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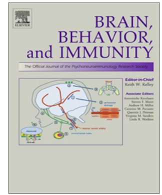

The effects of moderate exercise on chronic stress-induced intestinal barrier dysfunction and antimicrobial defense

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ABSTRACT

The purpose of this study was to examine the effect of moderate exercise on repeated restraint stress (RRS)-induced intestinal barrier dysfunction and explore possible mechanisms in a mouse model. Male Balb/c mice (6 weeks) were randomized into 7 groups: CON functioned as controls with no intervention; RRS was subjected to 6 h per day RRS for 7 consecutive days; RRS+SWIM received 30 min per day of swimming prior to RRS; CON+SWIM only received 30 min per day of swimming; and the other groups received one session of 30 min swimming prior to sacrifice at 1-, 3- and 6 h recovery. Intestinal permeability was quantified with FITC-dextran. Bacterial translocation was determined by quantification of bacterial colony forming units (CFUs) in cultured mesenteric lymph nodes (MLN), and with fluorescence in situ hybridization (FISH). Antimicrobial related gene expression at baseline and 1 h after one session of 30 min swimming was tested by quantitative real-time polymerase chain reaction (Q-PCR) in small intestinal segments. Protein expression of 5 genes with statistically significant increase was measured at baseline, and 1-, 3- and 6 h post-swimming using enzyme-linked immunosorbent assay (ELISA). Thirty minutes per day of swimming before RRS attenuated bacterial translocations and maintained intestinal permeability. Gene expression and protein levels for four antimicrobial peptides (α-defensin 5, β-defensin 1, RegIIIβ and RegIIIγ) were significantly increased after one 30 min swimming session. In conclusion, moderate exercise attenuated chronic stress-induced intestinal barrier dysfunction in mice, possibly due to augmentation of antimicrobial responses in the small intestine.

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1. Introduction

Chronic psychological stress induces a variety of changes in gut homeostasis. Experiments using animal models demonstrate that chronic psychological stress induces intestinal barrier dysfunction leading to increased permeability (Mazzon et al., 2002; Yang et al., 2006) and enhanced uptake of noxious luminal material (Velin et al., 2004). Enhanced bacterial uptake after chronic stress induces an increased antigen load in the intestinal mucosa, leading to both local and widespread intestinal inflammation (Zareie et al., 2006). Stress causes bacterial translocation in healthy animals even without epithelial barrier dysfunction (Jorge et al., 2010).

Given the role of chronic stress in the pathophysiology of intestinal disorders, reducing stress-induced barrier changes may have a therapeutic benefit (Groschwitz and Hogan, 2009). Control of bacterial interactions with the intestinal mucosal surface is a critical first line of host defense (Duerkop et al., 2009). Intestinal epithelial cells (IEC) and intraepithelial lymphocytes (IEL) have specialized antimicrobial functions, and produce antimicrobial proteins (AMP) by engagement of various pattern recognition receptors (PRR) that recognize pathogens (Medzhitov, 2007). Mucins produced by IEC also help to prevent the attachment and entry of pathogens (Johansson et al., 2008).

Moderate physical activity has protective effects on the gastrointestinal tract, reducing risk of colon cancer, cholelithiasis, gastrointestinal haemorrhage, Inflammatory Bowel Disease (IBD), diverticular disease, and constipation (Peters et al., 2001). Regular moderate exercise exerts anti-inflammatory effects (Gleeson et al., 2011), enhances immunosurveillance (Nieman, 2012) and is associated with lowered risk for multiple diseases. Therefore, we hypothesized that moderate exercise would have a protective effect on chronic stress induced-intestinal barrier dysfunction. The aim of our study was to examine the effect of 30-min of swimming on repeated restrain stress (RRS) induced intestinal barrier

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damage. Potential mechanisms were explored using outcome measures for intestinal permeability, bacterial translocations, and antimicrobial-related gene expressions for antimicrobial responses in the small intestine.

2. Methods

2.1. Animals

Eighty Balb/c mice (6-weeks) were purchased from Taconic Farms (Hudson, NY), and randomized into 7 groups: CON ($n = 18$) functioned as controls with no intervention; RRS ($n = 18$) were subjected to 6-h per day of RRS for 7 consecutive days; RRS+SWIM ($n = 18$) received 30-min per day of swimming prior to RRS; CON+-SWIM ($n = 8$) received 30-min per day of swimming; and the other 3 groups received one session of 30-min swimming prior to being sacrificed at 1 h ($n = 6$), 3 h ($n = 6$) and 6 h ($n = 6$) post-exercise. Mice were bred separately under specific pathogen-free conditions, given ad libitum access to irradiated diet and sterile water, housed on irradiated bedding under a reverse light-dark cycle (lights on at 8 PM, lights off at 8 AM) and kept in individually ventilated and filtered cages. Mice were handled by the same person, acclimated to the facility (restraint devices and swimming pools) for 1 week prior to the experiments and weighed every day, with body weight change expressed as a percentage of weight in the day before RRS. All animal experiments were performed in compliance with and approved by the Shanghai University of Sport ethical review board.

