Mitigation of heat stress-related complications by a yeast fermentate product

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Abstract

Heat stress results in a multitude of biological and physiological responses which can become lethal if not properly managed. It has been shown that heat stress causes significant adverse effects in both human and animals. Different approaches have been proposed to mitigate the adverse effects caused by heat stress, among which are special diet and probiotics. We characterized the effect of the yeast fermentate EpiCor (EH) on the prevention of heat stress-related complications in rats. We found that increasing the body temperature of animals from 37.1 ± 0.2 to 40.6 ± 0.1 °C by exposure to heat (45 °C for 25 min) resulted in significant morphological changes in the intestine. Villi height and total mucosal thickness decreased in heat-stressed rats pre-treated with PBS in comparison with control animals not exposed to the heat. Oral treatment of rats with EH before heat stress prevented the traumatic effects of heat on the intestine. Changes in intestinal morphology of heat-stressed rats, pre-treated with PBS resulted in significant elevation of lipopolysaccharides (LPS) level in the serum of these animals. Pre-treatment with EH was effective in the prevention of LPS release into the bloodstream of heat-stressed rats. Our study revealed that elevation of body temperature also resulted in a significant increase of the concentration of vesicles released by erythrocytes in rats, pre-treated with PBS. This is an indication of a pathological impact of heat on the erythrocyte structure. Treatment of rats with EH completely protected their erythrocytes from this heat-induced pathology. Finally, exposure to heat stress conditions resulted in a significant increase of white blood cells in rats. In the group of animals pre-treated with EH before heat stress, the white blood cell count remained the same as in non-heated controls. These results showed the protective effect of the EH product in the prevention of complications, caused by heat stress.

1. Introduction

Heat stress results in a multitude of pathological and physiological responses which can become lethal if not properly managed. It was shown that heat stress causes significant morphological changes in the gut (Bouchama et al., 2005; Chang et al., 2013). Data to support these observations were obtained in human as well as in animal studies. For example, it has been found that heat exposure in pigs caused marked injury to the tips of the intestinal villi, inducing epithelial cell shedding, exposing the intestinal mucosa lamina propria, as well as shortening the villus height and crypt depth in the small intestine (Yu et al., 2010). These morphological changes clearly alter the integrity of the gastrointestinal tract, which serves as a first line of defense to protect the host from the internal environment of the gut containing bacteria and endotoxins in the form of lipopolysaccharides (LPS) from Gram-negative bacteria. Dysfunction of this protective barrier results in increased intestinal permeability and diffusion of toxic bacterial components from the gut lumen into the blood. The immune system serves to actively remove LPS from the circulatory system through the reticuloendothelial system of the liver, high-density lipoproteins and anti-LPS antibodies. The precise mechanism of injury and death from heat stress are proposed to arise from endotoxemia which develops when the rate of LPS clearance is not consistent with the rate of LPS translocation from the gut (Lim and Mackinnon, 2006). This situation triggers a systemic inflammatory response that then leads to disseminated intravascular coagulation, necrosis of organ tissues, and multi-organ failure. It was previously reported that elevation of the body temperature during heat stress causes an increase of the shedding of erythrocyte membrane vesicles (Moore et al., 2013a, 2014). Vesicles constitute...
a heterogenic population of cell-derived microscopic size particles that participate in a wide range of physiological and pathological processes. More recently, it was shown that the aging erythrocyte is characterized by changes in the plasma membrane, particularly in vesiculation of the cell membrane. This process is termed as eryptosis or programmed erythrocyte death. So heat causes a deadly impact on the erythrocyte structure.

Different approaches are proposed for mitigation of heat stress adverse effects, among which are special diet and probiotics. But there is no data about the efficacy of prebiotics in mitigation of stress-induced complications. EpiCor (EH) is a yeast (Saccharomyces cerevisiae) fermentate. The fermentation produces a product that is high in yeast metabolites, including vitamins, polyphenols, sterols, phospholipids and polysaccharides such as betaglucan. Previous studies showed that EH exhibits strong prebiotic properties (Possemiers et al., 2011). This product possess significant anti-inflammatory activity, selectively enhanced butyrate production in vitro (Jensen et al., 2008a; Possemiers et al., 2013) and supports mucosal defense in clinical trials (Jensen et al., 2008b). We hypothesize that this yeast fermentate could be effective in the prevention of heat stress adverse effects.

