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ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

Regular swimming before the induction of ulcer ameliorates oxidative gastric damage in rats: The anxiolytic effect of exercise

Ülser indüksiyonundan önce yapılan düzenli yüzme egzersizi sıçanlarda oksidatif mide hasarını azalttı: Egzersizin anksiyolitik etkileri

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ABSTRACT

Objectives: To investigate the impact of exercise on ulcerogenesis.

Materials and Methods: Gastric ulcer was induced in Wistar albino rats that were either kept sedentary or had swimming exercise for 6 weeks. Under anesthesia, ulcer was induced by applying acetic acid on the stomach serosa, while control rats had sham-operation. On 3rd or 10th days of ulcer induction, anxiety levels were measured and rats were decapitated for measuring serum cortisol level, while gastric tissue samples were obtained for histopathologic analysis and for determination of malondialdehyde (MDA), glutathione (GSH) levels, myeloperoxidase (MPO) activity, luminol- and lucigenin-enhanced chemiluminescence (CL) and nitric oxide (NO) levels.

Results: In sedentary 3-day-ulcer group, gastric MPO activities were elevated with concomitant reductions in GSH content, while these changes were reversed in 10-day ulcer group. In exercised 3-day-ulcer group, all parameters were significantly reversed. Increased lucigenin-CL was suppressed, while NO levels were elevated by exercise. Histological analysis revealed lower lesion scores in exercised groups. Serum cortisol levels were not different among ulcer groups, but anxiety was suppressed by swimming exercise in 3-day-ulcer group.

Conclusion: Regular swimming exercise ameliorated acetic acid-induced oxidative damage of stomach via inhibition of neutrophil infiltration and through anti-stress effect of exercise. Regular exercise may be considered in facilitating the healing of gastric ulcers.

Keywords: Acetic acid, Gastric ulcer, Exercise, Myeloperoxidase, Anxiety

ÖZ

Amaç: Düzenli egzersizin ülserogenez üzerine olan etkisini araştırmaktır.

Gereç ve Yöntem: Wistar albino sıçanlar, sedanter ve egzersiz grupları olarak ikiye ayrıldı. Altı haftalık egzersizin sonunda, anestezi altında mide serozasına asetik-asit uygulanarak ülser oluşturulurken, kontrol gruplarına taklit-cerrahi yapıldı. Ülser uygulanmasının 3. ve 10. günlerinde, anksiyete düzeyleri belirlendi ve sıçanlar dekapite edildi. Serum kortizol seviyeleri ölçüldü; mide dokusu örnekleri malondialdehit (MDA), glutatyon (GSH), miyeloperoksidaz (MPO), nitrik oksit (NO), luminol-lusigenin probları ile kemiluminesans (KL) ölçümleri ve histopatolojik analiz için alındı.

Bulgular: Kontrol gruplarına kıyasla sedanter 3 ve 10 günlük ülser gruplarında MPO ve MDA seviyeleri artarken, GSH seviyeleri azaldı. Bu değişiklikler 10 günlük ülser gruplarında kontrol düzeylerine döndü. Egzersiz yapan 3 günlük ülser grubunda tüm parametreler tersine döndü. Ülser ile artmış bulunan lusigenin KL düzeyi egzersiz ile baskılanırken NO düzeyi egzersizle anlamlı olarak artmış bulundu. Histolojik incelemede egzersiz gruplarında lezyon skorları düşük bulundu. Ülser yapılan sedanter ve egzersiz gruplarında kortizol seviyeleri arasında farklılık görülmedi. Ülsere bağlı artan anksiyete, 3 günlük egzersiz yapan ülser gruplarında azalırken 10 günlük ülser grubunda değişmedi.

Sonuç: Düzenli yüzme egzersizi, dokuya nötrofil infiltrasyonunu engellemek, NO yapımını uyarmak ve anksiyeteyi azaltmak yoluyla midede oksidatif hasarı hafifletmektedir. Düzenli egzersiz, mide ülserinin gelişmesini yavaşlatan ve iyileşmesini kolaylaştıran bir yaklaşım olarak dikkate alınmalıdır.

