Malondialdehyde and SOD-induced changes of gastric tissues in acute gastric mucosal injury under positive acceleration

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ABSTRACT. The aim of this study was to investigate the impacts of positive acceleration (+Gz) on the gastric mucosal tissues in cases of acute gastric mucosal injury and to explore the role of oxygen free radicals. Thirty Sprague Dawley rats were randomly divided into the absolute ethanol control group (A group), absolute ethanol +5Gz group (B group), absolute ethanol +10Gz group (C group). Following centrifugation, the gastric tissues of each group were studied for the presence of gastric mucosal injuries and morphological changes. The concentrations of malondialdehyde (MDA) and superoxide dismutase (SOD) contents were simultaneously investigated. Degree of gastric mucosal injuries were as follows: C group (visually 49.080 ± 10.254, under light microscopy 9.400 ± 2.011) > B group (visually 23.654 ± 9.678, under light microscopy 5.000 ± 1.054) > A group (visually 11.410 ± 3.742, under light microscopy 3.800 ± 1.399). The gastric mucosal MDA content (0.376 ± 0.084 *vs* 0.235 ± 0.044) was significantly higher in the C group than in the A group, whereas the SOD content (8.852 ± 1.001 *vs* 10.694 ± 0.965) was lower than that in the A group. However,

the MDA and SOD contents did not change much in the B group. Our results suggest that the +Gz exposure might aggravate the acute gastric mucosal injury, and changes in MDA and SOD contents in the gastric tissues indicated that the oxygen free radicals play an important role in this regard.

Key words: Malondialdehyde; Mucosa; Oxygen free radicals; Positive acceleration; Stomach; Superoxide dismutase

INTRODUCTION

Positive acceleration (+Gz) generated by modern high-performance fighter jets during flight maneuvers is characterized by high G values, rapid acceleration rate, long duration and repeated occurrence; its acceleration overload far exceeds the pilot's physiological tolerance limits. Several studies have demonstrated that +Gz can affect such systems as the heart and blood vessels, bones and kidneys and even consciousness (Tripp et al., 2006; Di Rienzo et al., 2010; Wagstaff et al., 2012; Biernacki et al., 2013). The occurrence of digestive diseases, such as irritable gastritis and acute gastric mucosal lesions, during flight training, is a matter of great interest to aerospace medical workers. Typical activities such as smoking, drinking, high noise levels and an irregular lifestyle adversely affect the digestive functions of pilots. The additional impact of weightlessness and +Gz flight can lead to acute gastric mucosal injury (Vasil'ev et al., 1996). However, the effect of the exposure of preexisting gastric mucosa injuries to +Gz conditions has not been extensively examined. In order to determine the effect of +Gz on gastric mucosal damage in this study, rats with ethanol-induced acute gastric mucosal injury were exposed to different +Gz conditions. In addition, the gastric mucosal malondialdehyde (MDA) and superoxide dismutase (SOD) contents were measured, in order to further clarify the potential roles and mechanisms of free oxygen radicals in the +Gz effect, thus providing a theoretical basis for the prevention and treatment of gastrointestinal diseases in pilots.

MATERIAL AND METHODS

Experimental animals

Healthy SD SPF grade rats (30), weighing 200 ± 10 g were provided by the Experimental Animal Center of Academy of Military Medical Science, License No. SCXK -(Army) -2007 -004. This study was carried out in strict accordance with the recommendations detailed in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the General Hospital of Air Force, PLA China.

Grouping

SD rats (30) were randomly divided into three groups (10 rats in each group): A Group: absolute ethanol control group; B group: absolute ethanol +5Gz; C group: absolute ethanol +10Gz. Experiments were conducted after 10 days of adaptive feeding.

Rat acceleration-simulating model

+Gz exposure situations were simulated using a centrifuge (Air Force Institute of Aviation Medicine, Beijing, China). A special fixation cartridge was used to load the rat. The cartridge was then fixed on the centrifugal rotating arm with a centrifuge radius of 1m. The head of the rat was pointed towards the centrifuge axis, and each rat was assigned to a specific cartridge. Acceleration direction was ascertained and fixed. The Gz value acceleration rate was 1.0Gz/S, and was controlled by a computer program.

