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Original article

High activity of endogenous opioid system protects against gastric damage development in mouse models of gastric mucosal injury

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ABSTRACT

Background: Gastric mucosal injury appears when acid and pepsin production, simultaneously with inadequate mucosal response, overwhelms protective mechanism in stomach. Here we aimed to explore the linkage between gastric lesion formation and endogenous opioid system activity.
Methods: Two mouse lines bidirectionally selected for high (HA) and low (LA) swim stress-induced analgesia associated with high and low endogenous opioid system activity were used. Gastric mucosal injury was induced by ethanol (EtOH) and chronic mild stress. To investigate the anti-inflammatory effect of the endogenous opioid system macroscopic score, myeloperoxidase (MPO) activity, the expression of inflammatory molecules as well as oxidative stress markers were determined. Moreover, expression of opioid receptors μ (MOR), κ (KOR) and δ (DOR) at mRNA levels were determined in gastric tissue.
Results: High activity of the endogenous opioid system alleviated gastric lesions development in the EtOH-and chronic mild stress-induced mouse gastric mucosal injury models, as demonstrated by decreased macroscopic score in HA line compared to LA. Additionally, antioxidative stress defence mechanisms were positively modulated in both models of gastric mucosal injury. MOR and partially KOR receptors may be responsible for the gastroprotective effect.
Conclusion: To our knowledge this is the first study to show that increased activity of the endogenous opioid system prevents from gastric lesion formation by influencing – among others – the anti-inflammatory and anti-oxidant mechanisms in the mice stomach. Hence, we suggest that opioids may play an important role in gastric mucosal injury prevention.

Introduction

Gastric mucosal lesion, one of the most prevalent findings during upper gastrointestinal (GI) endoscopy, are characterized predominantly by dysregulation in acid and pepsin production and insufficient mucosal restoration leading to the development of gastric erosions and lesions. In the recent years, several additional factors have been associated with the development of gastric lesions, such as alterations in mucus-bicarbonate layer and gastric mucosal blood flow as well as impairment of the opioid system activity [1–4]. Endogenous opioid system may influence gastric mucosal lesion development through modulation of gastric acid secretion volume, acidity as well as pepsin output and gastric mucus production. Importantly, reports suggest that both central and peripheral opioid receptors may be responsible for inhibition of gastric secretion, whereas mucus synthesis may only be modulated by peripheral opioid receptors [5].

Current treatment of gastric mucosal lesion, including pump inhibitors and H_2-receptors antagonists, depends among others on inhibition of gastric acid production. These drugs, while often effective, possess many adverse effects [6]. Thus, focusing on new therapeutic targets such as endogenous opioid system may lead to better handling of gastric mucosal lesion.

Endogenous opioid system is composed of opioid peptides and cell surface receptors. Opioid peptides, namely β-endorphin, enkephalins, endomorphins [7] and dynorphins acts through opioid receptors (ORs), which are divided into three subtypes μ (MOR), δ (DOR), κ (KOR) [8]. In the human body ORs are

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distributed in the central and peripheral nervous system and non-neuronal tissues, mainly in the GI tract, where they can be found on smooth muscle cells, at the terminals of sympathetic and sensory peripheral neurons, and immune cells. In the GI tract ORs are involved in modulation of intestinal motility, fluid and electrolyte absorption and secretion as well as immune response [8,9]. Recent reports suggest that the endogenous opioid system may play a role in inflammatory response in inflammatory bowel disease as well as gastric mucosal lesion formation [4,8]. The role of opioids were investigated in several ulcer models and both protection and aggravation were described [4]. Nevertheless, the linkage between gastric mucosal lesion formation and endogenous opioid system is still poorly understood.

Our aim was to characterize the impact of increased activation of the endogenous opioid system on the development of gastric mucosal lesion. In the study we employed a unique mouse model of high and low activity of the endogenous opioid system (namely high (HA)/low (LA) stress-induced analgesia mice), which was previously validated for research on opioids in the GI tract [10,11]. To test our hypothesis, we investigated the role of opioids in two mouse models of gastric mucosal injury mimicking the gastric mucosal lesion formation in human, ethanol (EtOH)- and chronic mild stress- induced gastric lesion formation. In our study we assessed clinical parameters for gastroprotection, such as gastric lesion area. In order to elucidate the mechanism of gastroprotective action, inflammatory cytokines, such as TNF-α and IL-1β as well as oxidative stress defence mechanism, catalase and SOD activity and H_2O_2 were assessed in gastric tissues.

