Protective effect of dietary nitrate on stress-induced gastric mucosal injury via enhancing blood perfusion in Mongolian gerbils

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To the Editor: Gastric ulceration caused by stress is one example of stress-induced organ injuries. Stress-induced ulcer is commonly seen in critically ill patients and can result in significant upper gastrointestinal bleeding associated with a highly increased mortality. Water immersion and restraint stress (WIRS), which is a complex of physical and psychological stressors, causes damage that mimics the gastric lesions caused by sepsis, trauma, or surgery.[1] Notably, it is well established that adequate mucosal blood flow plays an important role in protecting the gastric mucosa from injuries. Inorganic nitrate exists in everyday diet especially in some vegetables. Dietary nitrate can be rapidly converted to nitrite by oral bacteria, and further reduced to nitric oxide (NO) in the acidic stomach. It has been reported that nitrate serves as biological reservoirs for NO in hypoxia or acidic conditions.[2] Dietary nitrate, as NO donor, has the potential to prevent cardiovascular disease, vascular damage, and high blood pressure in animal models and humans.[3,4] Our previous study has showed that dietary nitrate enhances blood flow in ischemic skin flap.[5] Here, we explore the role of nitrate therapy in stress-induced gastric mucosal injury in Mongolian gerbils.

Qualified male Mongolian gerbils (40–60 g) were randomly divided into three groups with random number table: Control (without WIRS), WIRS, and WIRS + nitrate (sodium nitrate), with six Mongolian gerbils in each group. All animal experiments were approved by the Committee of Animal Care and Welfare in the Affiliated Hospital of Qingdao University (No. AHQU20190107A). The nitrate-treated Mongolian gerbils were administered 5 mmol/L sodium nitrate dissolved in distilled water by drinking water 1 week before WIRS. The daily dose of nitrate intake in nitrate group was approximately 1 mmol/kg. The remaining animals were used as the control group and WIRS group and administered distilled water containing sodium chloride of the same dose. WIRS assay was conducted as follows. Mongolian gerbils were fasted for 18 to 20 h before the experiment and then fixed on the rat board and immersed in a 20 ± 2°C water for 4 h up to the depth of the xiphoid process. After general anesthesia, the stomach was removed and prepared for subsequent experiments. The blood flow of gastric mucosa in Mongolian gerbils was detected by laser Doppler flowmetry (LDF). For details, a LDF with a vascular monitor system (Moor Instrument, Axminster, Devon, UK) was used. The mucosal blood flow in the glandular stomach of Mongolian gerbils was determined as a voltage output and expressed as perfusion units. An optical probe was placed gently 0.5 mm above and perpendicular to the mucosal surface of the glandular stomach. When the blood flow was stabilized, three points were selected for measurement (one point for 3 min) and the average value was calculated. Statistical analyses were performed with SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). All data are presented as mean ± standard deviation. The comparison between groups was performed using Student's *t* test, and a value of $P < 0.05$ was considered statistically significant.

As shown in Figure 1, WIRS group displayed severe injuries in the glandular stomach appearing as elongated bands of hemorrhage and tissue edema, while the nitrate effectively inhibited the ulceration in the WIRS + nitrate group. Accordingly, ulcer index in WIRS group showed an obvious increase, while nitrate-pretreated group reversed this trend significantly (13.40 ± 6.95 *vs.* 32.60 ± 7.02, $t = -4.346$, $P = 0.002$) as compared with WIRS group. To further explore the underlying mechanism, we measured gastric mucosal blood flow in Mongolian gerbils. The gastric mucosal blood flow was decreased after WIRS induction as compared with the control group (32.31 ± 17.38 PU *vs.*