Anahtar kelimeler: Asetik asit, Mide ülseri, Egzersiz, Miyeloperoksidaz, Anksiyete

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Introduction

Even in developed countries, peptic ulcer disease (PUD) is a frequently diagnosed clinical disorder, and there is nearly a 10 % risk of developing PUD during lifespan of every person [1]. Epidemiological studies have suggested that physical inactivity is associated with increased risk of PUD [2], while risk for developing duodenal ulcer, but not gastric ulcer, was found to be relatively lower in exercising men [3]. In contrary, some studies have shown that high level of energy expenditure during work was positively associated with the risk of having a duodenal ulcer as compared to individuals having a sedentary occupation [4]. Similarly, intensively trained endurance horses [5], as well as human athletes running marathons have shown a high prevalence of gastric lesions [6]. In a more recent prospective cohort study, moderate energy expenditure at leisure time was shown to be correlated with reduced likelihood of PUD in adults [7]. When possible mechanisms that are likely to be involved in the advantageous effects of moderate exercise on the development of PUD were studied, exercise-induced reduction of gastric acid secretion was suggested to play an important role in inhibiting ulcerogenesis [8]. Since either onset or exacerbation of PUD occurs frequently following stressful life events [9, 10], well-described stress-relieving potential of physical activity [11] was suggested to be involved in its anti-ulcereogenic effect by augmenting one's ability to cope with the psychological stress [12]. It is also proposed that exercise-induced oxidative stress, by providing a pre-conditioning adaptive mechanism, may be partially responsible for the beneficial effects of physical activity [13], because oxidative stress is regarded as one of the major aggravating factors that participate in the development of PUD [14].

In oxidative stress, formation of reactive oxygen species (ROS) disturbs the integrity and fluidity of membrane and impairs ionic transport across the membrane [14], but at the same time stimulates antioxidant systems [15]. Moderate aerobic exercise may increase the resistance of all the target tissues to any subsequent ROS-induced damage by triggering the activity of antioxidant enzymes and housekeeping enzymes, reducing the increased oxidative stress associated with aging, inflammation and DNA damage [16, 17]. We have previously shown that stress-induced gastric injury is ameliorated by regular exercise via the maintenance of oxidant/antioxidant balance [18]. In regularly exercising people, basal levels of pro-inflammatory markers, interleukin IL-1, IL-6 and tumor necrosis factor α (TNF)-α were found to be relatively low in the circulation [19], while secretion of the anti-inflammatory IL-10 was significantly increased with regular exercise [20].

Although epidemiological studies have indicated a relationship between physical inactivity and ulcerogenesis, to our knowledge, no experimental studies have been conducted to study the mechanisms involved in exercise-induced protection against gastric ulcer. The aim of the present study was to elucidate the impact of a 6-week swimming exercise on the development and healing of acetic acid-induced gastric ulcer in rats by investigating the possible beneficial role of exercise on anxiety and on the maintenance of oxidant/antioxidant balance.

Materials and Methods

Animals

Adult female Wistar albino rats (n=56; 240–270 g) were obtained from the Marmara University Animal Center, Istanbul, Turkey (DEHAMER). Rats were housed in a light-and temperature-controlled room at 12 /12 h light–dark cycle, in which the temperature (22±2°C) and relative humidity (65–70%) were kept constant. The rats were fed standard rat pellet chow and tap water ad libitum. The study was approved by the Institutional Ethics Committee for the Care and Use of Experimental Animals.

Experimental design

The rats were randomly assigned to an exercise protocol or a sedentary condition (Fig. 1). Following a 2-week swimming training (30 min/day, 3 days/week), rats were submitted to exercise (60 min/day, 5 days/week; n=24) [21] or were allowed to remain sedentary (n=24) for 6 weeks. Swimming exercise was performed in a cylindrical glass pool (150 x 50 x 50 cm) filled with 35-cm high water kept at 32 ± 1°C. Sedentary groups were not subjected to swimming exercise, but they were daily placed in wet glass containers for 60 min at identical time points as the exercised rats.

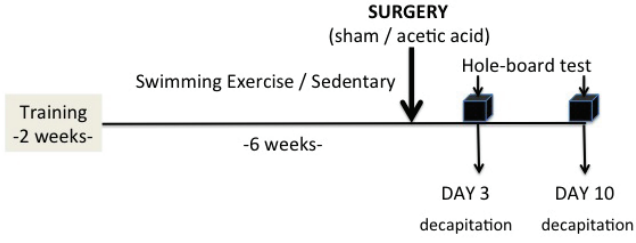

Fig. 1. Representative scheme showing the timescale of the applied procedures in the experimental groups.

At the end of the 6-week exercise or sedentary period, acetic acid-induced ulcer induction was performed under anesthesia, while saline was applied on the stomach of the control groups. The rats were then allowed to recover for either 3 or 10 days before they were decapitated, during which they were injected with intraperitoneal (ip) acetaminophen (Fentanyl Citrate®, Abbott, IL, USA; 0.1 mg/kg) for analgesia. Half of the sedentary or exercised control groups were decapitated on the 3rd day, while the other halves were followed for 10 days and their results were pooled. In all rats, hole-board test was accomplished to evaluate the level of anxiety at 3 hours before decapitation. Blood was withdrawn for the measurement of cortisol, and gastric tissue samples were stored at -80 °C for the determination of malondialdehyde (MDA), antioxidant glutathione (GSH) levels and myeloperoxidase (MPO) activity. Additional samples were obtained for histological evaluation and for the measurement of free radicals and nitric oxide (NO) in the gastric tissue samples.

