

EXPERIMENTAL STUDY

Prokinetics stimulate the increase of ghrelin in mice

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ABSTRACT

OBJECTIVES: Intestinal motility is regulated by several neurotransmitters and neuropeptides including dopamine and acetylcholine as well as ghrelin. Metoclopramide and domperidone are long-standing treatment options for dysmotility, and erythromycin is suggested in selected patients. In the present study, we aimed to investigate the effects of mentioned prokinetics on ghrelin levels.

METHODS: Serum ghrelin levels were estimated by using enzyme-linked immunoassay following a single administration of domperidone, metoclopramide, or erythromycin.

RESULTS: Our results showed that both antidopaminergic and cholinergic prokinetics increase the circulating ghrelin levels. There was no significant difference between enteral and parenteral control groups. Also, statistical analysis revealed that neither prokinetic was superior to the other in regard to its ghrelin stimulating effect.

CONCLUSION: Conclusively, the present study demonstrated that the circulating levels of ghrelin increase by the administration of antidopaminergic and cholinergic prokinetics. Hence, this effect on ghrelin may partly be responsible for the motility-stimulating actions of domperidone, metoclopramide, and erythromycin (Fig. 2, Ref. 39).

Text in PDF www.elis.sk.**KEY WORDS:** domperidone, erythromycin, ghrelin, metoclopramide, prokinetics.**Introduction**

The enteric nervous system (ENS), which regulates the gastrointestinal behavior independently of but in cooperation with the central nervous system (CNS), is the most complex part of the peripheral nervous system. The intrinsic neural cell population is allocated to the myenteric and submucosal plexuses. Dopaminergic neurons reside in both plexuses (1, 2) and all five types of dopamine receptors (D₁₋₅) have been identified in the gastrointestinal tract (2). Dopamine possesses properties of regulating the intestinal circulation (3) and motility (4). It decreases the motility mainly through D₂ receptors (2, 5), although D₁ receptors seem to gain dominance aborally (6). Hence, it is comprehensible why the drugs acting through D₂ antagonism, such as metoclopramide and domperidone, bear a net prokinetic effect on the gastrointestinal system (5). These drugs, as well as the cholinergic prokinetic erythromycin, are in clinical use against dysmotility disorders [reviewed by Acosta and Camilleri (7)].

Besides the enteric neurotransmitters, the intestinal motility is under the modulatory control of several neuropeptides. Among these, ghrelin has been identified in 1999 as the ligand for the growth hormone secretagogue receptor (GSH-R1a) (8).

Ghrelin has been shown to exhibit orexigenic, prokinetic, insulinostatic, adipogenic, vasodilatory, and immunomodulatory properties (9, 10). Ghrelin-producing (X/A-like) cells have been located throughout the intestinal tract in rodents, while the stomach is the richest for these cells (11). In physiological conditions, ghrelin levels increase before meals and decrease postprandially, making it to be termed as ‘hunger hormone’. However, it is accepted today that this terminology is a result of oversimplification, and ghrelin works as a nutrient-load detector which responds to optimize the energy balance and growth signals (12). As to the particular interest of the present study, ghrelin predominantly recruits extrinsic and intrinsic cholinergic pathways to exhibit its prokinetic effect (13–15). Even so, a recent study by Mondal et al (16) demonstrated the participation of adrenergic (via $\alpha 1$ receptors) and serotonergic (via 5-HT₃ receptors) neurons. The regulation of ghrelin secretion is a highly intricate process as there are numerous spiraling factors including neurotransmitters and hormones [reviewed by Iwakura et al (17)]. Considering the interaction between dopamine and ghrelin, the medical literature is far from offering a lucid explanation. This is because dopamine has been reported to increase ghrelin levels in the ghrelinoma cell line (18), while neither *in vivo* (19), nor *in vitro* (20) effects have been shown in gastric mucosal cells.

In the present study, we aimed to investigate *in vivo* effects of clinically used dopamine type 2 receptor antagonists, domperidone and metoclopramide, on the levels of circulating ghrelin to see if the dopaminergic regulation occurs via inhibition rather than activation. To compare the effect widths of dopaminergic antagonism, we also used a cholinergic agent, erythromycin, which previously reported to provoke the intestinal motility.

