NOTE TO THE EDITOR

Effects of a synbiotic product on blood antioxidative activity in subjects colonized with *Helicobacter pylori*

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Introduction

*Helicobacter pylori* colonizes the human gastric epithelium and can lead to chronic gastritis and peptic ulceration (Dunn et al. 1997). *Helicobacter pylori* infection locally induces the infiltration of the subepithelial gastric lamina propria by phagocytes, which produce excessive amounts of reactive species that induce the oxidative damage of lipids, proteins, nucleic acids and carbohydrates (Pignatelli et al. 2001). In *H. pylori*-positive individuals with gastric complaints the local excess of the pro-oxidants is reflected in blood (Mashimo et al. 2006) and defined as oxidative stress (OxS) (Sies 1997).

The human body has evolved an integrated antioxidant defence system consisting of nonenzymatic antioxidants, such as reduced glutathione (GSH), vitamin E, C, Q10, blood albumin, uric acid, bilirubin and enzymatic antioxidants (e.g. superoxide dismutase, glutathione peroxidase, catalase and haem oxygenase) (Gutteridge and Halliwell 2000). A decreased level of GSH has been detected in the gastric mucosa of patients with *H. pylori* infection (Beil et al. 2000; Jung et al. 2001).

Abstract

**Aim:** To evaluate the impact of the consumption of a synbiotic product on the antioxidative activity markers of blood in asymptomatic *H. pylori*-colonized persons.

**Methods and Results:** Fifty-three healthy adult volunteers without gastric symptoms participated in a randomized, double-blind placebo-controlled study. The crossover consumption of the enterocoated capsules containing antioxidative *Lactobacillus fermentum* ME-3, *Lact. paracasei* 8700:2 and *Bifidobacterium longum* 46 with Raftilose P95 lasted for 3 weeks and did not change the *H. pylori* colonization. In *H. pylori*-positive subjects the sera values of total antioxidative status (TAS) were significantly lower compared to *H. pylori*-negative subjects (0.97 vs 1.05 mmol l−1, *P*=0.008). After the consumption of the synbiotic, TAS values (0.97 vs 1.03 mmol l−1, *P*=0.004) increased, while the ratio between oxidized and reduced glutathione (0.035 vs 0.030, *P*=0.016) decreased in *H. pylori*-positive subjects.

**Conclusions:** The consumption of a synbiotic containing an antioxidative probiotic strain improved the reduced systemic antioxidative activity in *H. pylori*-colonized asymptomatic subjects.

**Significance and Impact of the Study:** A synbiotic product containing an antioxidative probiotic strain may be useful in the reduction of systemic oxidative stress in *H. pylori* infection.
Hence, we aimed to evaluate the antioxidative capacity of blood in healthy H. pylori-colonized individuals and the possibility of improvement through the consumption of a synbiotic product containing three selected probiotic strains and a prebiotic. The selected strains expressed moderate antagonistic activity towards H. pylori NCTC 11637, while Lact. fermentum ME-3 expressed high total antioxidative status (TAS) (Hütt et al. 2006). The inulin-based prebiotic Rafilose P95 has been shown to improve gastrointestinal health by markedly stimulating the bifidobacteria (Gibson et al. 2005).

Materials and methods

Fifty-three healthy adult volunteers (41 women and 12 men) without gastric symptoms completed the randomized, double-blind placebo-controlled crossover mechanistic study (EU-funded project, 'EU and Microfunction', QLRT-2001-00135; ISRCTN43435738). The inclusion criteria were: a desire to participate, an age between 20 and 60 years and no known health problems. Participants were asked to continue their normal diet except for the consumption of probiotic products. The exclusion criteria included: a history of gastrointestinal disease, food allergy and acute infection; the usage of any antimicrobial agents and acid-suppressive drugs within the preceding month and acute infection; the usage of any antimicrobial agents, vitamins; and pregnancy and breastfeeding. All participants signed their written informed consent and were given the possibility of withdrawing from the study at any time. The Ethical Committee of Tartu University approved the study protocol.

All volunteers were randomly allocated to receive enterocoated capsules containing the freeze-dried probiotic (Lact. fermentum ME-3, Lact. paracasei 8700:2 and B. longum 46) 3 × 10⁹ CFU per capsule and 1 sachet of prebiotic (6.6 g Rafilose P95, Orafti) or a placebo (maltodextrin) twice a day for 3 weeks. The viability of the probiotic strains in the capsules was confirmed before the trial. After a 2-week washout period, volunteers were crossed over to another 3 weeks of synbiotic/placebo administration. Helicobacter pylori colonization was tested from faecal samples at the baseline and at the end of the study applying the HpSA-test (ImmunoCard STAT HpSA, Meridian Bioscience Europe, Milan, Italy) (Andreson et al. 2003; Krogfelt et al. 2005).

Samples of fasting blood were collected at recruitment and after placebo and synbiotic consumption. Blood sera samples were analysed for TAS and de-proteinized whole blood, plasma and erythrocyte lysate for oxidized (GSSG) and reduced (GSH) glutathione.