Materials and methods

Animals

Male Swiss-Webster mice (32–35 g) were selectively bred for 78 generations for high (the HA line) and low (the LA line) swim stress-induced analgesia (SSIA). At the origin, outbred Swiss-Webster mice of either sex, 2 min after completion of 3 min swimming in 20 °C water, were screened for the latency of a nociceptive reflex on a hot plate at 56 °C. Those displaying the longest (50–60 s) and the shortest (5–10 s) post-swim latencies of the hindpaw flick or lick response (whichever occurred first) were selected as progenitors of the HA and the LA lines. Similar procedure was repeated in each offspring generation, but only subjects displaying the longest and the shortest post-swim hot plate latencies, respectively, were mated to maintain the lines.

Mice were maintained under a 12-h light/dark cycle in groups, five per cage, at a constant temperature (22–23 °C) and 55 ± 5% relative humidity, in sawdust-lined plastic cages with free access to chow pellets and tap water ad libitum.

The study was carried out in strict accordance with the institutional recommendations. The protocol was approved by the Local Ethical Committee for Animal Experiments (Protocol #17/LB654/2013).

Mouse model of EtOH-induced gastric mucosal lesions

The experiment was performed according to the well-established protocol [1]. Mice (10 per group) were randomly divided in groups and fasted for 12 h before experiment but had access to water ad libitum. Then, all mice from EtOH group received 80% solution of EtOH to induce gastric lesions. After thirty minutes, the animals were sacrificed; the stomachs were removed, opened along the greater curvature, gently rinsed with PBS to remove the gastric contents and blood clots, and then photographed. The quantification of the ulceration induced by EtOH was performed in a blinded manner. The gastric mucosal lesion index, expressed as a percentage of damaged area in relation to the area of the corpus of the stomach was calculated using the ImageJ software (National Institutes of Health, Washington D.C., USA).

Mouse model of stress-induced gastric mucosal lesions

The experiment was performed according to the method described by Nassar et al. with some modifications [12]. In the chronic mild stress model animals (10 per group) were subjected over five weeks to various kinds of stressors, which were changed in 12 h cycles. Each week the stress regimen consisted of: two periods of food deprivation (8 h), two periods of 45° cage tilt (12 h), one period of soiled cage (200 ml water in sawdust bedding, 12 h), two periods of paired housing (1 h), two periods of low-intensity stroboscopic illumination (8 h), two periods of overnight illumination, one period of removed bedding (12 h), one period of noise emitted by a radio receiver tuned out of the station (white noise combined with cage tilt, 12 h), one period of restraint in a plastic tube 11.5 cm long and 3 cm in diameter (15 min), and two periods of no stress (12 h). The paired-housing stress consisted of exposing one mouse to another stressed mouse of the same line. After 5 weeks, when mice developed gastric mucosal lesion, the animals were sacrificed. The quantification of the gastric mucosal lesions induced by stress was performed in the same manner as in EtOH-induced gastric mucosal lesion model.

Myeloperoxidase (MPO) activity assay

The activity of MPO (expressed mainly in neutrophil granulocytes) is linked to immune cell infiltration in inflamed tissue. To evaluate the severity of gastric inflammation, the sections from corpus of stomach (15–25 mg) were isolated, washed using PBS and homogenized in hexadecyltrimethylammonium bromide (HTAB) buffer (0.5% HTAB in 50 mM potassium phosphate buffer, pH 6.0; 50 mg of tissue/ml) using Ika Ultra Turrax Disperser T25 Digital 2 (Sigma Aldrich, Poznań, Poland). Then, homogenates were centrifuged (15 min, 13 200 x g, 4 °C) and supernatants were transferred to other test tubes. Seven μl of supernatant was added on a 96-well plate, followed by 200 μl of 50 mM potassium phosphate buffer (pH 6.0), containing 0.167 mg/ml of O-dianisidine hydrochloride and 0.05 μl of 1% hydrogen peroxide. Each sample was prepared in triplicate. Absorbance was measured at 450 nm after 30 and 60 s (iMARK Microplate Reader, Biorad, UK).

MPO activity was expressed in milliunits per gram of wet tissue, 1 unit defined as the quantity of enzyme needed to convert 1 μmol of hydrogen peroxide to water in 1 min at room temperature. To calculate the units of MPO activity per 1 min, a standard curve using purified peroxidase enzyme was performed.