The main objective of this work was to study the efficacy of the prebiotic-like product EH, in the prevention of complications related to heat stress in rats.

2. Material and methods

2.1. Ethics statement

All animal procedures were approved by the Auburn University Institutional Animal Care and Use Committee. The study was performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

2.2. Animals

Adult male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN, USA) weighing 250–300 g were used in this study. The animals were housed two per cage under specific pathogen-free conditions and were acclimatized for 2 days at a temperature (20 ± 1 °C), humidity (50 ± 5%) and lighting (12-h day/12-h night) with free access to water and standard food (2018 Teklad Global 18% Protein Rodent Diet, Harlan, Indianapolis, IN, USA).

2.3. Yeast fermentate

The yeast fermentate product EpiCor (EH) was provided by the manufacturer (Embria Health Sciences, Ankeny, IA, USA) in a powder form. For oral treatment of rats, suspension of the yeast fermentate in PBS was prepared at the rate 7 mg per kg of animal weight in 1 mL of phosphate buffered saline (PBS).

2.4. Experimental design

A total of 32 rats was used in this study. One group (16 rats) was fed a standard diet (basal diet) and the other group (16 rats) received the same diet, but they were treated by oral gavage with 1 mL of yeast fermentate suspension. Animals were treated once a day in the morning, every day for 14 days prior to the start of heat stress (Fig. 1). At the same time control rats received 1 mL of PBS by oral gavage. On day 15, rats in each group were subdivided (8 rats in each group), these groups were: 1) control (PBS/25 °C, 25 min), 2) EH (yeast fermentate/25 °C, 25 min), 3) stress (PBS/45 °C, 25 min), and 4) EH + stress (yeast fermentate/45 °C, 25 min). Animals from group 3 and 4 were exposed to 45 °C heat stress, relative humidity 55% for 25 min in a pre-heated climatic chamber (Environmental Chamber 6020-1, Caron, OH, USA). These conditions were previously described to achieve acute heat stress in rats (Moore et al., 2013a, 2014; Sachidhanandam et al., 2002). Control animals (groups 1 and 2) were exposed to identical conditions as the heat stressed animals, but at 25 °C. The animals had access to food and water during and after heating. Rectal temperature was measured for each rat before and immediately after heat stress using an electronic digital thermometer, inserted to a depth of 4 cm (Moore et al., 2013a). Four hours after the heat stress experiments, rats were anesthetized with isoflurane (2–4%) and euthanized by rapid decapitation. Trunk blood was collected from each rat into sterile aprotic tubes to obtain serum. Samples of blood were immediately taken for microscopic examination. Samples of small intestine from each rat were taken for morphological analysis.

2.5. Blood serum preparation

Blood in the tubes was allowed to clot for 30 min in a refrigerator. Tubes were centrifuged at 20 °C, 7000g for 10 min. Serum was collected and stored in 50 μl aliquots at −20 °C until assay.

2.6. Histological analysis

2.6.1. Sample preparation

Samples of small intestine (0.5–2 cm in length) were immediately fixed in Bouin’s solution (Electron Microscopy Sciences, Hatfield, PA, USA) for 10–15 min. Tissue samples were cut to proper size (5–7 mm in length) and transferred to fixative to make sure all tissues were completely immersed in fixative. The volume of fixative was 20–30 times the tissue volume. After 48 h of fixation at room temperature, the excess fixative was washed out in 70% alcohol until most of the yellow color was removed. Washed samples were put into tissue embedding cassettes (VWR, Radnor, PA, USA) and kept in 70% alcohol until processing in the Automated tissue processor (Tissue-Tek VIP, Miles/Sakura, Torrance, CA, USA). After processing, samples were embedded in paraffin blocks using embedding center (Tissue-Tek TEC, Sakura, Torrance, CA, USA). Embedded tissue was sectioned at 6 μm using the microtome (Reichert-Jung 2040 Autocut, Leica Biosystems Nussloch GmbH, Heidelberger Straße 17-19, 69226 Nussloch, Germany).