Specific experimental procedures

Prior to the experiments, the rats were deprived of food for 24 h, and water for 12 h; at which point absolute ethanol (0.4 mL/100 mg) (Beijing Chemical Company, Beijing, China) was administrated using the intragastric gavage method. An hour later, the A group rats were placed in the centrifuge for 3 min, without acceleration; B group rats were exposed to +5Gz acceleration for 3 min consecutively; C group rats were exposed to +10Gz acceleration for 3 min consecutively. The rats from the three groups were anesthetized immediately following the experiments, and gastric mucosal specimens and tissue samples were obtained using either the naked-eye or light microscopy. Next, the MDA and SOD levels in these gastric tissues was measured.

Specimen processing

1) Gastric mucosal damage index: subsequent to being placed in the centrifuge, the rats were anesthetized via an intraperitoneal injection of 3% sodium pentobarbital (Sigma Company, Shanghai, China) at a final concentration of 0.6 mL/200 g. The rats were then dissected and their stomachs were resected; the stomach was cut along the gastric greater curvature, the cavity was washed with saline, and then dried with filter paper. The resected stomach was then spread on a glass slide. The degree of gastric mucosal damage was expressed using a damage index based on the Guth Scoring method: 0 point: normal gastric mucosa; 1 point: strip-like damage measuring 1mm in length; 0.5 point: spotty bleeding (length and width were <1 mm); for damage with width >1 mm, the score was doubled. In cases of plaque-like bleeding (length and width were >2 mm), a specific area for scoring was first determined. 2) Pathological observations: after determining the mucosal damage index, the most damaged part of the specimen (0.5 x 0.5 cm) was set aside for tissue fixation and dehydration, followed by paraffin sectioning; after staining with hematoxylin (eosin), the mucosal specimen with pathological changes were examined using a light optical microscope. The Whittle scoring system was used to evaluate the degree of gastric mucosal damage: 1 point: epithelial cell damage; 2 points: the gastric gland was interrupted, and the upper mucosa exhibited edema; 3 points: the middle and lower mucosa exhibited hemorrhagic damage; 4 points: deep necrosis or ulceration of the tissue. The histological damage index was defined as the cumulative score of each section; the highest histological damage index awarded was 10 points. 3) Detection of gastric MDA and SOD contents: subsequent to measuring the gastric mucosal damage index and allocation of specimens for pathological examination, a razor was used to gently scrape the mucosa, then placed into an EP tube and stored at -80°C. The MDA and SOD contents were measured by the ELISA method: the sample was first weighed, 10% of the tissue was homogenized with 4% saline; the tissue homogenate was then centrifuged at 4°C and 4000 r/m

for 10 min, and the supernatant was used for detection, following the specific procedures detailed in the MDA and SOD enzyme immunoassay kits (Beijing Huaying Institute of Biological Technology, Beijing, China). To ensure the reliability of the results, the measurements for each specimen were repeated, and positive and negative controls were used for comparison.

Statistical analysis

The SPSS17.0 statistical software was used for data analysis, and variance analysis was performed. The results are reported as means ± SD with *t* test. P < 0.05 was considered to be statistically significant.

RESULTS

General structural changes of gastric mucosa after exposure to different +Gz levels

The surface of gastric mucosa in the A group exhibited mild edema (11.410 ± 3.742); while +Gz exposure aggravated the mucosal injury, the gastric mucosal surface of B group demonstrated hyperemia and edema, with scattered spotty and long-strip-like erosion and bleeding (23.654 ± 9.678). This difference was statistically significant when compared with the A group; the gastric mucosal damage of C group was the most severe. The gastric mucosal surface exhibited diffused congestion and edema, with multiple spotty and foci-like erosions, a large bleeding, and even some mucosal ablation (49.080 ± 10.254). This difference was statistically significant (P < 0.05) compared with the A and B groups (Table 1).

Table 1. Gastric mucosal damage indices acquired following naked-eye and light microscopy analysis for different group cases (data are reported as means ± SD).

Group cases	Under the naked-eye (point)	Light microscopy (point)
A group 10	11.410 ± 3.742[c]	3.800 ± 1.399[c]
	t = 3.732	t = 2.167
B group 10	23.654 ± 9.678[a]	5.000 ± 1.054[a]
	t = 5.703	t = 6.128
C group 10	49.080 ± 10.254[b]	9.400 ± 2.011[b]
	t = 10.914	t = 7.230

[a]For the comparison between groups A and B for the naked-eye and the light microscopy analysis, P < 0.05; [c]for the comparison between groups A and C, P < 0.01; [b]and for the comparison between groups B and C, P < 0.01.

