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Effects of pro-inflammatory cytokines and antioxidants expression in the jejunum of mice induced by hydrogen peroxide

Zhijie Cui [a,b,1], Jie Yin [a,b,1], Lijian Wang [a,b], Liuqin He [a,b], Yulong Yin [a,c], Tiejun Li [a,*]

[a] Scientific Observing and Experimental Station of Animal Nutrition and Feed Science in South-Central, Ministry of Agriculture, Hunan Provincial Engineering Research Center of Healthy Livestock, Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, Hunan 410125, China
[b] University of Chinese Academy of Sciences, Beijing 100039, China
[c] Southwest Collaborative Innovation center of swine for quality & safety, 211#211Huiming Road, Wenjiang district, Chengdu, China

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ABSTRACT

Inflammation-induced jejunal cell death and oxidative stress have been observed in inflammatory bowel disease (IBD), but now, there is still no systematic animal model of jejunal oxidative stress for the evaluation of potential therapies. Thus, the purpose of this study was to evaluate the dynamic changes of pro-inflammatory cytokines and antioxidant expression on the jejunal inflammation. In this study, Hydrogen peroxide(H_2O_2, 10 ml/kg)was administrated intragastrically to mice in a single dose of 1%, 3%, or 5%. The incidence of death, histomorphometry, inflammatory cytokines (including TNF-α,IL-1β,and IL-5), and antioxidant genes were measured via RT-PCR. During the whole period, a massive infiltration of neutrophils was observed in the jejunum. Intragastric administration of H_2O_2 significantly up-regulated the expression of TNF-α,IL-1β, and IL-5 ($P < 0.05$). Meanwhile, 5% H_2O_2 induced an acute stress in mice which lasted up to 3 days, while 3% H_2O_2 induced a chronic injury in the jejunum that lasted 7 days. In the early stage, H_2O_2 markedly enhanced expression of antioxidant genes and all of the doses used progressively decreased the expression of antioxidant genes in a time-dependent manner. These findings suggested that intragastric administration of 3% H_2O_2 induces relatively stable oxidative stress in the jejunum.

1. Introduction

Oxidative stress is considered to be an imbalance of ROS (reactive oxygen species) generation and antioxidant defense. In our lab, we previously found that various forms of stress contribute to oxidative stress and the antioxidant system plays an important role in animal growth and development [1–3]. In addition, excessive ROS can interact with many macromolecules, including proteins, lipids, and nucleic acids and mediate several signaling pathways [4–6]. Meanwhile, other compelling evidence has indicated that oxidative stress and ROS are closely associated with various pathological conditions, including inflammation, aging, diabetes, cancer, and neurodegenerative diseases,as well as pathogenesis of mucosal lesions in the gastrointestinal tract [4,7,8].

H_2O_2, the non-radical 2-electron reduction product of oxygen, is a highly reactive oxygen species and serves as a signaling molecule in the regulation of a wide variety of biological processes [9–11]. Normally, the liver produces H_2O_2 at a rate of 50 nm/min/g, and the intracellular H_2O_2 can maintained at about 10 nm under scavenging by catalase, glutathione peroxidases, and the Fenton reaction [4,11]. However, a disturbance in peroxidases and various stresses can lead to the accumulation of H_2O_2, and excessive H_2O_2 is capable of diffusing throughout mitochondria and across cell membranes, where it can cause many types of cellular oxidative injury [12,13]. A latest study shows that H_2O_2 can induce ubiquitination of RCAN1 and may promote P53 degradation [14].

In recent reports, H_2O_2 is widely used in models of oxidation in vitro [15,16]. However, there is no specific model for investigations of oxidative stress in animals. And the current study shows that H_2O_2 promote the production of ROS and result in the imbalance of pro-inflammatory factors and antioxidants [17]. Therefore, we explored the pro-inflammatory and oxidative effects of the intragastric administration of H_2O_2 in mice to provide a novel model of oxidative stress in vivo.

2. Material and methods

2.1. Animals and reagents

Eighty female ICR (Institute for Cancer Research,Changsha,Hunan) mice (23-25 g), 78 days of age, were purchased from SLAC Laboratory Animal Central (Changsha, China). All mice were housed in polycarbonate and wire covered-cages in a temperature (25 ± 3 °C)-and humidity

* Corresponding author at: Hunan Provincial Engineering Research Center of Healthy Livestock, Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences.
E-mail address: tjli@isa.ac.cn (T. Li).
[1] Contributed equally.

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(50 ± 5%)-controlled room with a 12 h dark- light cycle for at least 3 day(s) prior to treatment and had free access to water *ad libitum* and standard diet(Purina Chow)throughout the experimental period. H_2O_2 was purchased from Beijing Chemical Plant (China), and dissolved in purified water. Drugs were prepared immediately before administration.

2.2. Experimental design

Mice were randomly divided into four groups (all mice received distilled water and basal diet): A) Mice received a single intragastric administration of sodium chloride (10 ml/kg; n = 20); B) Mice were treated with 1% H_2O_2 via intragastric administration (10 ml/kg; H_2O_2 solution was prepared fresh daily, n = 20); C) Mice were treated with 3% H_2O_2 (10 ml/kg; n = 20); D) Mice were treated with 5% H_2O_2 (10 ml/kg; n = 20). Infusion started at 9:00 before feed in the morning on the first day of the experiment. Body weight was recorded on day(s) 0 and 7. Six mice were randomly selected from each group for sacrifice on day(s) 3, 5, and 7. Tissues from the jejunum of each mice were harvested, immediately frozen in liquid nitrogen and stored at −70 °C for subsequent gene expression analysis. One jejunal piece from each mouse (3 cm) was immediately placed in a 50 ml sample tube with 4% neutral buffered formalin for histomorphometry determination.