Induction of gastric ulcer

Gastric ulcers were induced based on the model described originally by Okabe and Pfeiffer [22]. Following an overnight fasting and anesthesia (100 mg/kg ketamine and 0.75 mg/kg chlorpromazine, ip), stomach of rats was exteriorized by a midline laparotomy. Using the barrel of a 3-ml syringe, a 0.5 ml volume of acetic acid (80%, v/v) was applied onto the serosal surface of corpus for 60 seconds. Excess fluid was then aspirated and the stomach surface was washed with saline. Similar surgical procedures were followed in the control groups, but acetic acid was replaced by saline. Okabe *et al*. [22] have previously shown that ulcers induced with this model become chronic within 2-3 days and completely heal within 2-3 weeks, making this model suitable for studying the healing period of gastric ulcer.

Evaluation of anxiety level

Hole-board test is widely used to investigate the exploratory behavior of rats, which is reduced when the anxiety level is increased [23]. A hole-board apparatus (100 x 100 x 50 cm) consists of a wooden box having 16 holes with 3.8 cm diameters, distributed equally on its floor. At 3 hours before decapitation, each rat was placed individually on the hole-board and allowed to freely explore on the box for 5 min during which the test was recorded by a video camera. Then, an observer blinded to the group names counted the head-dips during the video-taped 5-min period. After each test, the box was wiped thoroughly with alcohol to reduce the odor.

Serum cortisol measurement

Serum cortisol levels were measured by an electrochemiluminescence immunoassay using cortisol-specific biotinylated antibody and streptavidin coated microparticles using an automated analyzer (Modular Analytics E170, Roche Diagnostics, Germany). Within-run and total precision values given by the manufacturer for 7.53–46 μg/dl concentration levels were between 1.1–1.3% and 1.6%, respectively. The measuring range was between 0.018 and 63.4 μg/dl.

Malondialdehyde and glutathione assays in the gastric tissue

Gastric tissue samples were homogenized in 10 volumes of ice-cold 10% trichloracetic acid and centrifuged at 3,000 rpm for 15 min at 4°C. The supernatant was then re-centrifuged at 15,000 rpm at 4°C for 8 min. The lipid peroxide levels were expressed in terms of MDA equivalents as nanomoles of MDA per gram of tissue [14]. GSH level was measured by Ellman procedure [24].

Measurement of gastric myeloperoxidase activity

The myeloperoxidase (MPO) activity was assessed as an indicator of neutrophil infiltration into the inflamed tissue [25]. Gastric tissue samples (0.2-0.3 g) were homogenized in 10 volumes of ice-cold potassium phosphate buffer (20 μM K_2HPO_4, pH7.4) and then centrifuged at 12,000 rpm for 10 minute 4°C. The pellet was homogenized again with K_2HPO_4 containing hexadecyltrimethylammonium bromide (HETAB; 0.5%). The MPO activity was assessed by measuring the H_2O_2-dependent oxidation of o-dianizidine·2HCl. One unit of enzyme activity is defined as the amount of MPO present that causes a change in absorbance of 1.0 unit/min at 460 nm and 37°C, and it is expressed in units per gram of tissue [25].

Chemiluminescence assay and the measurement of nitric oxide activity in the gastric tissue

The assessment of the free radicals in the gastric tissue samples was done by a chemiluminescence (CL) method using Mini Lumat LB 9509 luminometer (EG&G Berthold, Germany). Measurements were made at room

temperature in the presence of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione; Sigma, St. Louis, MO) or lucigenin (bis-N–methylacridiniumnitrate; Sigma) probes, each added at a 0.2 mmol/L concentration. Luminol detects hydroxyl, hydrogen peroxide and hypochlorite radicals, while lucigenin is selective for superoxide radical. CL measurement of NO level is based on the reaction of hydrogen peroxide and NO to peroxynitrite. In this assay, K_2CO_3 (0.4 mM), desferrioxamine (60 mM), H_2O_2 (4 mM) and luminol-sodium salt (3.6 mM) was added to the tube containing gastric tissue sample [26, 27]. All counts were obtained at 1 min intervals for a counting period of 5 min. Results are expressed as the area under curve (AUC) of relative light unit for wet tissue weights (rlu/mg tissue).

