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Human phagocytic cell response to histamine derived from potential probiotic strains of *Lactobacillus reuteri*

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ABSTRACT

Histamine derived from lactobacilli isolates is considered to be a potential immunomodulator able to interact with the host immune system. We tested the effect of pure histamine (0.413 mM) together with the effect of cell-culture supernatants (CCS) containing different concentration of histamine produced by two of *Lactobacillus reuteri* isolates on the activities of antioxidant enzyme, as well as on the phagocytic activity of human leucocytes (HL). Phagocytic activity represents the non-specific immune response of HL homogenate, *in vitro*. Analysed histamine-producers were represented by a goatling isolate named *L. reuteri* KO5 and a lamb isolate named *L. reuteri* E and histamine production was determined using HPLC method connected with UV detection. Concretely, the samples contained the mixture of isolated HL and the addition of lactobacilli CCS at three different final concentrations of histamine ~ 0.1, 1.8 and 5.4 mM. It was found that pure histamine (0.413 mM) did not significantly influence the oxidant-antioxidant balance in HL demonstrated by unchanged degree of HL lipid peroxidation. However, at the same time, the final activity of catalase and superoxide dismutase were significantly changed ($p \leq 0.001$).

Moreover, the phagocytic index ($p \leq 0.01$), lysozyme ($p \leq 0.05$) and peroxidase activity ($p \leq 0.001$), and production of IL-1β significantly decreased. CCS containing different concentration of produced histamine were also able to modulate the host non-specific immune response together with the enzymatic activity of SOD and catalase too. However, our findings indicated that the impact of lactobacilli histamine is strictly strain-dependent and concentration dependent. Moreover, it seems that histamine is not the only one lactobacilli metabolite, which may play an important role in overall immunomodulatory and antioxidant potential of tested lactobacilli.

1. Introduction

Lactobacillus genera have long been used in fermentation processes to preserve the nutritive qualities of various foods. Over the past decades a large number of lactobacilli strains has been generally recognised as safe (GRAS) (Hanlon et al., 2017) and classified as probiotics, live microorganisms which when administrated in adequate amounts confer a health benefit on the host. Owing to their ability to interact with the host immune system, lactobacilli are capable of affecting mucosal immune system (also epithelial cells that cover mucosa) to activate its specific mechanisms. Three main signalling mechanisms that enable the innate immune system to recognize the lactobacilli comprise Toll-like receptors (TLR), nucleotide oligomerization domain (NOD) – like receptors and C-type lectin receptors. Lactobacilli influence the host immune system *in vivo* and *in vitro* through the carbohydrates present on the cell surface, enzyme changing the structure of lipoteichoic acid and metabolites (Wells, 2011). Biogenic amines (e.g. histamine, tyramine), basic nitrogenous low molecular weight compounds, belong to such lactobacilli metabolites able to modulate the host immune system (Tripathi et al., 2010; Babusyte et al., 2013). Their production is highly strain-specific and in the first place depends on the stage of the growth of particular lactobacilli strain and on the presence of an adequate amount of precursors in the environment. In addition, aminogenic activity of lactobacilli is influenced by the appropriate pH and temperature values of cultivation environment and by the presence of glucose, salts and enzyme cofactors (Linares et al., 2012). Production of biogenic amines enables lactobacilli to protect themselves against acid environment formed as a consequence of their metabolic processes, especially of sugar fermentation and to acquire energy via proton motive force (Molenaar et al., 1993).

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Histamine derived from *Lactobacillus* species was described as a primary immunomodulatory compound. It is well known for its pro-inflammatory effect in allergy and anaphylaxis, but after all, several studies have demonstrated its anti-inflammatory and immunoregulatory functions (Thomas et al., 2012; Vannier et al., 1991; Dohlsten et al., 1988; Elenkov et al., 1998). How does histamine exercise both pro- and anti-inflammatory effect on host cells? One possibility is that a structural modification discerns histamine as a pro- and anti-inflammatory compound, but fragmentation pattern of histamine revealed that it produced by *L. reuteri* is not covalently modified (Thomas et al., 2012). Another possible hypothesis is that the pro- or anti-inflammatory effect of histamine is concentration dependent, but this idea seems unlikely in the host since histamine is rapidly degraded by intestinal enzymes (Aschenbach et al., 2009). It is known, that locally produced histamine from *L. reuteri* activating H2 receptors on immune cells to suppress host mucosal immunity *via* inhibition of proinflammatory cytokines (Thomas et al., 2012).

Besides the microflora, intestine has a well-developed lymphatic system that is closely related with its functions, such as mucosal immunological defence or absorption of nutrients. The one key player in intestinal immunity are lymphocytes, the other key player is natural immunocompetent cells (phagocytes - neutrophils, monocytes, macrophages, dendritic cells) which responding to stimuli from the digestive tract lumen and processing the information. Host defence relies on innate immune mechanisms. Innate immunity is the first line of defence, instrumental in keeping invading microorganisms under control. Innate immune components also participate in activating antigen-specific adaptive immune responses. They include number of constitutively expressed phagocytosis and inducible humoral factors such as antimicrobial peptides, proteins, e.g. the defensins, the cathelicidins and lysozyme (Yang et al., 2001). Fahlgren et al. (2003) found that the lysozyme activity and expression of defensins increase under the impact of chronic inflammation e.g. epithelial cells with phagocytes. Toxic stress mediated by reactive oxygen species (ROS), which are generated by phagocytes, contributes significantly to the inflammatory process in cells. Histamine is known 100 years and the regulatory interactions between histamine and the immune response in host organism are still not fully understood.

