Research in Veterinary Science 96 (2014) 355–364

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Effect of simulated transport stress on the rat small intestine: A morphological and gene expression study

Changrong Wan [a], Peng Yin [a], Xiaolong Xu [a], Mingjiang Liu [a], Shasha He [a], Shixiu Song [a], Fenghua Liu [b,*], Jianqin Xu [a,*]

[a] CAU-BUA TCVM Teaching and Researching Team, College of Veterinary Medicine, China Agricultural University (CAU), Beijing 100193, PR China
[b] College of Animal Science and Technology, Beijing University of Agriculture (BUA), Beijing 102206, PR China

ARTICLE INFO

Article history:
Received 10 July 2013
Accepted 26 January 2014

Keywords:
Transport stress
Intestinal damage
Microarray analysis
Morphological study

ABSTRACT

The present study investigated the effects of simulated transport stress on morphology and gene expression in the small intestine of laboratory rats. Sprague Dawley rats were subjected to 35 °C and 0.1 ×*g* on a constant temperature shaker for physiological, biochemical, morphological and microarray analysis before and after treatment. The treatment induced obvious stress responses with significant decreases in body weight ($P < 0.01$), increases in rectal temperature, serum corticosterone (CORT), serum glucose (GLU), creatine kinase (CK) and lactate dehydrogenase (LDH) levels ($P < 0.01$), as well as expression of Hsp27/70/90 mRNA ($P < 0.05$; $P < 0.01$). The rat jejunum was severely damaged and apoptotic after mimicking transport stress, which may mainly be related to cell death, oxidation reduction and hormone imbalance determined by microarray analysis. The bioinformatics analysis from the present study would provide insight into the potential mechanisms underlying transport stress-induced injury in the rat small intestine.

1. Introduction

It is well recognized that the gastrointestinal (GI) tract is very sensitive to a wide range of physical and chemical stressors that can induce GI oxidative stress, mucosal injury (Bagchi et al, 1998) and small intestinal dysfunction by inhibiting small intestinal transit, causing small intestinal motility disorder, mucosal damage, and dysbacteriosis (Wang and Wu, 2005). Transport is considered a synthetic procedure that exposes animals to a series of adverse stimuli responsible for several physiological and biochemical responses. These stimuli include capture, collision and scrape, heat and cold, thirst and hunger, and fear (Obernier and Baldwin, 2006). Superposition of these various stimuli influences animal growth, attenuates immunity and causes tissue damage (e.g., susceptible GI), and even leads to death (Warriss and Brown, 1994). Previous studies have evaluated transport stress with target animals through behavioral, physiological, immunological, and hematological variables (Bao et al, 2008; Kadim et al, 2006; Minka and Ayo, 2007; Tateo et al, 2012). However, a real transport environment is unstable for studying the molecular mechanisms of tissue damage. In this study, laboratory animals were subjected to artificial vibration at 35 °C on a constant temperature shaker to mimic transport stress in summer. Changes in physiological and biochemical indexes, hormone and HSP mRNA concentrations were recorded. Microstructures of the small intestine were observed by light and electron microscope, and the rat jejunum was also stained with TUNEL to detect apoptosis after mimicking transport treatment. Gene expression profiles of rat jejunum were assessed by DNA microarray. Using hierarchical clustering, gene ontology (GO) analysis, and pathway analysis of differentially expressed genes, we systematically and quantitatively explored the relevant main biological processes and pathways to provide insight into the potential mechanisms underlying transport stress-induced injury in the rat small intestine.

2. Materials and methods

2.1. Animal care and experimental groups

All experimental protocols were approved by the Committee for the Care and Use of Experimental Animals, China Agricultural University. Twenty-four male Sprague Dawley rats weighing

* Corresponding authors. Address: College of Animal Science and Technology, Beijing University of Agriculture, No. 7, Beinong Road, Beijing 102206, PR China. Tel.: +86 10 8079 4699; fax: +86 10 8079 9147 (F. Liu). Address: CAU-BUA TCVM Teaching and Researching Team, College of Veterinary Medicine, China Agricultural University, No. 2, West Yuanmingyuan Road, Beijing 100193, PR China. Tel./fax: +86 10 6273 4111 (J. Xu).