2.6.2. Sample staining

Hematoxylin – eosin (H&E) staining was performed according to the standard protocol (Stevens, 1990), using Mayer’s haemotoxylin and Eosin Y (Electron Microscopy Sciences, Hatfield, PA). Briefly, sections were deparaffinized in Hemo-De × 3 changes for 8 min, 5 min and 5 min, cleared in 100% ETOH × 2 changes – 2 min each, followed by 95% ETOH × 2 – 2 min each and 80% ETOH – 2 min.
Sections were rinsed with distilled water and stained with hematoxylin for 2–3 min. Samples were washed in running tap water for about 5 min, put in 80% ETOH for 2 min and stained in eosin for 1–2 min. After staining, sections were put in 70% ETOH – 2 min, 95% ETOH × 2 – 2 min each, 100% ETOH × 2 – 2 min each, Hemo-De × 2 changes until clear (5 min, 10 min or longer). Mounting of the samples was performed using Eukitt Mounting Medium (Electron Microscopy Sciences, Hatfield, PA).

2.6.3. Measurements

Intestinal villi height and total mucosal thickness were measured for each sample using a high resolution microscope system (Vainrub et al., 2006). Only stained sections in which the mucosal villi were cut along their longitudinal axis were analyzed. The villi height was measured as a distance from the apex of the villus to the base of the crypt; total mucosal thickness – from the top of the villus to the muscularis mucosae. Twenty measurements of each parameter in each sample were taken and expressed in micrometers. An average of these measurements was expressed as a mean villi height and total mucosal thickness for one treatment group.

2.7. Lipopolysaccharide (LPS) assay

Serum concentration of LPS was analyzed by the Pierce LAL Chromogenic Endotoxin Quantitation Kit (Thermo Scientific, Rockford, IL) using the Limulus Amebocyte Lysate (LAL) assay according to the manufacturer’s recommendations. The sensitivity of the assay was 0.1 EU mL⁻¹ (0.01 ng endotoxin per mL). Sterile pyrogen-free plastic and glassware were used throughout the assay.

2.8. Cortisol assay

Cortisol concentration in serum of animals in all groups was analyzed by Rat Cortisol ELISA Kit (NeoBioLab, Cambridge, MA) according to the manufacturer’s instruction. The sensitivity of this assay was 1.0 ng mL⁻¹. Cortisol ELISA Assay Kit is a competitive immunoenzymatic colorimetric method for quantitative determination of cortisol concentration in serum.

2.9. IL-10 cytokine assay

Serum level of IL-10 cytokine was measured using the commercial rat cytokine-specific sandwich ELISA kit for IL-10 (Invitrogen Corporation, Carlsbad, CA, USA) according to the manufacturer’s instructions. Optical density values were measured at 450 nm using a microplate reader (Bio-Tek, Winooski, VT). The cytokine content of each sample was determined by comparison of mean experimental values with curve generated using standards, supplied with the kit. The minimum detectable dose of IL-10 kits was < 5 pg/mL.

2.10. High resolution light microscopy of the blood

The images of fresh blood were recorded with a super-resolution light microscope system described in Vainrub et al. (Vainrub et al., 2006). Test images were calibrated using a Richardson slide (Richardson, 1988). A small droplet (7 μl) of freshly drawn blood was placed on a glass slide, coverslipped and positioned on the microscope stage with oil contacts between condenser and objective lenses. Ten image frames of 72 × 53.3 μm² in each sample were videotaped, and concentrations of white blood cells and vesicles count and diameter were measured by Image-Pro Plus software (Media Cybernetics), providing high-resolution direct-view optical images in real time. The samples were observed in an aqueous environment and required no freezing, dehydration, staining, shadowing, marking or any other manipulation. At least 20 image frames were analyzed for each animal. Each frame contained between 50 and 200 vesicles (depending on conditions).