Determination of TNF-α and IL-1β levels by western blotting

Gastric tissue (10–15 mg) were isolated, rinsed with PBS and kept at −80 °C until further analysis. The tissue homogenates were prepared according to a standard protocol [1]. Primary antibodies: mouse monoclonal TNF-α (1:1000; sc-133192 C-4; Santa Cruz Biotechnology, Santa Cruz, CA, USA), mouse monoclonal IL-1β (1:1000; cs-32294; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and glycerylaldehyde-3-phosphate dehydrogenase (GAPDH; 1:15 000; MAB374; Merck Millipore, Warszawa, Poland) as reference protein, diluted in 1% non-fat dry milk in PBS with Tween 20 (0.1% m/v) were used to probe the membranes. Appropriate horseradish-peroxidase (HRP)-conjugated secondary antibody at a concentration of 1:6000 was applied and then bands were visualized using Super Signal West Pico western blotting substrate (Thermo Scientific, Rockford, IL, USA) as a substrate for the localization of HRP activity. Qualitative and quantitative

analysis was performed by measuring integrated optical density (IOD) by ImageLab v.5.2.1 for WindowsTM program (Bio-Rad SA, Warszawa, Poland). For determination of protein weight, we have used 5 μl/lane of Precision Plus Protein Standards (Bio-Rad SA, Warszawa, Poland).

Determination of H_2O_2

The assay was performed according to the methodology described by Salaga et al. [13]. Briefly, 50 mg of gastric tissue fragments were homogenized with 2 ml of 1.15% potassium chloride. Aliquots of tissue homogenates (10 μl) were mixed with 90 μl of PBS (pH 7.0) and 100 μl of horseradish peroxidase (1 U/ml) supplemented with 400 μmol of homovanillic acid (HRP + HVA solution) or 90 μl of PBS and 100 μl of 1 U/ml horseradish peroxidase (HRP solution). Homogenates were incubated for 60 min at 37 °C. Subsequently, 300 μl of PBS and 125 μl of 0.1 M glycine-NaOH buffer (pH 12.0) with 25 mM EDTA were added to each homogenate sample. Excitation was set at 312 nm and emission was measured at 420 nm (Perkin Elmer Luminescence Spectrometer, Beaconsfield, UK). Readings were converted into H_2O_2 concentration using the regression equation: Y = 0.012X-0.007, where Y=H_2O_2 concentration in homogenate (μM); X = intensity of light emission at 420 nm for HRP + HVA solution reduced by HRP solution emission (arbitrary units, AU). The regression equation was prepared from three series of calibration experiments with 10 increasing H_2O_2 concentrations (range 10–1000 μM).

Determination of catalase (CAT) activity

CAT activity in the gastric tissue was determined using the Catalase Assay Kit (Cayman Chemicals, Ann Arbor, MI, USA). Briefly, tissue samples were homogenized in ice-cold buffer containing 50 mM potassium phosphate, pH = 7.0 and 1 mM EDTA per gram of tissue. Homogenates were centrifuged at 10 000 *g* for 15 min at 4 °C. Supernatants were put on ice and underwent the procedure described in the manufacturer's protocol. Absorbance was measured at 540 nm (iMARK Microplate Reader, Biorad, UK). All measurements were performed in triplicate. CAT activity was expressed in milliunits per gram of wet tissue, with 1 unit being the amount of enzyme that causes the formation of 1.0 nmol of formaldehyde per minute at room temperature. Units of CAT activity were calculated from a standard curve using purified bovine liver CAT enzyme.

Determination of superoxide dismutase (SOD) activity

Superoxide Dismutase Assay Kit (Cayman Chemicals, Ann Arbor, MI, USA) was used to determine SOD activity in the gastric tissue. Briefly, tissue samples were homogenized in cold 20 mM HEPES buffer, pH = 7.2, containing 1 mM ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA), 210 mM mannitol and 70 mM sucrose per gram of tissue and centrifuged at 1500 *g* for 5 min at 4 °C. Supernatants were collected and underwent the procedure described in the manufacturer's protocol. Absorbance was measured at 450 nm (iMARK Microplate Reader, Biorad, UK). All measurements were performed in triplicate. SOD activity was expressed in milliunits per gram of wet tissue, with 1 unit being the quantity of enzyme needed for 50% dismutation of the superoxide radical at room temperature. Units of SOD activity were calculated from a standard curve using purified bovine erythrocyte SOD enzyme.

mRNA analysis

RNA was isolated from the sections of stomach (weighing 20–30 mg) according to manufacturer's protocol using Total RNA Mini Plus kit (A&A Biotechnology, Gdańsk, Poland). RNA was eluted from ion exchange columns by diethyl pyrocarbonate (DEPC)-treated water (40 μl). The purity and quantity of isolated RNA, was

