NOTE TO THE EDITOR

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

P. Hütt[1], H. Andreson[1], T. Kullisaar[2], T. Vihalemm[2], E. Unt[3], J. Kals[2], P. Kampus[2,4], M. Zilmer[2] and M. Mikelsaar[1]

1 Department of Microbiology, Medical Faculty, University of Tartu, Tartu, Estonia
2 Department of Biochemistry, Medical Faculty, University of Tartu, Tartu, Estonia
3 Institute of Exercise Biology and Physiotherapy, University of Tartu, Tartu, Estonia
4 Department of Cardiology, Medical Faculty, University of Tartu, Tartu, Estonia

Keywords
blood antioxidative activity, *H. pylori* colonization, synbiotic.

Correspondence
Pirje Hütt, Department of Microbiology, University of Tartu, Ravila 19, Tartu 50411, Estonia. E-mail: pirje.hutt@ut.ee

2008/1615: received 20 September 2008, revised 3 February 2009 and accepted 9 February 2009

doi:10.1111/j.1472-765X.2009.02607.x

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}, $P = 0{\cdot}008$). After the consumption of the synbiotic, TAS values (0·97 *vs* 1·03 mmol l^{-1}, $P = 0{\cdot}004$) increased, while the ratio between oxidized and reduced glutathione (0·035 *vs* 0·030, $P = 0{\cdot}016$) 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.

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, Q_{10}, 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).

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 Raftilose 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 or the use of any regular concomitant medication, including nonsteroidal anti-inflammatory drugs and antioxidant 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) 3×10^9 CFU per capsule and 1 sachet of prebiotic (6·6 g Raftilose 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-proteinated 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 H_2O_2.

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 $P < 0{\cdot}05$. 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*

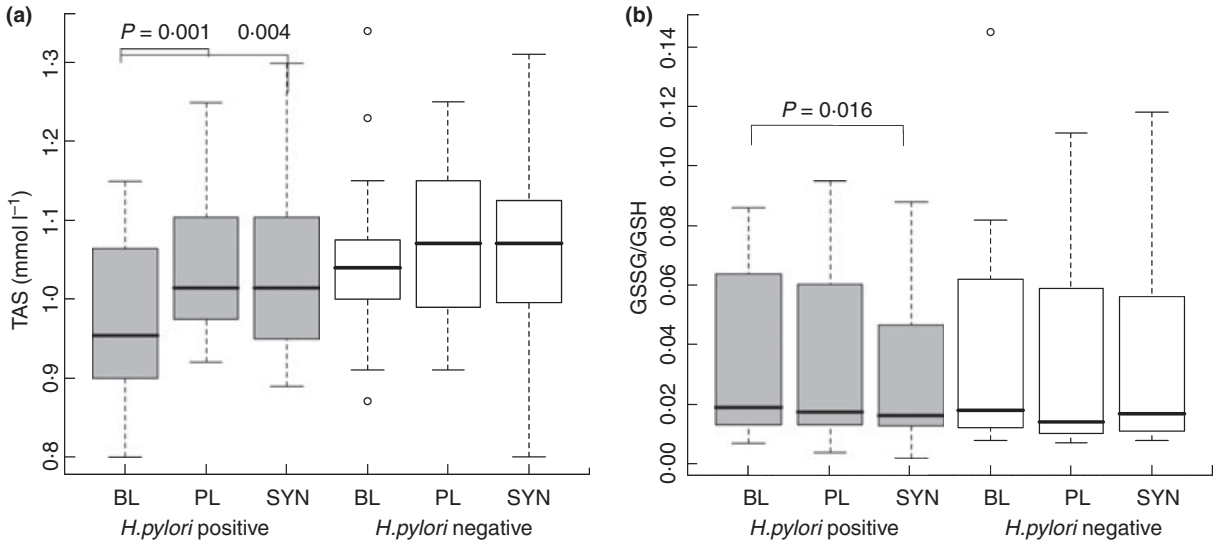

Figure 1 The different antioxidative activity markers measured in asymptomatic individuals. The comparison of altered TAS (a) and GSSG/GSH values (b) during the trial in *Helicobacter pylori* positive (n = 28) and *H. pylori* negative (n = 25) individuals. Statistical difference was tested by paired t-test.

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.

Acknowledgements

The study was supported by a grant from the Estonian Science Foundation (basic funding 0182555s03 and 6588), by Estonian Technology Agency funding (01103, 7480) and the EU Research Commission Grant QLRT-2001-00135.

References

Andreson, H., Maaroos, H.I. and Mikelsaar, M. (2003) Reliability of non-invasive *Helicobacter pylori* antigen test ImmunoCard STAT! HpSA in peptic ulcer patients after eradicative treatment (Abstract). *Helicobacter* **8**, 490.

Beil, W., Obst, B., Sewing, K.F. and Wagner, S. (2000) *Helicobacter pylori* reduces intracellular glutathione in gastric epithelial cells. *Dig Dis Sci* **45**, 1769–1773.