Histopathological analysis

For light microscopic investigations gastric tissue samples (n=6-7 in each group) were fixed in 10% buffered formaldehyde and prepared for routine paraffin embedding. Tissue sections at 5-µm thickness were stained with hematoxylin and eosin (H&E), examined and photographed with a digital camera (Olympus C-5060) attached to a photomicroscope (Olympus BX51, Tokyo, Japan). Based on pre-determined criteria, gastric injury was evaluated semiquantitatively (*0, None; 1, Mild; 2, Moderate; 3, Severe)*: desquamation of surface epithelium (0-3), hemorrhage, focal necrosis and mucosal congestion (0-3) and degeneration of glandular cells (0-3), Inflammatory cell infiltration (0-3) with a maximum score of 12 [28].

Statistical analysis

All data are expressed as means ± S.E. The analysis was performed using GraphPad Prism 6.0 software. Groups of data were compared with one-way ANOVA, Mann–Whitney U-test and Student's t-test. The results were considered significant when p values were less than 0.05.

Results

Effects of exercise on the anxiety and serum cortisol levels

Ulcer induction had no effect on the head-dipping behavior of the sedentary rats. Compared to sedentary control group, number of head-dipping on the hole-board test was increased in pre-exercised control rats (Fig. 2a), suggesting a reduction in anxiety level. Similarly, head-dipping number was higher in the pre-exercised rats with 3-day ulcer as compared to respective sedentary rats ($p<0.05$), but it was lower (increased anxiety) with respect to pre-exercised non-ulcer group ($p<0.05$). However, on the 10th day of ulcer, anxiety levels of both the sedentary and pre-exercised groups were not different from that of the sedentary control group. No significant difference was observed among the serum cortisol levels of the sedentary or pre-exercised rats with either ulcer induction or sham operation (Fig. 2b).

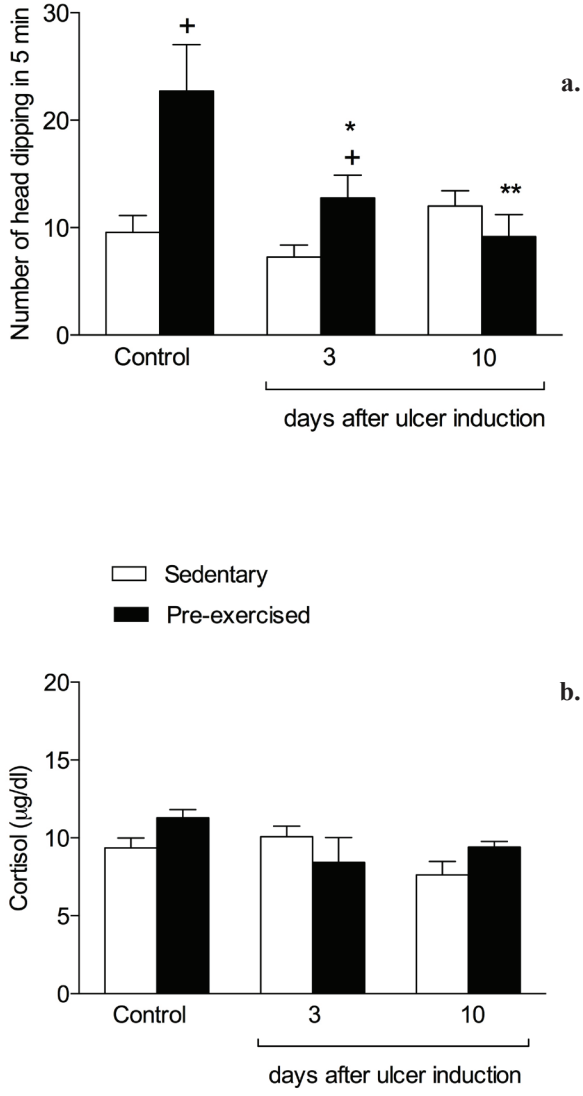

Fig. 2. a) Number of head-dipping behavior on the hole-board test, and b) serum cortisol levels of rats that have exercised or left sedentary before they had surgery for sham-operation (control) or acetic acid application. The rats were decapitated on the 3rd or 10th day of surgery. *$p<0.05$; **$p<0.01$, compared to respective control group; + $p<0.05$, compared to respective sedentary group.

Effects of exercise on ulcer-induced alterations in oxidative parameters

Among the sedentary rats, gastric MDA levels indicative of lipid peroxidation were not different in the acetic acid-applied

or sham-operated groups (Fig. 3a). On the other hand, having exercised before the sham-operation or acetic acid application significantly depressed the gastric MDA levels ($p<0.05$-0.01). MPO activity in the gastric tissue, representing neutrophil recruitment, was significantly elevated on the 3rd day of ulcer induction ($p<0.001$), but the increase in the MPO activity of the 10-day ulcer group was not statistically significant (Fig. 3b). Although exercise suppressed ulcer-induced elevation in gastric MPO activity ($p<0.01$), the measured MPO activities of both ulcer groups were still higher than that of the control group. Intracellular antioxidant GSH level was significantly diminished in the gastric tissues of exercised control rats as compared to that of the non-exercised controls ($p<0.05$; Fig 3c). Reduced GSH in the 3-day ulcer group ($p<0.001$) was preserved when the rats have previously exercised ($p<0.05$). Either sedentary or exercised, gastric GSH levels measured on the 10th day of ulcer were not different than their respective control values.