The TAS of the serum was measured with a commercially available kit (TAS, Randox Laboratories Ltd, Ardmore, UK) as described elsewhere (Kullisaar et al. 2002), with water-soluble vitamin E (Trolox) as a standard. The method is based on the inhibition of the absorbance of the ferrylmyoglobin radicals of 2,2′-azinobis-ethylbenzothiazoline 6-sulfonate (ABTS+*) generated by the activation of metmyoglobin peroxidase with H₂O₂.

The total glutathione and oxidized glutathione were measured by an in-house modified method (Griffith 1980; Kullisaar et al. 2003) using a GSH assay kit (Cayman Chemical Company, Michigan, USA). The glutathione content was calculated on the basis of a standard curve generated with a known concentration of glutathione. The amount of GSH (µg ml⁻¹) was calculated as the difference between the total glutathione and GSSG.

Statistical analysis was performed by using R 2.6.2 (A Language and Environment, http://www.r-project.org). For comparing the differences in antioxidative markers between H. pylori-positive and negative subjects, the Student's t-test was used. Baseline values of antioxidative markers (TAS, GSSG/GSH) were compared after synbiotic and placebo treatment in the H. pylori-positive and H. pylori-negative groups, with the paired t-test or the Wilcoxon test selected according to the distribution of the data. Differences were considered statistically significant if the value was P < 0.05. Logistic regression models adjusted by age, body mass index (BMI) and sex were applied to calculate odds ratios (ORs) for TAS values in H. pylori-positive and H. pylori-negative groups (with H. pylori-negative as a reference group).

Results and discussion

In our study the prevalence rate of H. pylori was 53%. H. pylori-negative subjects were significantly younger than H. pylori-colonized persons, which is in accordance with a study of Estonian children (Oona et al. 2004). Our previous in vitro experiments showed the moderate inhibitory activity of the currently used capsulated probiotic strains against the reference strain of H. pylori (Hütt et al. 2006). In the therapy of H. pylori infection, probiotics supplementation has been applied to increase the eradication rates of the pathogen and to decrease therapy-related side effects (Tong et al. 2007). In our study, the prevalence of H. pylori did not change after synbiotic consumption. Seemingly, this was associated with the lack of inhibitory activity of the probiotic bacteria to H. pylori in the stomach as the enterocoated capsules were soluble only in the small intestine.

However, we have found a reduction in systemic antioxidative activity in asymptomatic H. pylori-colonized persons. This widens the understanding of H. pylori...
infection accompanied by systemic OxS (Mashimo et al. 2006). The systemic antioxidative marker, the serum TAS, was significantly lower (0.97 vs 1.05 mmol l⁻¹, P = 0.008) in H. pylori-positive subjects than in negative ones; between the two groups the OR was 5.4 (95%CI 1.62–18.01) when we divided the TAS values either for ≥1.0 or <1.0. After adjusting the OR for age, gender and BMI, this relationship remained significant: OR = 4.14 (95%CI 1.02–16.83).

After the consumption of the synbiotic, TAS values increased in H. pylori-positive persons (Fig. 1a). The similarly increased values of TAS in the placebo period could be due to the carryover effect on synbiotic consumption in the crossover study. The designed frequency of blood sampling and the missing second baseline samples represent the limitation of our study.

A second positive change in the antioxidative markers was the reduction of the ratio between oxidized and reduced glutathione (0.035 vs 0.030, P = 0.016) after the consumption of the synbiotic in H. pylori-positive subjects, while in H. pylori-negative subjects no changes were found (Fig. 1b). The decrease in the ratio was mainly due to the increase of the GSH (972.1 vs 1018.1 μg ml⁻¹, P = 0.063) in H. pylori-positive subjects. To date, the GSH and GSSG/GSH are applied to detect OxS (Karelson et al. 2002; Zilmer et al. 2005; Sies and Jones 2007). GSH is characterized as the cellular redox buffer acting as a scavenger of free radicals and toxic substances, and serving as a cosubstrate for detoxification enzymes (Hansen et al. 2009).

Evidently, the increase of the TAS and the GSH values are mainly due to the high antioxidative properties of Lact. fermentum ME-3, alleviating OxS- and inflammation-related damages in the intestinal cells (Truusalu et al. 2004). High antioxidative activity has also been detected in probiotic cheese with ME-3 (Songisepp et al. 2004). Moreover, the consumption of the probiotic Lact. fermentum ME-3 in fermented goat milk (Kullisaar et al. 2003) and capsules (Songisepp et al. 2005) increased the TAS in healthy subjects.

In conclusion, we demonstrated that the consumption of a synbiotic containing an antioxidative probiotic strain improved the reduced systemic antioxidative activity in H. pylori-colonized asymptomatic subjects. Synbiotic products containing antioxidative probiotic strain may be useful in the reduction of systemic OxS.

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References