Gastric mucosal changes in the different experimental groups observed using light microscopy

The epithelia in A group was relatively integrated, with partial gastric mucosa demonstrating interstitial hemorrhage, and a low level of inflammatory cell infiltration (3.800 ± 1.399); the gastric mucosal epithelia in B group was relatively incomplete, and demonstrated partial disordering of glandular structures, with a low level of submucosal neutrophil infiltration (5.000 ± 1.054). When compared with A group, the difference was statistically significant; the damage in the C group was the most serious; most of glandular structures were disordered, the tissue stroma appeared flaky congested and eroded, the submucosa, muscularis exhibited

neutrophil infiltration, even involved in the serosa (9.400 ± 2.011). These difference were statistically significant (P < 0.05) (Table 1) compared with the A and B groups.

MDA and SOD content determination in different groups

Compared with A group, the MDA of gastric mucosa increased in B and C group, the difference was not significant in the B group (0.255 ± 0.074) (P > 0.05); while the MDA content in the C group increased significantly (0.255 ± 0.074), showing a statistically significant difference when compared with the A group (P < 0.05), indicating that +Gz exposure might aggravate gastric mucosal injury; the higher the +Gz level, the greater the damage. Compared with A group, the SOD in the gastric mucosa decreased in B and C groups, the difference was not significant in the B group (10.000 ± 1.067) (P > 0.05); while the SOD content in the C group decreased significantly (8.852 ± 1.001), showing a statistically significant difference when compared with A group (P < 0.05), indicating that +Gz exposure might cause decrease to gastric mucosal protective factors; the higher the +Gz level, the greater the effects (Table 2).

Table 2. Malondialdehyde (MDA) and superoxide dismutase (SOD) content of gastric mucosa of different group cases (data are reported means ± SD).

Group cases	MDA (nmol/mg)	SOD (U/mg)
A group 10	0.235 ± 0.044[c]	10.694 ± 0.965[c]
	t = 4.930	t = 4.198
B group 10	0.255 ± 0.074[a]	10.000 ± 1.067[a]
	t = 0.747	t = 1.534
C group 10	0.376 ± 0.084[b]	8.852 ± 1.001[b]
	t = 3.529	t = 2.482

[a]For comparison between groups A and B, P > 0.05; [c]for comparison between groups A and C, P < 0.05; [b]and for comparison between groups B and C, P < 0.05.

DISCUSSION

+Gz is defined as the head-to-foot acceleration generated during flight, with high +Gz characterized as levels greater than 7. Several studies have shown that high +Gz exposure can lead to acute and chronic gastric mucosal lesions (Vasil'ev et al., 1996). Under conditions of +Gz exposure, including centrifugal force of inertia and the enhanced sympathetic activities, results in gastric mucosal vessels undergoing spasms and contractions, the mucosal blood supply decreases, resulting in local tissue ischemia, hypoxia and the decrease of defense functions. Our experimental results demonstrate that +Gz exposure can aggravate gastric mucosal injury, and that the higher the +Gz value, the more severe the resulting damage is. The MDA and SOD levels in the gastric tissues revealed that oxygen free radicals are one of the important mechanisms by which this damage is created.

Under physiological conditions, the gastrointestinal tract itself generates and releases a small amount of oxygen free radicals; the NADPH oxidase and dioxidase in the epithelial cells generates the superoxide anion (O_2-) and hydrogen peroxide (El Hassani et al., 2005; Ha et al., 2005; Kuwano et al., 2006). At the same time, the body's defense system scavenges the free oxygen radicals, to prevent them reaching tissue-damaging levels. MDA is the final product of the lipid peroxidation by these free radicals, it can lead to cross-linking of such

large molecules as protein and nucleic acids, and is therefore cytotoxic. Reduced glutathione (GSH), SOD and catalase are important antioxidant damage-defense system *in vivo* (Oh et al., 2001; Li et al., 2006); MDA and SOD are now recognized as good indicators reflecting changes in free radical levels.