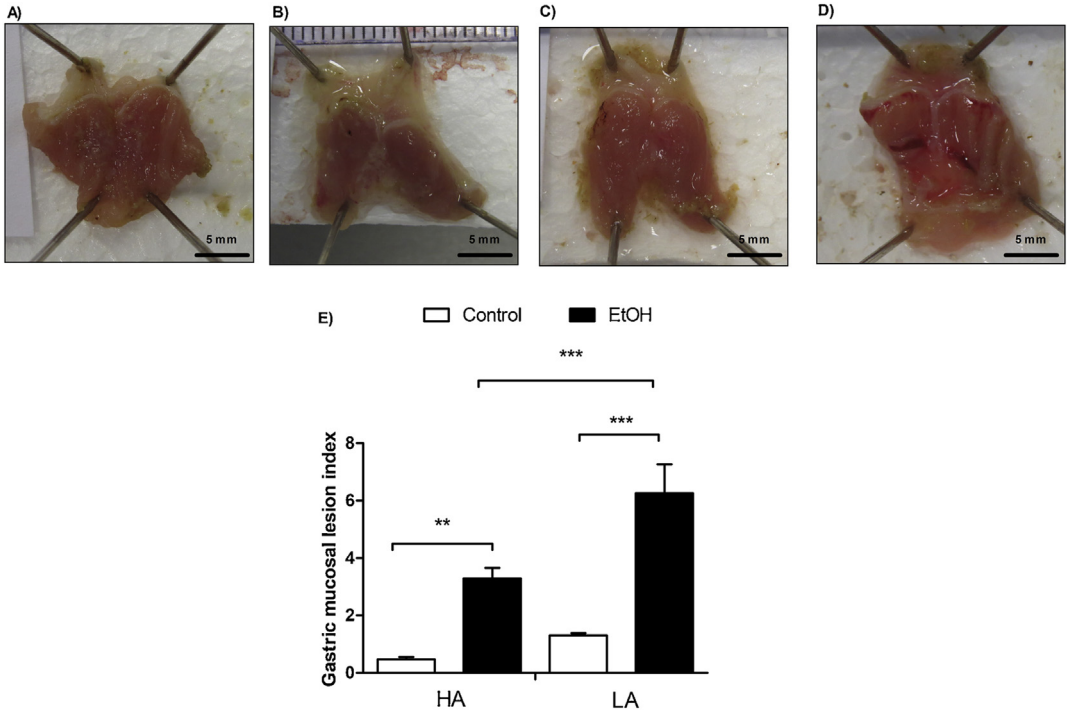

Fig. 1. Macroscopic score and representative images of the mouse stomach from (A) Control group HA line, (B) EtOH treated-group HA line, (C) Control group LA line, (D) EtOH treated-group LA line. The differences in the development of EtOH-induced model of gastric lesions between HA and LA mouse lines observed in macroscopic score ** $p < 0.01$ and *** $p < 0.001$, respectively. Scale bar: 5 mm. Data represent mean ± SEM of 10 mice per group.

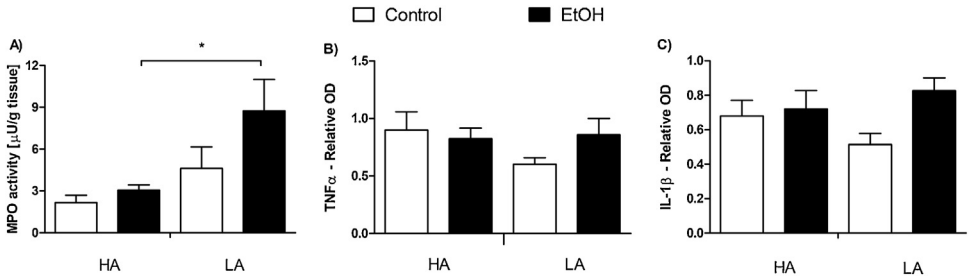

Fig. 2. A) The differences in the MPO activity in EtOH-induced model of gastric lesions between HA and LA mouse. B) Quantitative analysis by Western blot of expression of TNF-α and IL-1β (C) in specimens from the stomach collected from control and EtOH-treated mice of HA and LA strain. * $p < 0.05$. Relative OD – relative optical density. Data represent mean±SEM of 6–8 samples per group (1 sample/animal).