Gastric luminol CL level, indicative of ROS generation, was elevated in the pre-exercised control rats as compared to that of the non-exercised control group ($p<0.001$; Fig. 4a). Luminol CL in the sedentary ulcer group was increased on the 10th day ($p<0.01$), but not on the 3rd day; while having exercised before ulcer induction had no additional impact on luminol CL. Similar to luminol CL, lucigenin CL level was also elevated in exercised control rats with respect to sedentary group ($p<0.01$; Fig. 4b). Lucigenin CL in the sedentary ulcer groups was elevated on both the 3rd and 10th days of ulcer, while having exercised before ulcer induction depressed the CL level back to control level only in the 3-day ulcer group. As observed in the luminol and lucigenin CL levels, NO level in the exercised control group was significantly greater than that of the sedentary control group ($p<0.05$; Fig.4c). This exercise-induced elevation of NO was also evident in the 3-day ulcer group ($p<0.01$), but not in the 10-day ulcer group. On the other hand, ulcer induction had no effect on the gastric NO levels of the non-exercised rats.

Microscopic examination of the gastric tissues of pre-exercised and sedentary control groups revealed regular mucosal and submucosal layers. In the sedentary 3-day ulcer group, severe damage of surface mucous and glandular epithelium with maximum inflammatory cell infiltration and mucosal hemorrhage were evident (score: 12±0.0). On the other hand, in the gastric tissues of 3-day ulcer group that has pre-exercised, mild damage of surface mucous cells, moderate damage of glandular epithelium accompanied with moderate level of inflammatory cell infiltration and vascular congestion were noticed (score: 10.0±0.3; $p<0.05$). In the