NSAIDs, alcohol, alkali and physical factors (Das and Roy, 2012) can damage the gastric mucosal barrier, causing gastric mucosal lesions. The mechanisms of the ethanol-induced gastric mucosal lesions are complex, among which one important point is related to oxygen free radicals (Ismail et al., 2012). Several studies have reported (Olaleye and Farombi, 2006; Alvarez-Suarez et al., 2011) that in the ethanol-induced gastric mucosal injury model, the SOD activity decreased. This study shows that the gastric mucosal SOD levels of all groups decreased, when compared with A group, the SOD significantly decreased after +Gz exposure, and was negatively correlated with the +Gz values, the higher the +Gz values, the more the SOD content decreased, indicating that +Gz exposure could cause a decrease in the gastric mucosal antioxidant protective factors. Olaleye et al. (2007) pointed out that ethanol-induced gastric mucosal lipid peroxidation was enhanced, and that MDA could be used to measure the extent of cell membrane damage (Eslami et al., 2014). In this experiment, compared with A group, the MDA content in gastric mucosa significantly increased after +Gz exposure, and was positively correlated with the +Gz values, indicating that +Gz value could increase the gastric mucosal injury. The possible mechanism for this might be through the mediation of lipid peroxidation. The changes of MDA and SOD contents indicated that the oxygen free radicals played an important role in the increased gastric mucosal injury after +Gz exposure. In addition, we found that, compared with A group, the changes of MDA and SOD contents in B group had no statistically significant difference, indicating that the low value of +Gz exposure did not severely affect gastric mucosal injury, while high +Gz exposure could significantly increase the damage, thus high +Gz value exposure would have greater significance with regards to mucosal injury protection.

In addition, +Gz conditions would increase the hydrostatic pressure gradient, therefore the organic arterial blood pressure would decrease; when blood flow decreases below a certain threshold, the organs might experience acute ischemia and hypoxia. Neutrophils are the most active inflammation cells *in vivo*, and would be largely generated in such pathological environments as infection and ischemia, many studies have found that the neutrophil infiltration played an important role in gastric mucosal injury (Ichikawa et al., 2002; Jiménez et al., 2004; Sener et al., 2004). In the early stages of inflammation, neutrophils migration to the inflammation sites play an important defensive role (Cassatella, 1999), but when the ratio of neutrophils and the target cells achieves the non-physiological level (greater than 20:1), it can result in the gastric epithelial cell injuries, and the mechanism might be through activation of the non-active coenzyme II (reduced nicotinamide-adenine dinucleotide phosphate II, NADPH II) inside the cell membrane, thus inducing the neutrophil respiratory burst, releasing large amounts of oxygen free radicals, and resulting in damage of normal tissue (Kim et al., 2012). In this experiment, it was observed that the pathological section of gastric mucosa exhibited a large amount of inflammatory cell infiltration after +Gz exposure, indicating that +Gz exposure could induce the gastric mucosal injury, which might be related with the inflammatory chemotaxis cell-promoted lipid peroxidation. During the gastric mucosal ischemia, the blood flow is reduced, the cells might suffer from necrosis, dysfunction and toxic metabolites generation (de Groot, 2005). Furthermore, when the cellular energy is insufficient, the intracellular Ca^{2+} would increase, which all could cause the xanthine dehydrogenase to rapidly

and irreversibly transfer to the xanthine oxidase, while the gastric vessels themselves had the adaptive protective diastolic function, when the gastric mucosal blood flow restored, the large amount of oxygen would flow into the ischemic tissues with blood, the xanthine oxidase would produce large amounts of oxygen free radicals during catalyzing the conversion of hypoxanthine into xanthine and uric acid, which would then result in the ischemia-reperfusion injury (Zamora Rodríguez et al., 2007). Moreover, the gastric acid increased returning would also increase the gastric mucosal injury. So, the +Gz exposure might also possibly increase the gastric mucosal injury through making the gastric vascular ischemia and then reperfusion, thus mediating the large generation of oxygen free radical.

In summary, exposure to high +Gz might aggravate the acute gastric mucosal damage, increase oxidative stress levels, while reducing the antioxidant capacity, which might be one of the important causes of the aggravation to the gastric mucosal injury. These experiment provide new experimental evidence for the clinical use of antioxidation treatment in the prevention and treatment of gastrointestinal diseases in pilots.

Conflicts of interest

The authors declare no conflict of interest.

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