2.2. Experimental protocols

2.2.1. Experiment 1: moderate exercise on chronic stress-induced intestinal barrier dysfunction

2.2.1.1. Repeated restraint stress. The RRS protocol was based on the procedures described by Feng et al. (2012). Briefly, a mouse flat bottom holder (RSTR541; Kent Scientific, Torrington, CT) was used for chronic restraint. The holder was well ventilated and allowed restrained mice access to food and water. Mice were restrained for 6 h daily (10:00 AM–4:00 PM) for 7 consecutive days. Restrained mice (RRS and RRS+SWIM) were maintained horizontally in their home cages during the restraint sessions and released into the same cage during the free sessions. CON and CON+SWIM were unrestrained and left undisturbed in their home cages during the same period.

2.2.1.2. Moderate exercise. Moderate exercise consisted of swimming for 30 min prior to RRS. Swimming is thought to be a natural behavior of mice, and it is less stressful than, and avoids the foot injuries associated with, other forced exercise protocols (Kregel et al., 2006). The mice in RRS+SWIM and CON+SWIM group swam separately in pools ($18 \times 38 \times 25$ cm) filled with warm sterile water (36 ± 1 °C) to a depth of 10 cm. Swim training was performed at 9:00 AM for 30 min. Then the mice were dried carefully by soft towels and left in empty cages on a 37–40 °C auto-heated platform for 30-min. The RRS and CON mice were also put in a swimming pool (same size) but without water around 9:00 AM for 30 min, then on the 37–40 °C auto-heated platform for another 30 min.

2.2.2. Experiment 2: one session of moderate exercise on antimicrobial response-related gene and protein expressions

The mice were subjected to one session of 30-min swimming in the same condition as in the Experiment 1. The intestinal tissue was collected. Antimicrobial response-related gene expressions were examined at the baseline (CON served as the baseline) and 1 h (6/group) after swimming. Antimicrobial response-related protein were screened from the statistically significant increased genes, their expressions were detected at the baseline (CON served as the baseline), 1 h (6/group, the same group for detecting gene expressions), 3 h (6/group) and 6 h (6/group), respectively.

2.3. Tissue collection

The mesenteric lymph nodes (MLNs) and small intestines were dissected. The small intestine was divided into three parts: the duodenum section (within 1–3 cm distal to the pylorus), the jejunum section (within 2–4 cm distal to the ligament of Treitz), and the ileum section (within 1–3 cm proximal to the ileocecal valve). The pieces of the small intestine collected for subsequent Q-PCR and ELISA studies were 1 cm of each section, frozen at −80 °C. 0.5 cm of each small intestinal section was immediately placed in 4% formaldehyde-saline or Carnoy's fixation for histological analysis.

2.4. Fluorescence in situ hybridization (FISH) and immunostaining

Paraffin-embedded 4% formaldehyde-saline fixed sections were dewaxed and stained with hematoxylin and eosin (H&E). Paraffin-embedded Carnoy's solution fixed sections were dewaxed and stained with Alcian blue-periodate acid schiff (AB-PAS) or hybridized with a general bacterial 16S rRNA probe (EUB338, 5′-GCTGCCTCCGTAGGAGT-3′) and immunostained for DNA by 4′,6-diamidino-2-phenylindole (DAPI). Images were obtained with a fluorescence microscope (IX70, Olympus, Tokyo, Japan). Three H&E or AB-PAS staining slides per section (duodenum, jejunum and ileum) were examined and scored to determine the histological status of small intestines and mucus layers. Three FISH slides per section were numbered the positive probe signal in villi or crypt separately.

2.5. Histology score, goblet cell and mucus layer measurements

H&E stained small intestinal tissues (3 slides per section) were scored in a blinded fashion to quantify small intestine damage (Saunders et al., 2010; Welz et al., 2011). A maximum combined score of 10 was determined from the severity of inflammatory cell infiltration (0, none; 1, increased presence of inflammatory cells between the crypts; 2, inflammatory infiltrate extending into the villi; 3, extension of inflammatory infiltrate throughout the lamina propria); extent of tissue damage (0, none; 1, mucosal; 2, mucosal and submucosal; 3, transmural), and crypt damage (0, none; 1, 0–10% of crypts affected; 2, 10–40% of crypts affected; 3, 40–70% of crypts affected; 4, more than 70% of crypts affected). Number of goblet cells was counted for a defined distance (100 μm) from the surface epithelium of longitudinally cut crypts (Johansson et al., 2013). A total of 12 crypts (4 crypts per section) were analyzed for each mouse. Normal goblet cell distribution with densely filled goblet cells was assigned a value of 0. 5–20% decrease in number of densely filled goblet cells was assigned a value of 1. 20–40% decrease was assigned a value of 2, 40–50% decrease was assigned a value of 3, and more than 50% reduction was assigned a value of 4. Mucosa thickness decrease (0–4): normal thickness was assigned a value of 0, 20–40% decreased thickness was assigned a value of 1, 40–60% decreased thickness was assigned a value of 2, 60–80% decreased thickness was assigned a value of 3, and 80–100% decreased thickness was assigned a value of 4.

2.6. Intestinal permeability in vivo

Intestinal permeability was examined using 4000 Da fluorescent FITC-labeled dextran (FD4, Sigma–Aldrich, St. Louis, MO) as

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