E-mail addresses: liufenghua1209@126.com (F. Liu), xujianqincau@126.com (J. Xu).

http://dx.doi.org/10.1016/j.rvsc.2014.01.008
0034-5288/

200 ± 20 g (Beijing Vital River Laboratory, Animal Technology Co., Beijing, PR China) were housed [25 °C, 60% relative humidity (RH)] for 7 days. On the 8th day, the rats were randomly divided into the following four groups: control (C), 1-day stress, 2-day stress, and 3-day stress (S1d, S2d, and S3d) groups. Six rats in each group were housed in plastic cages (400 mm × 300 mm × 180 mm) with a layer of soft woodchips and provided free access to food and water.

2.2. Treatment and sampling

Rats in the control group were housed in a controlled environment (25 °C, 60% RH); rats in stress groups were housed under control group conditions but subjected to vibration at 0.1×*g* (relative centrifugal force, RCF) at 35 °C on a constant temperature shaker (DHZ-CA, TAICANG, China) from 9:00 to 11:00 daily to simulate transport stress for 1, 2, or 3 days respectively. Body weights and rectal temperatures were measured before and after each stress treatment. Rats from the control and stress groups were given anesthesia by inhaling Ether absoluter ($CH_3CH_2OCH_2CH_3$, PR China), exsanguinated immediately after anesthesia, and then sacrificed. Blood samples were collected and centrifuged at 2000×*g* (relative centrifugal force, RCF) for 10 min at 4 °C, and the serum samples were stored at −20 °C for the analysis of CORT, GLU, CK, LDH and ALP. Sections of the jejunum were rapidly excised and washed with physiological saline and divided into the following three parts: (1) a 2-cm section was fixed for 48 h in 10% buffered formalin phosphate for paraffin embedding; (2) a 1 mm^2 sample was fixed for 48 h in 4% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for electron microscopy; and (3) a 3-cm section was minced and separated into four sample tubes, snap frozen in liquid nitrogen, and stored at −80 °C for DNA microarray and RT-PCR analysis.

2.3. Morphological and biochemical indicator analysis

Formalin-fixed samples were embedded in paraffin and transversely sectioned (5-μm thickness). After deparaffinization and dehydration, some paraffin sections were stained with hematoxylin and eosin (Sigma, St. Louis, MO, USA) for microstructures of the jejunum observed using a BH2 Olympus microscope (DP71, Olympus, Tokyo, Japan) and analyzed using an Olympus Image Analysis System (version 6.0). Others were stained with *In Situ* Cell Death Detection Kit, Fluorescein (Roche, version 16.0, Philadellphia USA) for apoptosis visualization using an excitation wavelength in the range of 450–500 nm and detection in the range of 515–565 nm (green) under a fluorescence microscope (DP71, Olympus). The glutaraldehyde-fixed samples were washed in the same buffer and fixed for 1 h in cold 1% osmium tetroxide in cacodylate buffer. After dehydration in graded ethanol solutions, the preparations were embedded in Araldite (EPON812, Emicron, Shanghai, PR China). Ultra-thin sections were stained with saturated uranyl acetate in 50% ethanol and lead citrate and examined by transmission electron microscopy (JEM, 1230, JEOL, Tokyo, Japan).

Concentrations of serum GLU, CK, LDH and ALP were detected by an automated biochemical analyzer (TBA-40FR, Toshiba, Tokyo, Japan), in which the performance rate method was used for CK, LDH, and ALP; the glucose oxidase method was used for GLU. Serum CORT samples were assayed respectively with radioimmunoassay according to the instructions of reagent kits (RS49011, IBL, RIA, Germany) according to the manufacturer's specifications.