Dunn, B.E, Cohen, H. and Blaser, M.J. (1997) *Helicobacter pylori*. *Clin Microbiol Rev* **10**, 720–741.

Gibson, G.R., McCartney, A.L. and Rastall, R.A. (2005) Prebiotics and resistance to gastrointestinal infections. *Br J Nutr* **93** (Suppl. 1), S31–S34.

Griffith, O.W. (1980) Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Anal Biochem* **106**, 207–212.

Gutteridge, J.M. and Halliwell, B. (2000) Free radicals and antioxidants in the year 2000. A historical look to the future. *Ann NY Acad Sci* **899**, 136–147.

Hansen, R.E., Roth, D. and Winther, J.R. (2009) Quantifying the global cellular thiol-disulfide status. *PNAS* **106**, 422–427.

Hütt, P., Shchepetova, J., Lõivukene, K., Kullisaar, T. and Mikelsaar, M. (2006) Antagonistic activity of probiotic lactobacilli and bifidobacteria against entero- and uropathogens. *J Appl Microbiol* **100**, 1324–1332.

Jung, H.K., Lee, K.E., Chu, S.H. and Yi, S.Y. (2001) Reactive oxygen species activity, mucosal lipoperoxidation and glutathione in *Helicobacter pylori*-infected gastric mucosa. *J Gastroenterol Hepatol* **16**, 1336–1340.

Karelson, E., Mahlapuu, R., Zilmer, M., Soomets, U., Bogdanovic, N. and Langel, U. (2002) Possible signaling by glutathione and its novel analogue through potent stimulation of fontocortical G proteins in normal aging and in Alzheimer's disease. *Ann NY Acad Sci* **973**, 537–540.

Krogfelt, K.A., Lehours, P. and Mégraud, F. (2005) Diagnosis of *Helicobacter pylori* infection. *Helicobacter* **10** (Suppl. 1), 5–13.

Kullisaar, T., Zilmer, M., Mikelsaar, M., Vihalemm, T., Annuk, H., Kairane, C. and Kilk, A. (2002) Two antioxidative lactobacilli strains as promising probiotics. *Int J Food Microbiol* **72**, 215–224.

Kullisaar, T., Songisepp, E., Mikelsaar, M., Zilmer, K., Vihalemm, T. and Zilmer, M. (2003) Antioxidative probiotic fermented goats milk decreases oxidative stress-mediated atherogenicity in human subjects. *Br J Nutr* **90**, 449–456.

Mashimo, M., Nishikawa, M., Higuchi, K., Hirose, M., Wei, Q., Haque, A., Sasaki, E., Shiba, M. *et al.* (2006) Production of reactive oxygen species in peripheral blood is increased in individuals with *Helicobacter pylori* infection and decreased after its eradication. *Helicobacter* **11**, 266–271.

Oona, M., Utt, M., Nilsson, I., Uibo, O., Vorobjova, T. and Maaroos, H.I. (2004) *Helicobacter pylori* infection in children in Estonia: decreasing seroprevalence during the 11-year period of profound socioeconomic changes. *Helicobacter* **9**, 233–241.

Pignatelli, B., Bancel, B., Plummer, M., Toyokuni, S., Patricot, L.M. and Ohshima, H. (2001) *Helicobacter pyl*ori eradication attenuates oxidative stress in human gastric mucosa. *Am J Gastroenterol* **96**, 1758–1766.

Sies, H. and Jones, D.P. (2007) Oxidative stress. In *Encyclopedia of Stress*, 2nd edn, Vol. 3, ed. Fink, G. pp. 45–48. San Diego: Elsevier.

Songisepp, E., Kullisaar, T., Hütt, P., Elias, P., Brilene, T., Zilmer, M. and Mikelsaar, M. (2004) A new probiotic cheese with antioxidative and antimicrobial activity. *J Dairy Sci* **87**, 2017–2023.

Songisepp, E., Kals, J., Kullisaar, T., Mändar, R., Hütt, P., Zilmer, M. and Mikelsaar, M. (2005) Free in PMC evaluation of the functional efficacy of an antioxidative probiotic in healthy volunteers. *Nutr J* **4**, 22.

Tong, J.L., Ran, Z.H., Shen, J., Zhang, C.X. and Xiao, S.D. (2007) Meta-analysis: the effect of supplementation with probiotics on eradication rates and adverse events during Helicobacter pylori eradication therapy. *Aliment Pharmacol Ther* **25**, 155–168.

Truusalu, K., Naaber, P., Kullisaar, T., Tamm, H., Mikelsaar, R-H., Zilmer, K., Rehema, A., Zilmer, M. *et al.* (2004) The influence of antibacterial and antioxidative probiotic Lactobacilli on gut mucosa in a mouse model of *Salmonella* infection. *Microbial Ecol Health Dis* **16**, 180–187.

Zilmer, M., Soomets, U., Rehema, A. and Langel, Ü. (2005) The glutathione system as an attractive therapeutic target. *Drug Design Rev – Online*, **2**, 121–127.

