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Oral consumption of cinnamon enhances the expression of immunity and lipid absorption genes in the small intestinal epithelium and alters the gut microbiota in normal mice

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ABSTRACT

Because cinnamon is orally ingested, its biological activity is likely to influence the small intestinal epithelium and the microbiota along the intestinal tract. We investigated small intestinal epithelial gene expression associated with immunity and lipid metabolism and measured IgA level in the small intestines and sera of mice fed cinnamon. We also analyzed microbial changes in the small and large intestines and feces using pyrosequencing of the 16 s rRNA gene. Cinnamon increased the gene expression of *Muc2*, *RegIIIγ*, and *Pigr*, which are necessary for defense against gut bacteria. It also increased the gene expression of *GATA4* and *Slc27a2*, which are implicated in lipid absorption. Cinnamon increased luminal IgA level but not serum IgA and decreased the class *Gammaproteobacteria* (classified within the phylum *Proteobacteria*), particularly the genus *Pseudomonas,* in the large intestine. Cinnamon thus affected immunity and lipid absorption in the small intestinal epithelium and microbial composition in the intestines.

1. Introduction

The small intestinal epithelium absorbs nutrients and water and performs the immune function of preventing invasion of gut bacteria (Okumura & Takeda, 2017). Gut bacteria reside in the upper surface of this one layered tissue with immune cells including B cells located in the underlying connective tissue (the lamina propria). The small intestinal epithelium produces mucins and antimicrobial peptides to inhibit microbial access to the epithelial surface (Ramanan & Cadwell, 2016) and makes polymeric immunoglobulin receptor (pIgR), which is used to deliver the IgA produced by the B cells to the lumen (Gommerman, Rojas, & Fritz, 2014). IgA binds to commensals, pathogens, and food antigens to prevent them from adhering to the epithelium (Gommerman et al., 2014). Interestingly, the small intestinal epithelium seems to combine its immune function with lipid absorption, as demonstrated by Shulzhenko et al., who showed that the jejunum of B cell- or IgA-deficient mice upregulates genes involved in immunity to mount a defense against gut bacteria but has impaired lipid absorption due to repression of GATA4, a transcription factor necessary for maintenance of morphology and function, including lipid absorption, in the proximal small intestine (Battle et al., 2008; Bosse et al., 2006; Shulzhenko et al., 2011). This helps to explain why patients with common immunodeficiency or HIV infection exhibit defective lipid absorption (Greer, Morgun, & Shulzhenko, 2013).

In recent years, knowledge of the beneficial functions and composition of gut bacteria has been rapidly expanding due to the development of next-generation sequencing technology for microbial analysis. Nutritionally, gut bacteria generate short-chain fatty acids, which host cells use for energy, and take part in the production of vitamins and metabolism of endogenous and xenobiotic substances (Zhang et al., 2015). Furthermore, studies of germ-free mice have elucidated the role of gut bacteria in the development and maturation of the immune

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system (Noverr & Huffnagel, 2004; Round & Mazmanian, 2009). It is now well accepted that immune cells have evolved to accommodate commensals while preventing invasion of exogenous pathogens (Cerf-Bensussan & Gaboriau-Routhiau, 2010).

The microbial composition of individuals is unique and the overall diversity of microbiota is relatively stable throughout life (Lozupone, Stombaugh, Gordon, Jansson, & Knight, 2012). Currently, less than 30% of gut bacteria can be cultured because the anaerobic conditions and carbon sources required for the rest are unknown (Maccaferri, Biagi, & Brigidi, 2011). As shown by culture-independent 16 s rRNA gene sequencing analysis, human microbiota contain more than 1000 species, and they exhibit low diversity, unlike other ecosystems (Lozupone et al., 2012). Of the 52 phyla represented, *Firmicutes* and *Bacteroidetes* dominate the mammalian intestinal tract, accounting for approximately 90%, and *Proteobacteria*, *Actinobacteria*, and others are minor groups (Shin, Whon, & Bae, 2015). Among them, facultative anaerobes that belong to *Proteobacteria* have been reported to increase during dysbiosis, a state in which the composition of the gut-associated microbial community is disturbed (Litvak, Byndloss, Tsolis, & Baumler, 2017).

Due to the convenience of sampling, feces are used in most studies of changes in gut microbiota. However, microbial community composition varies along the length of the intestine because of environmental differences between the small and large intestines (Gevers et al., 2014; Gu et al., 2013). The small intestine is more acidic and aerated and produces more antimicrobial substances than the large intestine. Bile acids, secreted at the proximal small intestine, are bactericidal to certain species (Donaldson, Lee, & Mazmanian, 2016). These features allow fast-growing facultative anaerobes to dominate in the small intestine (Donaldson et al., 2016). Furthermore, a short transit time and the availability of simple sugar in the small intestine contribute to a microbial community different from that in the large intestine (Donaldson et al., 2016). Analyses of the bacterial community along the mouse gastrointestinal tract after feeding with different diets show that changes in the microbiota of the small intestine differ significantly from those in the large intestine (Asano, Yoshimura, & Nakane, 2013; Onishi et al., 2017).