2.11. Statistics

All results were presented as a mean and standard deviation. The difference between groups were analyzed by the one-way ANOVA, followed by the Bonferroni test. The significance level was set at 0.05 to define statistical significance. Statistical calculations and graph plotting were carried out using Microcal™ Origin version 9.0 (Northampton, MA) and 2010 Microsoft Excel.

3. Results

3.1. Body temperature

Exposure of rats to high temperature resulted in a significant increase of body temperature. The mean body temperature (°C) of rats before and immediately after heat stress was 37.4 ± 0.2 and 40.6 ± 0.2 (p < 0.05) respectively in group 4 (EH) and 37.1 ± 0.2 and 40.4 ± 0.4 (p < 0.05) respectively in group 3 (PBS). No central nervous system abnormalities such as convulsions, or coma in animals during or after heat stress, as well as after 4 h recovery were noticed. Body temperature of rats in control groups (groups 1 and 2) was stable during the experiment.

3.2. Morphometric analysis of the intestine

Villi height and total mucosal thickness in control rats, not exposed to heat were 612.8 ± 8.3 μm and 739.9 ± 8.0 μm respectively (group 1 – PBS/25 °C) and 613.4 ± 7.1 μm and 740.6 ± 7.7 μm respectively (group 2 – EH/25 °C) (Fig. 2A and B; Fig. 3). Exposure of rats pre-treated with PBS to heat stress conditions (group 3 – PBS/45 °C) significantly decreased villi height and total mucosal thickness – 394.1 ± 7.5 μm and 526.5 ± 8.7 μm accordingly. Treatment of rats with EH before heat stress prevented the damage effect of heat on intestinal morphology. In this group of rats (group 4 – EH/45 °C) parameters for villi height and total mucosal thickness were similar to the control rats.

3.3. Microscopic evaluation of the blood

The concentration of free vesicles in the blood of control rats was (1.70 ± 0.07) × 10⁹ particles mL⁻¹ (PBS treated) and (1.80 ± 0.07) × 10⁹ vesicles mL⁻¹ (EH treated) – Fig. 4A. Significant increase of vesicle concentration was found in heat-stressed rats, pre-treated with PBS (Figs. 4A and 5). No change of free vesicle concentration was found in rats treated with EH and exposed to heat stress conditions (Fig. 4A). The diameter of vesicles also significantly increased only in the blood of rats treated with PBS before heat stress (Fig. 4B). Pre-treatment with EH prevented the formation of large vesicles in the blood. We also found a significant increase of white blood cell (WBC) concentration in the blood of rats exposed to heat after treatment with PBS (Fig. 6). No change in the number of WBC (in comparison with control groups) was found in the blood of heat-stressed rats pre-treated with EH.

3.4. Serum LPS concentration

Levels of LPS significantly increased in serum of heat-stressed animals which received PBS before heat exposure in comparison with EH-treated heated rats and rats in both control groups (Fig. 7). Concentration of LPS in the serum of rats pre-treated with
EH before heat stress was not different from the LPS level in serum of animals from control groups.

3.5. Serum cortisol level

The serum cortisol level was stable in the rats of the different groups. Cortisol level in heat stressed rats pre-treated with PBS in comparison with control PBS-treated rats was 23.30 ± 1.48 ng mL⁻¹ and 20.30 ± 1.48 ng mL⁻¹ respectively. The concentration of serum cortisol in rats pre-treated with EH before exposure to heat stress conditions was comparable with the level registered in the serum of control EH-treated rats (19.30 ± 1.47 mL⁻¹ and 22.80 ± 1.48 ng mL⁻¹ respectively).

3.6. IL-10 in serum

No significant change in the level of serum IL-10 of rats in different groups was found (3.40 ± 0.14–6.82 ± 0.38 pg mL⁻¹ in heat stressed and control EH groups respectively and 4.60 ± 0.34–4.04 ± 0.18 pg mL⁻¹ in stressed and control PBS groups respectively).