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Materials and methods

Animals and chemicals

A total of 40 adult (> 10 weeks) male Balb/c mice were purchased from the Mustafa Kemal University Application and Research Center for Experimental Researches (Hatay, Turkey). The animals were housed in polycarbonate cages under standardized conditions (22 ± 2 °C temperature, 55 ± 10 % relative humidity, 12:12-h light/dark cycle). Tap water and mouse chow were provided *ad libitum*. Domperidone (Janssen Pharmaceutica, Belgium) and erythromycin thiocyanate [eq. to 92.54 % (w/w) erythromycin base] (Vetas, Turkey) were ground to fine powder and suspended in distilled water. Metoclopramide (Sifar, Turkey) was obtained as a ready-to-use solution. All experimental procedures were approved by the Local Ethics and Animal Care Committee of Mustafa Kemal University (#2015/6-6) and performed in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals (Publication No. 86-23, revised 1996).

Experimental design

The animals were randomly assigned to five groups as Enteral Control (ECon; n = 8), Parenteral Control (PCon; n = 8), Domperidone (Dom; n = 8), Metoclopramide (Met; n = 8), and Erythromycin (Eryt; n = 8). All animals were fasted overnight (12 h) and then given either the vehicle or respective drug depending on which group the animals belonged to. A volume of 0.5 mL distilled water was orally given to ECon group. Dom and Eryt groups received 20 mg/kg domperidone and 6 mg/kg erythromycin thiocyanate, respectively, by oral gavage. PCon animals were intraperitoneally injected with 0.9 % sodium chloride solution (0.2 mL), while 20 mg/kg, i.p. metoclopramide was administered to the animals in Met group. The animals were anesthetized with 2 % isoflurane in a ventilation chamber immediately before the cardiac puncture to acquire blood. Considering the diverse pharmacokinetics of the drugs, the blood was drawn at time points differing as for the groups: following the drug administration, at 5th min for PCon and Met, 15th min for ECon, 30th min for Dom, and 90th min for Eryt. Collected blood was decanted into serum tubes and left to clot for 2 hours at room temperature. Following the centrifugation for 15 minutes at 1000 g, the supernatant was pipetted into microcentrifuge tubes for immunochemical analysis. Active ghrelin levels in the serum were measured by using an ELISA kit (Elabscience Biotechnology, China) following the instructions of the manufacturer. The optical density was measured spectrophotometrically at a wavelength of 450 nm and ghrelin concentrations (ng/dL) were determined by comparing the optical density of the samples to the standard curve.

Statistical analysis

Data for enteral treatment groups (ECon vs Dom vs Eryt) were analyzed by one-way ANOVA test followed by post hoc Fisher's least significant difference (LSD) test. The two-tailed unpaired Student's t-test was used for parenteral treatment groups (PCon vs Met) and control groups (ECon vs PCon). The normality of data distribution was determined by using Shapiro-Wilk normality

test. The analyses were performed using Prism v.6.0 (Graphpad software Inc.). The statistical significance was considered as $p < 0.05$. Data are represented as means \pm standard errors of means.

Results

As illustrated in Figure 1, serum ghrelin concentration was increased (one-way ANOVA test; $F(2,21) = 3.542$, $p = 0.047$) by the administration of either domperidone or erythromycin compared to orally treated controls (post hoc LSD test; $p = 0.032$ and $p = 0.032$, respectively). Although Eryt group displayed a higher level of ghrelin than Dom group, the difference was not statistically significant (post hoc LSD test; $p > 0.05$). There was no significant difference between enteral and parenteral control groups after receiving the respective vehicle (Student's t-test; $t = 0.699$, $df = 14$, $p = 0.496$) (Fig. 2A). In comparison to PCon animals, the administration of metoclopramide raised the circulating ghrelin levels (Student's t-test; $t = 2.539$, $df = 14$, $p = 0.024$), as depicted in Figure 2B. The multiple comparison of Met, Dom, and Eryt groups showed no statistically significant difference (one-way ANOVA test; $F(2,21) = 0.828$, $p = 0.451$).

Discussion

Domperidone and metoclopramide, the antidopaminergic prokinetics, are commonly prescribed against a variety of intestinal motility disorders, as well as for the prevention of nausea and vomiting (5). In recent years, erythromycin, the first macrolide discovered, has been recommended to be used for its prokinetic properties, which is through cholinergic recruitment, although with cautions (21, 22). Additionally, ghrelin, an endogenous neuropeptide, is known to enhance the motility and so, ghrelin mimetics are proposed to be a novel line of the treatment strategy in intestinal motility disorders (23). However, to date, the interactions of the investigated prokinetics, namely domperidone, metoclopramide and erythromycin, with ghrelin secretion have remained occult. The results of the present study indicated a significant increase in ghrelin with both types of prokinetics.