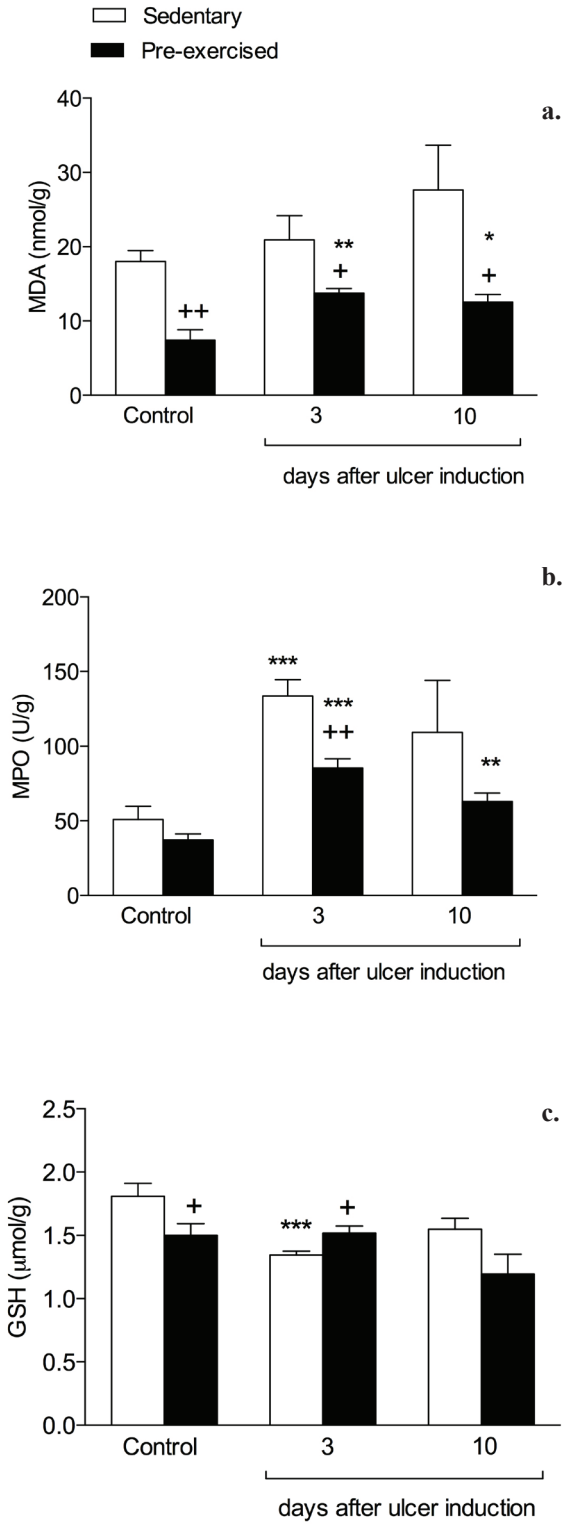

Fig. 3. a) Malondialdehyde (MDA) levels, b) myeloperoxidase (MPO) activity and c) glutathione (GSH) levels in the gastric tissues of rats that have exercised or left sedentary before they had surgery for sham-operation (control) or acetic acid application. The rats were decapitated on the 3rd or 10th day of surgery. *$p<0.05$; **$p<0.01$, ***$p<0.001$, compared to respective control group; + $p<0.05$, ++$p<0.01$, compared to respective sedentary group.

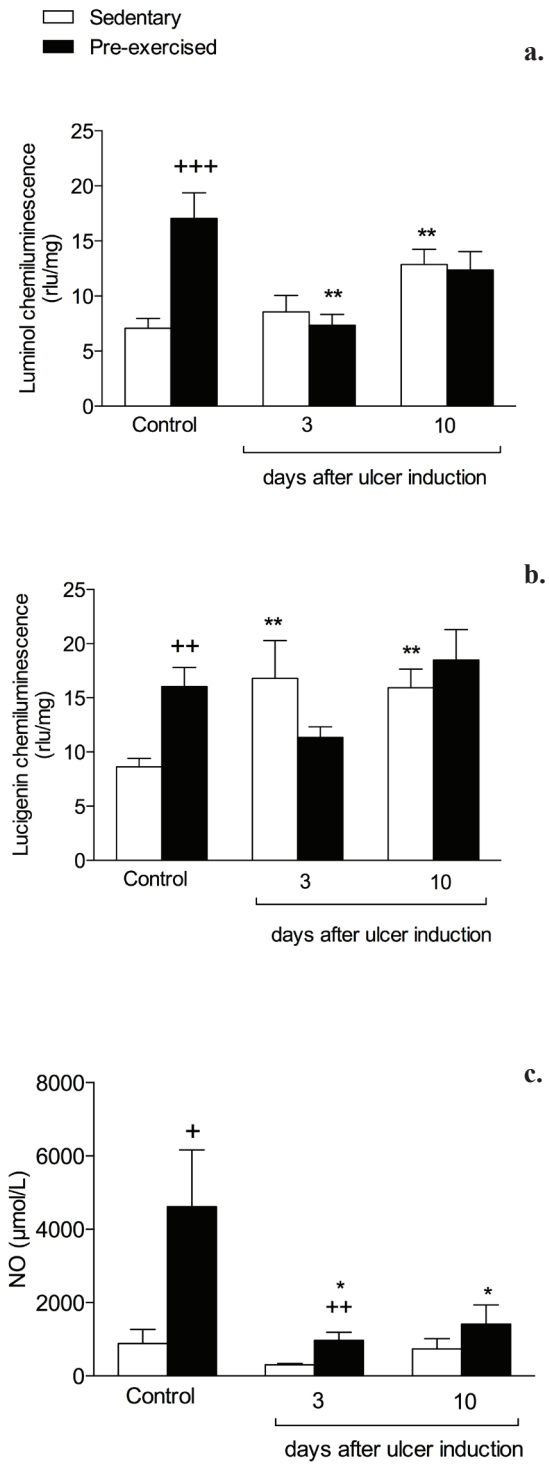

Fig. 4. a) Luminol- and b) lucigenin-enhanced chemiluminescence (relative light unit (rlu)/mg tissue), and c) nitric oxide (NO) levels in the gastric tissues of rats that have exercised or left sedentary before they had surgery for sham-operation (control) or acetic acid application. The rats were decapitated on the 3rd or 10th day of surgery. *p<0.05; **p <0.01, compared to respective control group; + p<0.05, ++p<0.01, +++p<0.001, compared to respective sedentary group.

sedentary 10-day ulcer group, quite regular surface mucous cells, mild degeneration of glandular epithelial cells and moderate inflammatory cell infiltration were observed (score: 5.1±1.2), demonstrating that gastric damage was significantly reduced on the 10th day of ulcer as compared to the 3-day ulcer group (p<0.001). Similarly, quite regular surface mucous and glandular epithelium with mild inflammatory cell infiltration was present in the pre-exercised 10-day ulcer group (score: 4.9 ± 2.4) presenting a better morphology, but no significant difference was observed between the scores of the 10-day ulcer groups (Fig. 5).