It is yet demonstrated, that histamine affects the blood phagocytes and decreases production of cytokine (Thomas et al., 2012). The goal of our research was, at first, to analyse the effect of histamine dihydrochloride (with chemical purity ≥99.0%) on the processes of non-specific immune response and antioxidant enzymatic activity of isolated human leucocytes (HL) as the reference experiment. Secondly, we treated isolated HL with lactobacilli cell-culture supernatants containing different histamine concentration. The histamine present in cell-culture supernatants together with the other metabolites were produced by goatling isolates named *L. reuteri* KO5 and lamb isolates named *L. reuteri* E and its quantity was analysed by using HPLC method connected with UV detection (Greif et al., 2006). The comparison with the reference experiment may help us to understand the influence of histamine produced by lactobacilli isolates on the various immune and enzymatic processes of HL and may contribute to this less explored area.

2. Material and methods

2.1. Bacterial strains

Two *Lactobacillus reuteri* strains producing histamine were used: lamb isolate *L. reuteri* E (Očová, Slovak republik) previously characterised by Bilková et al. (2008) and goatling isolat *L. reuteri* KO5 (Teplý Vrch, Slovak republic) previously characterised by Kiňová Sepová and Bilková (2013). Lactobacilli were stored in de Man, Sharpe and Rogosa (MRS) broth (MERCK, Germany) under aerobic conditions at 5 °C.

2.2. Production of biogenic amines

2.2.1. Samples preparations

Overnight lactobacilli cultures (18 h, 37 °C) were diluted to form 1% of inoculum. Histamine production was determined in cell-culture supernatants (CCS) obtained after anaerobic cultivation (48 h; test tubes with septum) of lactobacilli in two different MRS broth: MRSx (pure MRS broth without supplementation) and MRSy with the addition of biogenic amine precursors L-histidine (0.2% w/v; MERCK, Germany), L-tyrosine (0.2% w/v; MERCK, Germany), L-phenylalanine (0.1% w/v; MERCK, Germany) and L-lysine (0.1% w/v; MERCK, Germany). Each CCS were acquired after centrifugation (9000 RPM, 5 °C, 20 min).

2.2.2. Qualitative and quantitative analysis of production of biogenic amines

Qualitative and quantitative analysis of biogenic amines was realised using HPLC method described by Greif et al. (2006) with UV detection requiring pre-column derivatization with dansyl chloride.

2.3. Analysis of biological activities of isolated human leucocytes (HL)

2.3.1. Isolation and treatment of human phagocytes

Human leucocytes (HL) from healthy blood donor (Health Centre Mýtna, Bratislava, Slovak republic) were isolated and purified using solution for erythrocytes lysis (1:4) according to Böyum (1968). Blood sample with HL was extracted from a vein in the arm using a hypodermic needle according to study on realization biomedical research which has been approved in Decision 6/2017 by the Ethic committee for biomedical research, Faculty of Pharmacy, Comenius University in Bratislava, Slovak Republic (blood for scientific purpose according to Helsinki ethics committee guidelines). HL were suspended in RPMI medium (Biochrom GmbH, Germany) supplemented with Fetal bovine serum (10% v/v) (SIGMA, USA) and Tobramycin solution (0.5% v/v) (TEVA, Hungary) to 2×10^6 cells/ml and checked for viability using 0.2% of trypan blue exclusion method (SIGMA, USA). Isolated HL were subsequently cultivated at 37 °C in 5% CO_2 over 18 h exposed to given amounts of lactobacilli CCS containing different concentrations of histamine. nOvernight lactobacilli cultures (18 h, 37 °C) were diluted to form 1% of inoculum, then cultivated in two different broths: MRSx and MRSy broth under anaerobic cultivation (37 °C; 48 h; test tubes with septum). Corresponding CCS were acquired after centrifugation (9000 RPM, 5 °C, 20 min) and histamine production was determined. According to previously defined ratios of HL suspensions to different CCS of *L. reuteri* KO5 or E containing histamine, three sorts of sample suspensions were prepared: (a) HL treated with CCSa containing histamine obtained after 48 h anaerobic cultivation of lactobacilli in unsupplemented MRSx broth (3:1, 25% CCSa v/v); (b) HL treated with CCSb containing histamine obtained after 48 h anaerobic cultivation of lactobacilli in MRSy broth supplemented with biogenic amine precursors (3:1, 25% CCSb v/v) and (c) HL treated with CCSb containing histamine obtained after anaerobic cultivation of lactobacilli in MRSy broth supplemented with biogenic amine precursors (1:3, 75% CCSb v/v). As control sample, untreated HL suspension together with HL treaded with MRS broth (25% v/v) were used. Additionally, HL were treated with pure MRS broth (47% v/v) containing Histamine dihydrochloride with chemical purity ≥99.0% (AT); 2-(4-Imidazolyl)ethylamine dihydrochloride (SIGMA, USA).

2.3.2. Phagocytic activity and phagocytic index

For phagocytic activity and index, treated and untreated HL (2×10^6 cells/ml) were incubated (1 h; 37 °C) with heat-inactivated *Enterococcus faecalis* (2.5×10^7 CFU) in total volume 150 µl. Phagocytic activity and index were determined microscopically immediately after Wright's staining which was performed according to conventional method by Wright (1902). Phagocytic activity was calculated as the percentage of leucocytes with ingested *Enterococcus faecalis* from the