2.4. Total RNA isolation and reverse transcription (RT)

Total RNA was isolated from the rat jejunum tissue using a phenol and guanidine isothiocyanate-based TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Concentration and purity were assessed using a spectrophotometer (SmartSpec plus, Bio-Rad Laboratories, Inc., Hercules, CA, USA) based on OD_{260}/OD_{280} and OD_{260}/OD_{230} ratios, respectively.

Total RNA was reverse transcribed as follows: 2.0 μg RNA isolated from each tissue sample was added to 25 μL reaction solution containing 2.0 μL oligo-dT_{18}, 5.0 μL dNTPs, 1.0 μL RNase inhibitor, 1.0 μL M-MLV transcriptase, 5.0 μL M-MLV RT reaction buffer (Promega, Wisconsin USA) and RNase-free water. The reverse transcription procedure was performed according to the manufacturer's instructions (Promega); the protocol was as follows: 70 °C for 5 min and 42 °C for 1 h. The RT products (cDNA) were stored at –20 °C.

2.5. Gene mRNA expression analysis by real-time PCR

Expression levels of Hsp27, Hsp70, Hsp90, Ppp3r1, Rela, Gpx2, Rhot2, Gsdma1, Bax, Ndufc2, and Nox1 were determined by real-time PCR (RT-PCR) analysis.

Quantitative PCR analysis was performed using the DNA Engine Mx3000P® (Stratagene, La Jolla, California, USA) fluorescence detection system against a double-stranded DNA-specific fluorescent dye (Stratagene, USA) according to optimized PCR protocols. β-actin was amplified in parallel with the target genes and used as a normalization control. The cycling conditions were as follows: 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s, 60 °C for 20 s, and 72 °C for 60 s. For the dissociation curve, we incubated the amplified products at 95 °C for 1 min and ramped the temperature to 55 °C at a rate of 0.2 °C/s while continuously measuring the fluorescence levels. Expression levels were determined using the relative threshold cycle (CT) method as described by the manufacturer (Stratagene, USA). Each gene was calculated by evaluating the expression $2^{-\Delta\Delta CT}$, where ΔΔCT is the result of the following: $[CT_{gene} - CT_{\beta\text{-actin}}]_{(transport\ stress)} - [CT_{gene} - CT_{\beta\text{-actin}}]_{(control)}$. The cDNA of each sample was subjected to RT-PCR using the primer pairs listed in Table 1. The PCR reaction (20 μL) contained 10 μL of SYBR Green PCR mix (Invitrogen, USA), 0.3 μL of reference dye, 1 μL of each primer (both 10 μmol/L), and 1 μL of cDNA template.

2.6. DNA microarray and data analysis

2.6.1. RNA extraction and target labeling

RNA extraction followed the method described earlier. RNA integrity of each sample was determined using RNA 6000 Lab Chip Kit and Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). RNA only had a 28S/18S ratio of ⩾1.8. RNA was purified using a QIAGEN RNeasy® Mini Kit (74106, QIAGEN, Valencia, CA, USA) and amplified using a Low RNA Input Linear Amplification Kit (5184-3523, Agilent). Each RNA sample was annealed with a primer containing a polydT and T7 polymerase promoters. Reverse transcriptase produced single and double-stranded cDNAs. T7 RNA polymerase was then used to create cRNA from the double stranded cDNA by incorporating cyanine-3-labeled cytidine 5-triphosphate. The quality of the labeled cRNA was verified, and the absolute concentration was detected using a spectrophotometer (Nanodrop ND1000, Wilmington, Delaware, USA).

2.6.2. Hybridization, scanning and feature extraction

The cRNA was hybridized using a Gene Expression Hybridization Kit (5188-5242, Agilent, USA). Hybridization was performed at 60 °C for 17 h on Agilent Whole Rat Genome Arrays (G4131F, Agilent, USA). The arrays were washed using a Gene Expression Wash Buffer Kit (5188-5327, Agilent, USA) before stabilization and dehydration were performed (5185-5979, Agilent, USA). The arrays were scanned on a microarray (G2565BA, Agilent, USA), and the subsequent data were compiled with Agilent feature