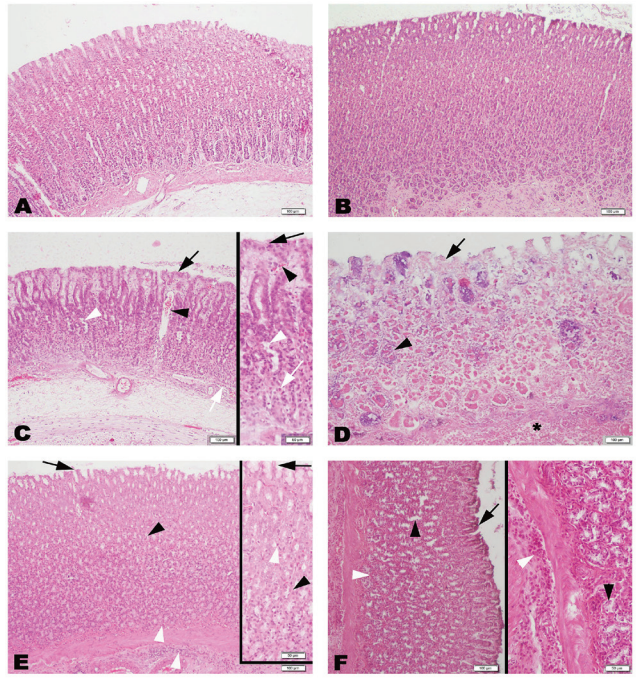

Fig. 5. Micrographs illustrating the histological appearances of gastric tissues in different experimental groups. Regular gastric surface mucous epithelium and glandular cells are observed in the pre-exercised **(A)** and sedentary **(B)** control groups. Regular surface mucous cells (black arrow), dilatation of glandular epithelium and moderate degeneration of glandular epithelial cells (white arrowhead), vascular congestion (black arrowhead) and moderately increased inflammatory cell infiltration (white arrow) are present in the pre-exercised 3-day ulcer group **(C).** Severe degeneration of surface mucous cells (arrow), glandular epithelium (arrowhead), mucosal hemorrhage and inflammatory cell infiltration (*) are seen in the sedentary 3-day ulcer group **(D).** Regular surface mucous cells (arrow) and glandular epithelium (black arrowhead), a few inflammatory cells (white arrowhead) in the lamina propria and submucosa are observed in the pre-exercised 10-day ulcer group **(E).** Regular surface mucous cells (arrow), moderate dilatation of glandular epithelium and moderate degeneration of glandular epithelial cells (black arrowhead) and moderately increased inflammatory cells (white arrowhead) in the lamina propria and submucosa are seen in the sedentary 10-day ulcer group **(F).** H&E staining.

Discussion

Regarding the lack of concrete mechanisms that can explain the beneficial effects of physical activity on the development and healing of gastric ulcer, as suggested by the epidemiological studies, the present study was aimed to elucidate the stress-relieving and anti-inflammatory effects of a 6-week swimming exercise on acetic acid-induced ulcer in rats. In the absence of gastric ulcer, exercising for 6 weeks reduced MDA as well as GSH levels along with elevations in the ROS and NO levels detected by CL. With the development of ulcer, MPO activity and superoxide level were elevated and gastric GSH stores were depleted in sedentary rats, but these oxidative changes were reversed when the rats have previously exercised. Similarly, severe damage of the ulcerated gastric tissue was also improved in the pre-exercised group. Although gastric ulceration did not affect the anxiety levels of the rats, a 6-week exercise before the induction of ulcer or sham-operation demonstrated an anxiolytic action. These data suggest that swimming exercise protected the gastric tissue against the oxidative damage, by reducing neutrophil infiltration and ROS generation, and by maintaining the antioxidant status, while the anxiolytic effect of exercise accompanied these anti-inflammatory mechanisms.

Regular physical activity is well known to support both physical and psychological health and reduce the risk of many chronic diseases with its beneficial effects on different systems [19, 29]. Several studies suggest an opposite relationship between physical activity and the chance of developing gastrointestinal disorders [30], such as colon cancer, diverticular disease [31], cholelithiasis [32], or constipation [32]. Although acute strenuous exercise has adverse effects on gastric mucosa [33], moderate exercise was suggested to have beneficial effects through several biologic mechanisms [34]. Exercise at a moderate intensity performed regularly was proposed to protect against infections by altering the activity of macrophages, neutrophils, and cytokines [35], while acute strenuous exercise may cause a short-lived reduction in the immune response, increasing the risk of infections [36]. In patients with duodenal ulcer, combining the conventional antiulcer treatment with bicycle exercise has accelerated the healing of ulcer [37]. Similarly, we have previously shown in rats that colonic damage induced by acetic acid was improved by regular exercise [38]. The current data also demonstrate that exercising regularly prior to the occurrence of ulcer has a protective and a healing-accelerating effect on acetic acid-induced oxidative damage.