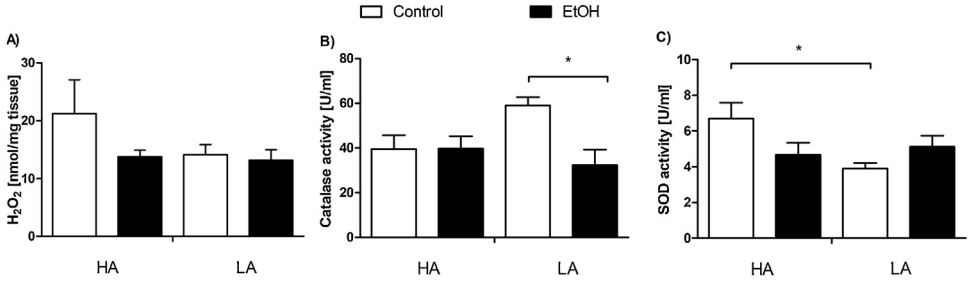

Fig. 3. The effect of increased activation of the endogenous opioid system on antioxidant mechanism in the mouse stomach in EtOH-induced gastric mucosal injury model. Figure shows differences in A) H_2O_2 levels, B) CAT activity and C) SOD activity between HA and LA mouse. In HA mice nonsignificant reduction in H_2O_2 levels as well as SOD activity were observed. Data represent mean±SEM of 6–8 samples per group (1 sample/animal), * $p<0.05$.

measured using Colibri Microvolume Spectrophotometer (Biocompare, San Francisco, CA, USA). Total RNA (2 μg) was transcribed onto cDNA with high capacity Reverse Transcriptase Kit (Life Technologies, Carlsbad, CA, USA). Quantitative analysis was performed using fluorescently labeled TaqMan probes MOR (Mm01188089_m1), KOR (Mm01230885_m1), DOR (Mm01180757_m1), and GAPDH (Mm99999915_g1) as endogenous control (Life Technologies, Carlsbad, CA, USA) on Mastercycler S realplex 4 apparatus (Eppendorf, Hamburg, Germany) and TaqMan Gene Expression Master Mix (Life Technologies, Carlsbad, CA, USA) in accordance with manufacturer's protocol. All experiments were performed in triplicate. The Ct (threshold cycle) values for studied genes were normalized to Ct values obtained for GADPH. Relative amount of mRNA copies was calculated using the following equation: $2\text{-}\Delta Ct \times 1000$.

Statistical analysis

Statistical analysis was performed using Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA). The data are expressed as means ± SEM. One-way ANOVA followed by Newman-Keuls *post-hoc* test was used for analysis. p Values < 0.05 were considered statistically significant.

Results

High activity of endogenous opioid system has gastroprotective and anti-inflammatory effect in the EtOH-induced gastric mucosal injury model in mice

To test the possible gastroprotective effect of the endogenous opioid system, we used a mouse model of EtOH-induced gastric mucosal injury. Our previous research demonstrated that administration of 80% solution of EtOH results in reproducible gastric lesions [1].

In the current study, in both lines HA and LA administration of EtOH induced gastric lesions, manifested by a significant increase in gastric mucosal lesion index. Of note, LA mice developed more severe gastric mucosal lesion, demonstrated by a 1.5-fold increase in gastric mucosal lesion index ($p < 0.001$) (Fig. 1).

Basal activity of MPO tended to be increased in LA *vs.* HA mice, but the difference was not significant (Fig. 2A). Administration of EtOH resulted in a non-significant increase in MPO activity in LA but not HA line compared to respective non-treated animals. Nevertheless, a statistically significant difference between HA and LA lines after EtOH administration was observed in MPO activity (Fig. 2A).

Interestingly, basal levels of TNF-α and IL-1β tended to be increased in HA line in comparison to LA line (Fig. 2B and C). Administration of EtOH in HA line did not result in an increase in TNF-α or IL-1β. Importantly, in LA mice, tendency to increase in those pro-inflammatory cytokines can be observed, however it was no significant (Fig. 2B and C).

High activity of endogenous opioid system modulates the oxidative-stress defence mechanism in the mouse stomach in the EtOH-induced gastric mucosal injury model in mice

Oral gavage of EtOH in HA but not LA line resulted in a non-significant decrease in H_2O_2 (Fig. 3A). In HA line no changes in CAT activity were observed, whereas in LA line CAT activity diminished after EtOH administration (Fig. 3B). As for SOD activity, there was a non-significant decrease after EtOH administration in HA but not LA mice (Fig. 3C). Importantly both H_2O_2 levels and SOD activity in control group were higher in HA than in LA line, whereas the values of H_2O_2 and SOD in ethanol treated groups did not differ in HA and LA lines.