2.3. Ethics statement

This study was conducted according to the guidelines of the Declaration of Helsinki and all procedures involving animal subjects were approved by the animal welfare committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences.

2.4. Histomorphometry determination

The samples that were placed in formalin were subjected to haematoxylin and eosin (HE) staining to observe and evaluate the changes in the jejunum morphology according to N'Da HA's report [18]. The harvested samples were further processed using routine histological methods and mounted in paraffin blocks. Six-micrometer-thick sections were cut and stained with haematoxylin and eosin (HE). All specimens were examined under a light microscope (Nikon, Japan). Villus height and crypt depth were measured using an image-analysis system (MIAS2000-P2, China).

2.5. Real-time quantitative (RT-PCR)

Total RNA was isolated from liquid nitrogen-frozen and ground jejunal tissue with TRIZOL reagent (Invitrogen, USA) and then treated with DNase I (Invitrogen, USA), according to the manufacturer's instructions. Primers (Table 1) were designed with Primer 5.0 according to the mouse gene sequence. β-actin was used as an internal control to normalize target gene transcript levels. Real-time PCR was performed as in our previous studies [19]. Briefly, 1 μl cDNA template was added to a total volume of 10 μl containing 5 μl SYBR Green mix, 0.2 μl Rox, 3 μl ddH2O, and 0.4 μl each of forward and reverse primers. We used the following protocol: (i) pre-denaturation program (10 s at 95 °C); (ii) amplification and quantification program, repeated 40 cycles (5 s at 95 °C, 20 s at 60 °C); (iii) melting curve program (60–99 °C with a heating rate of 0.1 °C/s and fluorescence measurement). Relative expression was expressed as a ratio of the target gene to the control gene using the formula $2^{-(\Delta\Delta Ct)}$, where $\Delta\Delta Ct = (Ct_{Target}\text{-}Ct_{\beta\text{-actin}})_{treatment} - (Ct_{Target}\text{-}Ct_{\beta\text{-actin}})_{control}$ [20]. Relative expression was normalized and expressed relative to the expression in the control group.

Table 1
PCR primer sequences: the forward primers (F) and the reverse primers (R) used in this study.

Table 1	Accession No.	Nucleotide sequence of primers (5′–3′)	Size (bp)
β-Actin	NM_007393.3	F:GTCCACCTTCCAGCAGATGT R:GAAAGGGTGTAAAACGCAGC	117
ZnCuSOD	NM_011434.1	F:CCACTGCAGGACCTCATTTT R:CACCTTTGCCCAAGTCATCT	216
Gpx1	NM_008160.6	F:GGTTCGAGCCCAATTTTACA R:CCCACCAGGAACTTCTCAAA	199
Gpx2	NM_030677.2	F:GTGTGATGTCAATGGGCAGAA R:ACGTTTGATGTCAGGCTCGAT	241
Gpx3	NM_008161.3	F:GATGTGAACGGGGAGAAAGA R:CCCACCAGGAACTTCTCAAA	152
Gpx4	NM_001037741.3	F:CTCCATGCACGAATTCTCAG R:ACGTCAGTTTTGCCTCATTG	117
IL-1β	NM_008361.3	F:CTGTGACTCGTGGGATGATG R:GGGATTTTGTCGTTGCTTGT	213
IL-5	NM_010558.1	F:ATGGAGATTCCCATGAGCAC R:GTCTCTCCTCGCCACACTTC	265
TNF-α	NM_013693.2	F:AGGCACTCCCCCAAAAGAT R:TGAGGGTCTGGGCCATAGAA	192

2.6. Statistical analysis

All statistical analyses were performed using SPSS 19.0 software. The survival rate of mice was analyzed by Fisher's test. Group comparisons were performed using the one-way analysis of variance (ANOVA) to test the homogeneity of variances via Levene's test followed by Turkey's multiple comparison test. Data are expressed as the mean ± standard error of the mean. *P* value of 0.05 and 0.01 was considered statistically significant.

3. Results

3.1. Survival and average weight gain

The survival data after exposure to H_2O_2 before 3 days are shown in Table 2. One mouse died in each of low- and medium-dose group, and 4 mice died after administration of 5% H_2O_2 before 3 days. In addition, one mouse died in the day 5, 2 mice died in the day 7, but only 1 mouse alive when we prepared to collect samples. While there were on significant differences in survival with the different doses of H_2O_2 ($P > 0.05$), the administration of 5% H_2O_2 tended to have the highest death rate. There were non-significant differences in average daily gain after exposure to H_2O_2 ($P > 0.05$) (Fig. 1). However, we did not collect weight-gain data from the 5% H_2O_2 group because of the high rate of death.

3.2. Histological evaluation

As shown in Fig. 2, while the jejunum from the control mice did not show any pathological changes, while all of the H_2O_2-treated groups showed marked neutrophilic infiltration in the jejunum. And the extent of neutrophilic infiltration increased with the increase of H_2O_2 concentration.

Hypereosinophilia is followed by cell shrinkage and chromatin condensation, resulting in hyperchromatic nuclei. Apoptotic intestinal

Table 2
Effect of administration with H_2O_2 on probability of survival before 3 days.

Dose	Death	Death rate
Control	0	0
1%	1	5%
3%	1	5%
5%	4	20%