Cinnamon is a herb long-used in traditional Asian medicine to treat colds, arthritis, and gynecological conditions. The biological activities of cinnamon, such as its anti-inflammatory, immunomodulatory, and anti-cancer effects have been investigated extensively (Hong et al., 2012; Lee, Kim, Cho, Sohn, & Kang, 2011; Zhang et al., 2017). Because all those effects are acquired after oral consumption of cinnamon, it is likely that cinnamon influences the small intestinal epithelium, and that its unabsorbed components interact with the intestinal ecosystem. In this study, we fed mice cinnamon water extract, analyzed small intestinal epithelial gene expression associated with immunity and lipid metabolism, and measured IgA level in the small intestine and serum. Furthermore, using pyrosequencing of the 16 s rRNA gene, we analyzed microbial changes in the small and large intestines and feces of normal mice fed cinnamon water extract.

2. Materials and methods

2.1. Preparation of cinnamon water extract

Cinnamon bark of Vietnamese origin was purchased from Omni Herb (Daegu, South Korea). The sample was ground and soaked in water for 72 h at 4 °C and further dissolved by sonication for 1 h. The supernatant was filtered, concentrated, and evaporated using a freeze dryer (Eyela, Japan). Coniferyl aldehyde, coumarin, cinnamic acid, and cinnamaldehyde were characterized by HPLC (data not shown).

2.2. Animals

Male Balb/c mice (seven-weeks of age) were purchased from Koatech (Pyungtek, South Korea) and given free access to rodent chow and water in a temperature-and humidity-controlled pathogen-free facility. Mice were maintained in accordance with the Guide for the Care and Use of Laboratory Animals issued by the US National Research Council (1996). All experiments were approved by the Kyung Hee University Medical Center Institutional Animal Care and Use Committee (KHMC-IACUC-2014-050). After acclimatization for one week, mice were randomly assigned to the control or cinnamon group, each containing 20 mice. The dose was determined based on our previous reports (Hong et al., 2012; Lee et al., 2011). Cinnamon water extract at 100 mg/kg was given to mice daily via oral gavage for seven days. Control mice received an equal volume of water. At the end of the experiment, mice were allocated to the following assays. Five mice in each group were used for isolation of small intestinal epithelium; 10 mice were used for determination of blood and intestinal IgA; the remaining five mice were used for DNA preparation from the small and large intestines and feces.

2.3. Epithelial tissue isolation, RNA isolation and real-time PCR

After the mice were sacrificed by cervical dislocation, the small intestines were harvested, and the mesentery, fat tissue, and Peyer's patches were removed. Epithelial cells were isolated as described (Goodyear, Kumar, Dow, & Ryan, 2014) with some modifications. In brief, one gram of the intestine was obtained and cleared of its contents by flushing with a syringe filled with cold phosphate buffered saline (PBS). The intestine was opened longitudinally, cut into 4–5- cm lengths, and incubated in Hank's Balanced Salt Solution (HBSS) (Sigma, St. Louis, MO, USA) with 2% fetal bovine serum (FBS) (Hyclone, Logan, Utah, USA) and 5 mM DTT (Sigma) for 20 min at 37 °C with continuous stirring. The solution was removed using a 100-μm cell strainer and the remaining tissue was incubated in HBSS with 2% FBS and 5 mM EDTA for 15 min at 37 °C. Cells were collected using the cell strainer. This step was repeated three times. The cells were then pooled and centrifuged at 300 × *g* for 5 min. RNA was extracted using an RNeasy mini kit (Qiagen, Germany) and reverse-transcribed into cDNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster, CA, USA). Real-time PCR was performed using SYBR Green Mix (Applied Biosystems) on a StepPlus One real-time PCR system (Applied Biosystems). Quantification of each cDNA copy number was based on the standard curve calculation method. Target genes were normalized to three housekeeping genes glyceraldehyde-3-phosphate dehydrogenase (GAPDH), beta-2-microglobulin (B2M), and hypoxanthine phosphoribosyl-transferase (HPRT). Primer sequences are shown in Suppplementary Table S1.

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jff.2018.08.013.

2.4. Analysis of IgA in mucus and luminal washing from the small intestine and serum

We obtained 15 cm of small intestine from the terminal ileum and collected luminal fluid by passing 1 mL of cold PBS through the intestine. The luminal wash was centrifuged at 10,000 × *g* for 20 min at