Expression of opioid receptors is increased after damaging stimuli in HA but not LA line in the EtOH-induced gastric mucosal injury model in mice

Expression of MOR were more abundant than KOR and DOR in mouse gastric tissue of both lines. Basal expression levels of MOR

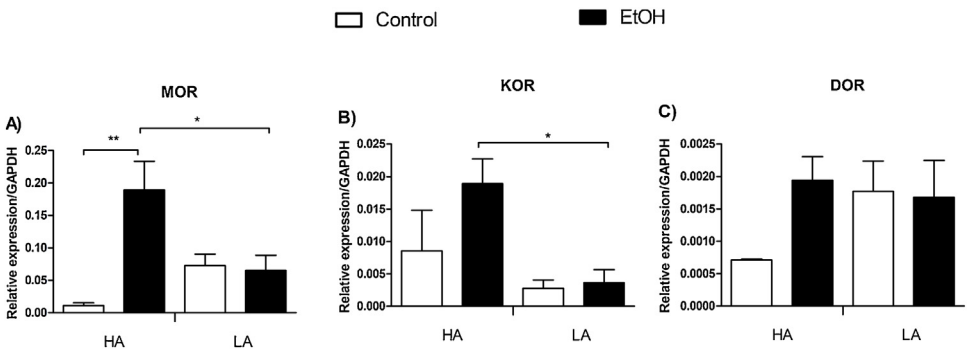

Fig. 4. Relative mRNA expression of MOR, DOR and KOR receptors in the stomach of control and EtOH-treated mice of HA and LA strain. * $p < 0.05$, ** $p < 0.01$. Data represent mean±SEM of 6–8 samples per group (1 sample/animal).

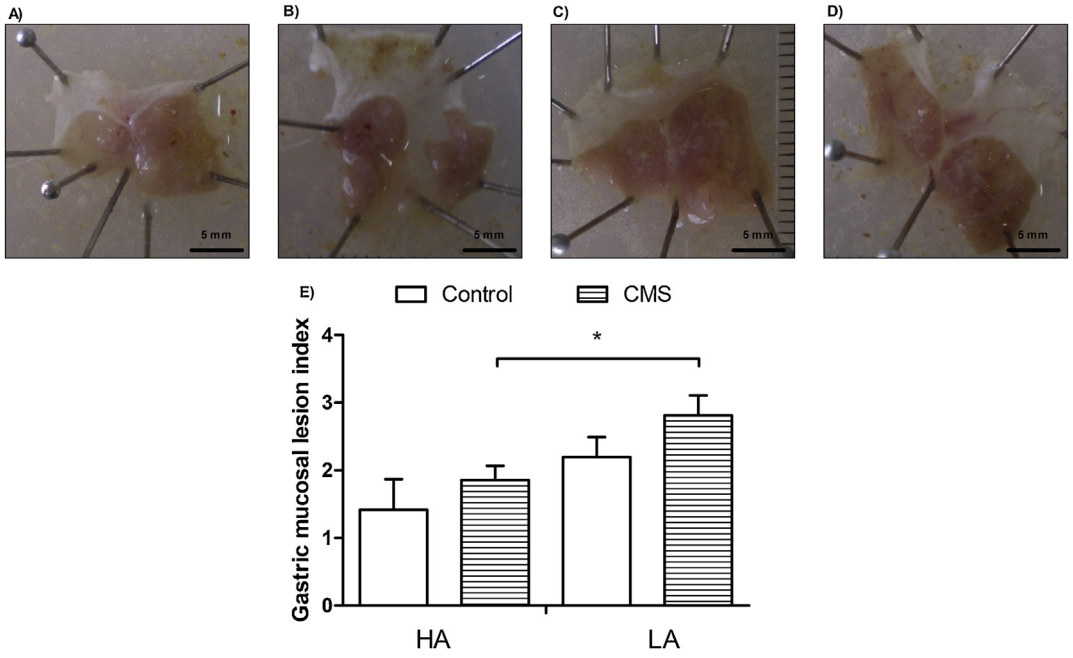

Fig. 5. Macroscopic score and representative images of the mouse stomach from (A) Control group HA line, (B) CMS group HA line, (C) Control group LA line, (D) CMS group LA line. The differences in the development of chronic mild stress (CMS)-induced gastric mucosal injury between HA and LA mouse lines observed in macroscopic score * $p < 0.05$. Scale bar: 5 mm. Data represent mean ± SEM of 10 mice per group.

and DOR tended to be increased in LA line in comparison to HA (Fig. 4A and C). KOR expression was non-significantly increased in HA line compared to LA line in control animals and a significant increase was observed in HA line compared to LA line following ethanol administration. Noteworthy, administration of EtOH resulted in a significant increase in expression of MOR and a non-significant increase in KOR and DOR in HA but not LA line, compared to control (Fig. 4).