Meta-analysis of outcome variables following exercise interventions has demonstrated that moderate-to-high intensities of exercise have greater therapeutic effects on anxiety as compared to low intensities [39]. The anxiolytic potential of exercise was also shown in rats performing voluntary wheel running [11]. Since rats have an instinctive ability for swimming, and the application of swimming method has advantage over treadmill running, we have preferred swimming exercise by expecting similar anxiolytic effects on the psychological state of the rats [8, 18]. The results revealed that swimming performance in rats without ulcer resulted in increased head-dipping behavior on the hole-board, indicative of an anxiolytic-like condition [40]. Despite that ulcer induction did not increase the anxiety level, the anxiolytic effect of exercise was still present following a 3-day ulcer, but it was abolished on the 10^{th} day. The results suggest that having exercised before ulcer development diminished the anxiety behavior, which would otherwise result in additional inflammatory effects on the acutely ulcerated gastric mucosa. Exercise-induced anxiolytic effect has disappeared on the 10^{th} day following the ulcer induction, because exercise sessions have ended during the post-ulcer period.

Although corticosteroids were indicated as ulcerogenic hormones in some studies [41], other studies have attributed a modulatory role for endogenous glucocorticoids in protecting against stress- or aspirin-induced gastric damage [42, 43]. Physical exercise, as a powerful stressor with physical, psychological, and physiological effects, is proposed to have a modifying role on the circulating levels of cortisol [44, 45]. Studies have shown that moderate to high intensity exercise increases circulating levels of cortisol, while low intensity exercise may even result in reduced cortisol levels [46]. However, in the present study, no significant differences were observed among the serum cortisol levels of the experimental groups that have exercised or have remained sedentary, suggesting that neither anxiolytic exercise sessions nor the ulcer induction had an impact on cortisol secretion. It appears that the moderate intensity swimming exercise in the present study, which was terminated by ulcer induction or sham-operation, did not have a prolonged effect on the serum cortisol level. Thus, circulating levels of cortisol are not involved in the gastroprotection afforded by exercise in the present study.

In physiological conditions, endothelial NO in the gastric tissue is well known to dilate the blood vessels, thereby increases gastric mucosal blood flow [47] and keeps the gastric mucosal barrier intact [48], while its depletion has an inhibitory effect on the maintenance of gastric mucosal integrity [49]. Although diminished blood flow to gastric mucosa during extensive exercise sessions may result in the breakdown of mucosal integrity [30,50], transient changes

in the gastric mucosal circulation by moderate exercise may have an adaptive preconditioning effect on the mucosal barrier [51] have demonstrated that regular physical exercise results in the up-regulation of endothelial NO synthase gene, and a positive correlation exists between exercise intensity and NO levels. Accordingly, in our study, elevated gastric NO levels in the exercised rats may be involved in the exercise-induced gastroprotection through the vasodilatory effect of NO.

Regular physical exercise was proven to decrease the incidence of diseases related with oxidative stress [13], which is one of the major contributors to ulcerogenesis [52]. This preventive effect of exercise was associated with a systemic adaptive response to repetitive oxidative challenges that involve increased levels of ROS in the skeletal muscle, liver and brain [13], but no study so far has investigated the contribution of ROS in exercise-induced protection against gastric ulcer. In the skeletal muscle, ROS were shown to act as messenger molecules in triggering adaptive homeostatic responses to keep the oxidant/antioxidant balance by enhancing the gene expression of antioxidant defense systems [15]. Although the 6-week exercise in the present study has resulted in depletion of gastric GSH levels of the control animals, ulcer-induced reduction in gastric GSH content was maintained when the rats have previously exercised. This exercise-induced preservation of gastric GSH content was also accompanied by significant decreases in the extent of microscopic damage and the levels of lipid peroxidation and superoxide radical generation, verifying that exercise that was performed earlier provides protection against ROS-induced gastric damage. Moreover, increased accumulation of neutrophils to the ulcerated gastric mucosa was also depressed when the rats have exercised before, suggesting that repetitive inflammation with exercise leads to a preconditioning and increases the endurance for a new physicochemical challenge via the inhibition of neutrophil accumulation. In another model of oxidative damage induced by psychological stress, we have also shown that swimming exercise suppressed MPO and MDA levels and elevated GSH levels in several organs of the stressed rats, including stomach [18].

There is extensive evidence verifying the therapeutic potential of habitual exercise as a lifestyle intervention for a life-long protection by lowering the risk of cardiovascular disease [53]. The findings of the current study suggest that regular moderate exercise, through its repetitious inflammatory nature, prepares the gastric mucosa for a forthcoming injurious event. By introducing post-ulcer exercise regimens, additional research targeting the effects of physical activity on gastric ulcer healing is needed to further elucidate the gastroprotective potential of exercise.

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