final fish oil dilution To study the dose response of Caco-2 cells to *B lactis* CFS, 20% *B lactis* CFS was used.

2.3. TEER measurements to study intestinal epithelial integrity

For differentiation of the cells for TEER measurement, a previously described short cell growth protocol [21, 23] with slight modifications was used. Briefly, 6.7×10^5 CaCo-2 cells per cubic centimeter were seeded on collagen (Rat tail collagen, Gibco; #A1048301)-coated Transwell ThinCert, 12W Multiwell Plate Inserts (Greiner Bio-One, Frickenhausen, Germany; #703-665640) with translucent membrane filters (growth area: 113.1 mm^2). Growth medium was changed every second day. After 6 days, cells were confluent as observed by light microscopy. In confluent Caco-2 cells, TJs, the integrity of which is measured by TEER, are already formed [23]. A TEER value exceeding 250 Ω indicates the formation of TJs within the cell layer [24]. The experiments were performed 6 days after differentiation of the cells on cell culture inserts. The TEER was measured with Millipore Millicell-ERS (Millipore, Billerica, MA, USA) before (time 0 hour) and after (time 24 hours) adding 1 mL of 10% CFS or fish oil (1 mmol/L DHA, 0.12 mmol/L EPA) in CGM to the apical side of the cells. Bacterial CFS and fish oil were tested separately. In addition, bacterial CFSs were tested in combination with fish oil. The results