High activity of endogenous opioid system alleviates gastric mucosal lesions formation in the chronic mild stress-induced gastric mucosal injury model in mice

In both lines chronic mild stress induced gastric lesions, manifested by an increased gastric mucosal lesion index. Notably, LA mice developed more severe gastric lesions, demonstrated by significantly increased gastric mucosal lesion index ($p < 0.05$) (Fig. 5).

Interestingly, adversely to EtOH-induced gastric mucosal injury model, no significant changes in MPO activity were observed (Fig. 6A).

Levels of H_2O_2, a marker of oxidative stress was decreased in chronic mild stress-group in comparison to control group in both lines (Fig. 6B).

Discussion

Numerous factors may play a role in gastric mucosa damage formation leading to local inflammatory state and ulceration of gastric wall. Stress, along with other aggressive factors like EtOH, *Helicobacter pylori* infection, NSAIDs and steroids, increase the possibility of gastric mucosal lesion formation [1,12]. In our study we investigated whether gastric lesions development is prevented by increased activation of the endogenous opioid system. Moreover, we also aimed to unravel the basic mechanism that may be involved in this antiulcerogenic effect. Consequently, we demonstrated that hyperactivation of the endogenous opioid system observed in HA line led to gastroprotective effect in two effective mouse models of gastric mucosal injury as evidenced by decrease in the gastric mucosal lesion area in the mouse stomach. Both anti-inflammatory and anti-oxidant defence mechanisms were involved.

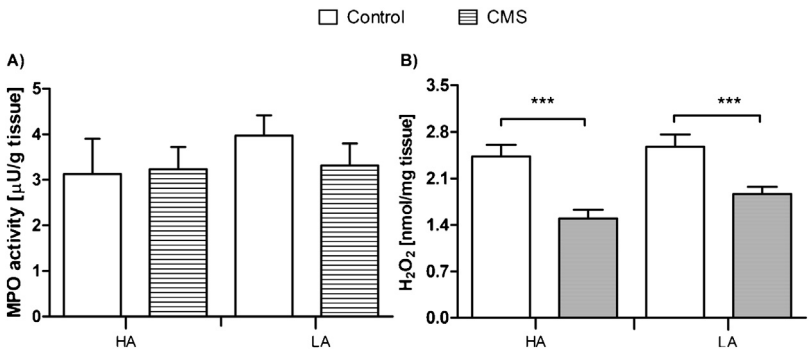

Fig. 6. A) The differences in the MPO activity in chronic mild stress (CMS)-induced gastric mucosal injury between HA and LA mouse. B) The effect of increased activation of the endogenous opioid system on H_2O_2 levels in the mouse stomach in CMS-induced gastric mucosal injury model. *** $p < 0.001$. Data represent mean ± SEM of 10 mice per group.

EtOH is a well-known risk factor for development of gastric lesions. Due to the ability to solubilize the protective mucus, it easily infiltrates the gastric mucosa and exposes the epithelium to pepsin and hydrochloric acid. EtOH also causes imbalances in antioxidant processes at cellular level, causing the release of superoxide anion and other ROS [14]. Additionally, EtOH produces necrotic lesions in the gastric mucosa of animals by a direct toxic effect thereby reducing the secretion of bicarbonates and dysregulating gastric mucus production. Importantly, the EtOH-induced gastric mucosal injury model is independent of gastric acid secretion and mimics acute mucosal injury in humans [15]. Summarizing, EtOH-induced mucosal injury model is useful for studying anti-gastric mucosal lesion drug candidates that have cytoprotective and anti-oxidant activities and thus has been chosen for this study.

Stress influences the mechanism of neurohormonal regulation, which results in stomach lesions [12]. Chronic mild stress is a state where animals are exposed to a combination of mild unpredictable stressors [16]. Several reports implicate the role of enhanced production of inflammatory mediators and reactive oxygen species in chronic mild stress-induced gastric mucosal injury model [17–21]. Stimulation of brain gut axis, reduction of mucosal blood glow and leucocyte infiltration are all associated with the pathogenesis of stress induced gastric mucosal lesion. Moreover, imbalance in the production of TNF-α is likely involved in gastric mucosal lesion formation *via* activation of caspase-3 dependent apoptotic pathway [18,22].