4. Discussion

This study was designed to evaluate the efficacy of yeast fermentate EH in the prevention of heat stress-induced adverse effects in rats. The rat model of acute heat stress described...
by Sachidhanandam et al. (2002) was used in our study. We explored this model in our previous experiments (Moore et al., 2013a, 2014). The conditions of the model (45 °C for 25 min) resulted in increase of body temperature of rats to 40.3 ± 0.2 °C. The standard deviation of body temperature after heat stress indicated small variability between animals. No central nervous system abnormalities such as convulsions, or coma in animals during or after heat stress, as well as after 4 h recovery were noticed. So we can assume that this model of heat stress is a humane model because the exposure to heat is very brief and does not result in mortality of animals, but leads to the manifestation of the acute heat stress symptoms. Thus, it was found that the increase of the body temperature of animals exposed to heat stress conditions resulted in significant morphological changes in the intestine. Villi height and...
total mucosal thickness decreased in heat-stressed rats pre-treated with PBS in comparison with control animals not exposed to the heat. Treatment of rats with EH before heat stress prevented the traumatic effect of heat on the intestine because the results of measurements of villi height and total mucosal thickness in this group of animals were the same as in the control groups. We can speculate that mucosal immune protection by EH reported previously (Possemiers et al., 2013) contributed to the protective effect of this yeast fermentate.

In our previous study, we showed that heat stress results in an increase in the shedding of erythrocyte membrane vesicles (Moore et al., 2013a, 2013b). Vesiculation of the cell membrane is related to the aging of erythrocytes (Rosen et al., 2008; Ghoshghaieinia et al., 2012) and is termed as eryptosis or programmed erythrocyte death (Lang and Lang, 2015). This study revealed that the elevation of the body temperature resulted in a significant increase of the concentration of vesicles in the blood of rats, compared to PBS-treated rats before heat exposure. This is an indication of a pathological impact of heat on the erythrocyte structure of this group of animals. Treatment of rats with EH completely protected their erythrocytes from the pathology, caused by heat stress. Our data agree in accordance with previously published results about beneficial effects of EH on erythrocyte health (Jensen et al., 2008b).

A significant shift to a higher vesicle diameter was also indicated as an adverse effect of heat stress (Moore et al., 2013a). The results of this study showed that treatment with EH prevented rats from this adverse effect of heat stress. Exposure to heat stress conditions resulted in a significant increase of white blood cell (WBC) counts in rats, pre-treated with PBS. This result is in accordance with the findings of other authors, showed elevated levels of WBC in stressed animals (Matur et al., 2016; Peli et al., 2013). In the group of animals treated with EH before heat stress, the white blood cell count remained the same as in control groups.

We did not find significant changes in serum IL-10 levels in different groups of rats. These results confirmed previously obtained data that consumption of EH does not alter the level of serum IL-10 (Jensen et al., 2008b).

The level of cortisol was consistent in all groups of animals. Literature data show that cortisol level can be stable during stress (Radahmadi et al., 2006) or even can be decreased during 2–4 h after heat stress (McMorris et al., 2006).

Our previous results showed that the thermodynamic data for rat environmental hyperthermia and human exercise-induced temperature increase are consistent with each other and agree well with thermodynamic literature results (Vodyanoy, 2015). Future directions of this study will help to understand the feasibility of our approach not only in environmental heat stress but also in heat stress, related to physical activity.

5. Conclusion

Our study showed a significant protective effect of EH against heat-stress–related adverse effects in a rat model. Pre-treatment of animals with EH before exposure to heat protected gut morphology, prevented increase of LPS in blood and pathological impact of heat stress on blood erythrocytes. Further study of EH efficacy during heat stress will lead to a better understanding of the mechanisms of this protection, and to develop new approaches for the prevention of heat stress–induced adverse effects in the gut.

Conflict of interest

No conflict of interest declared.
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References