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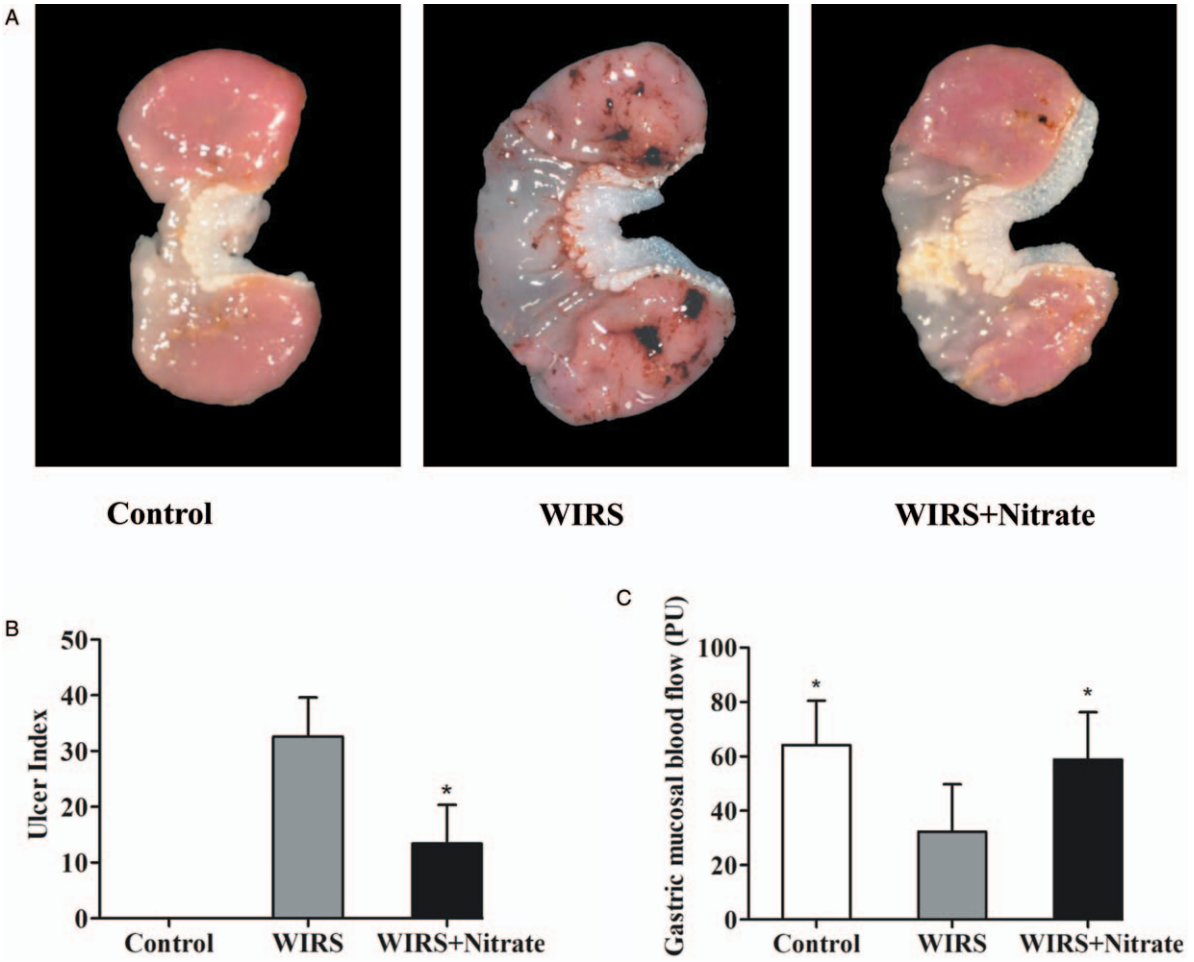

Figure 1: Gastroprotective activity of dietary nitrate on stress-induced gastric injury. (A) Macroscopic appearance of the stomach. (B) Ulcer Index of gastric mucosa. (C) Gastric mucosal blood flow in Mongolian gerbils. $^{*}P < 0.05$ *vs.* WIRS group. WIRS: Water immersion and restraint stress.

64.16 ± 16.26 PU, $t = -2.992$, $P = 0.017$). Whereas, oral administration of nitrate significantly restored gastric mucosal blood flow (58.77 ± 17.51 PU *vs.* 32.31 ± 17.38 PU, $t = -2.398$, $P = 0.043$) as compared with WIRS group.

The gastric mucosa is continuously exposed to plenty of noxious factors. To protect the gastric mucosa from aggressive stimulus, a mucosal defense has evolved, which includes an increase of the production of bicarbonate in surface mucus, regulating the gastric mucosal blood flow, and accelerating the epithelial regeneration. Among all the defense system, gastric mucosal blood flow plays a key role. Sufficient mucosal blood flow helps the gastric mucosa recover from injuries as soon as possible. In this study, we found that dietary nitrate exerted gastroprotective effect in Mongolian gerbils probably due to the increase of gastric mucosal blood flow. Adequate gastric mucosal blood flow supported by nitrate therapy contributes to the gastroprotective role. The findings may have implications for novel preventive strategies against stress-related organ injury.

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Conflicts of interest

None.

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