Finally, oxidative stress, depletion of antioxidants, neutrophil accumulation, increased numbers of inflammatory cytokines and reduced blood supply to the gastric mucosa have all been shown to be crucial in gastric mucosal injury formation. Reactive oxygen species (ROS), which are harmful to the gastric mucosa, are profusely produced in response to ulcerogenic agents administration, whereas the mucus layer and endogenous antioxidants are necessary in protecting against ROS-induced cytotoxicity [23]. Among ROS, superoxide, H_2O_2 and hydroxyl radicals are crucial in the tissue damage, whereas antioxidant enzymes, such as SOD, CAT and glutathione peroxidase (GPx), protect tissue against the toxic effects of ROS [24,25]. More specifically, SOD-produced H_2O_2 is converted to water by CAT or GPx. Furthermore, the level of GSH, the most abundant cell antioxidant, is sustained by its synthesis from cysteine and regeneration of oxidised glutathione (GSSG) [26]. Our results indicate that hyperactivation of endogenous opioid system may have modulatory role in cellular anti-ROS machinery in the stomach alleviating the gastric tissue damage induced by both EtOH and chronic mild stress. Our data suggest that increased activation of the endogenous opioid system after EtOH administration results in the tendency to decreased activity of SOD and thus to decreased production of hydrogen peroxide and related lesions. However, SOD also inactivates free radicals, thus decrease of SOD activity may lead to increase in the tissue damage through elevation of superoxide level. Accordingly, SOD reduced mucosal damage induced either by EtOH or aspirin [27]. On the other hand, gastroprotective agents may increase the activity of SOD in gastric mucosa, which may be responsible, at least partly, for their gastric mucosal protective action [28]. Further studies to fully elucidate potential impact of the endogenous opioid system on anti-ROS machinery are urgently needed as important for implementation in the treatment and prevention of upper GI disorders.

To further elucidate the involvement of ORs in gastroprotection we analysed MOR, KOR and DOR expression on mRNA levels. Of note, mobilization of MOR in mice with increased activity of the endogenous opioid system (HA line) were observed after administration of EtOH, as demonstrated by increased expression of those receptors in gastric tissue. Interestingly, in LA mice there was no increase in expression of ORs observed. Obtained data suggest that MOR are mainly responsible for gastroprotective effect in our experiments.

Previously, Tazi-Saad et al. tested the effect of morphine administration on gastric secretion, barrier mucus and mucosal lesions in pylorus-ligated model of gastric ulcers after administration of three different ulcerogenic agents: indomethacin, aspirin and taurocholic acid. Administration of morphine resulted in dose-dependent inhibition of the gastric acid secretion volume as well as pepsin output. Moreover, morphine increased barrier mucus levels as observed by increased amount of Alcian blue bound to the mucosa [29]. In a study performed by Gyires et al. MOR agonist [D-Ala^2, Phe^4, GlyT-ol] enkephlin (DAGO) attenuated the mucosal damage induced by both EtOH and indomethacin in picomolar dose range given centrally. Interestingly, the protective effect was reversed after bilateral cervical vagotomy in both ulcer models. Moreover, N^G-nitro-L-arginine, an inhibitor of NO synthase, reduced the protective effect in EtOH-induced gastric damage. Presented results suggest that activation of supraspinal ORs protects from gastric mucosal lesions development and that prostaglandins and nitric oxide are at least partially responsible for the protective action of opioid peptides in EtOH-induced gastric mucosal lesion model [4]. In study performed by Scoto et al. the effects if intraperitoneal and intracerebroventricular administration of MOR and DOR selective agonists (DAGO and DPDPE, respectively) on gastric lesions were investigated using cold-restraint-stressed rats. Both agonists, administered peripherally and centrally induced a significant gastric protection [30].

Finally, we used CMS-induced gastric lesion model to verify the observations made in the EtOH-induced model. Interestingly, our results indicate that increased activation of endogenous opioid system modulates gastric lesion formation in different manner in

ethanol and stress induced gastric damage, i.e. in acute and chronic conditions. Our results suggest that during acute phase of gastric lesion development represented by EtOH induced model the endogenous opioid system affects inflammatory response observed as decrease in MPO activation. In presence of chronic damaging stimuli the endogenous opioid system does not influence MPO activity, or modulates reactive oxygen species production in the gastric tissue.

Conclusion

This study is the first to show that increased activation of the endogenous opioid system prevents gastric lesion formation. The possible mechanism of action may involve enhancement of the anti-inflammatory pathways and defence mechanism against oxidative stress in the stomach. Thus, we suggest that the use of non-additive opioids and their derivatives might be beneficial for patients with gastric lesion, especially when gastric lesions are caused by EtOH or stress.

Author contributions

Study Design – HZ, MS, JF; Data Collection – HZ, MSal., MZ, AW, MSac.; Statistical Analysis – HZ, MSal., MZ; Data Interpretation – HZ, MSal., JF; Acceptance of final manuscript version - all; Literature Search - HZ; Funds Collection – AM, MSac., JF.

Conflict of interest

Authors declare no conflict of interest.

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