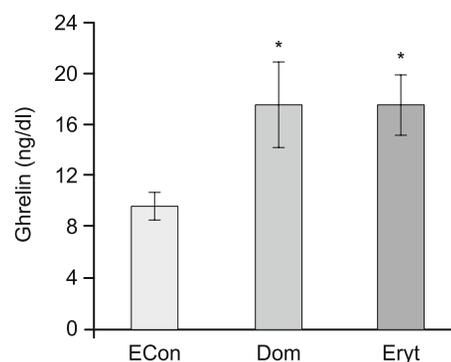


Fig. 1. The administration of either domperidone or erythromycin increased ghrelin concentrations. Asterisk (*) indicates the statistical significance ($p < 0.05$) versus control group.

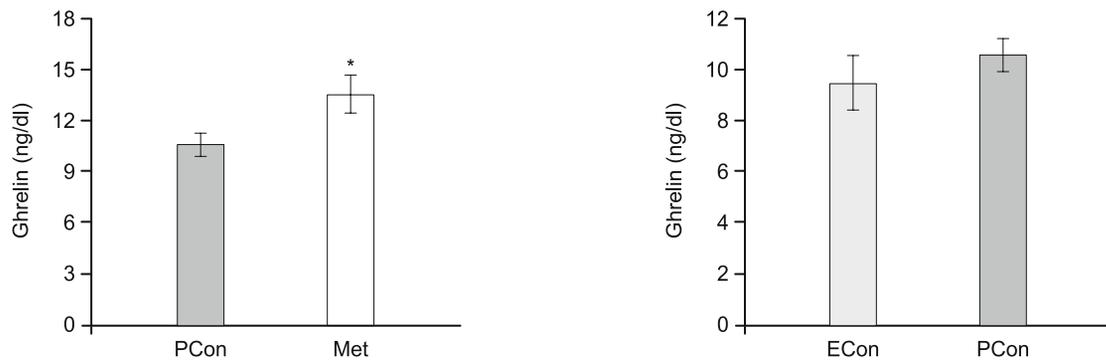


Fig. 2. (A) There was no statistically significant difference between enteral and parenteral controls ($p > 0.05$). (B) Compared to parenteral controls, the administration of metoclopramide resulted in an increase in ghrelin. Asterisk (*) indicates the statistical significance ($p < 0.05$) versus control group.

Erythromycin occupies the motilin receptors in human, which enhances intestinal motility through the cholinergic activity (24). Because it is a well-established fact that acetylcholine provokes the increase in ghrelin (25), the relation between erythromycin and ghrelin can be anticipated in humans. On the other hand, rodents lack the motilin receptors (26). Nevertheless, several authors have reported a prokinetic feature of erythromycin in rodents (27, 28). The present study demonstrated an increase in circulating ghrelin with the erythromycin treatment, although the mechanism of this increase, which is needed to be explained in future studies, seems to be independent of the motilin receptors. In regard to the results with antidopaminergic prokinetics, taking a look at Parkinson's disease can be elucidatory, because it is a quintessential prototype for central dopaminergic deficiency. Although about a half of the patients suffering from Parkinson's disease experience reduced intestinal motility (29), it is likely because of decreased cholinergic and increased catecholaminergic stimulation (30), and dopaminergic medication (31). The study by Unger et al (32) pointed out the decrease in ghrelin signaling under this catecholaminergic and dopaminergic overstimulation, whereas it should be also noted that Karasawa et al (33) reported conflicting findings in a similar experimental Parkinsonism model. Interestingly, no change in fasting ghrelin in vagotomized patients (34) suggests that disturbed cholinergic transmission is not a determinant for lowered ghrelin levels in Parkinson's disease. Moreover, an *in vitro* study by Iwakura et al (18) demonstrated that dopamine stimulates the ghrelin release via D_1 receptors in the mouse ghrelinoma cell line (MGN3-1). Since D_1 and D_2 receptors have antithetic characters as D_2 receptor antagonists potentiate D_1 receptor-associated events and *vice versa* in the CNS (35), the increment of ghrelin concentrations with D_2 antagonists may be related to the enhancement of D_1 receptor activity. It is of note that the interrelation of D_1 and D_2 receptors in this manner in the gastrointestinal system remains unknown. It is also not clear if intestinal dopamine influences ghrelin release *in vivo*; however, the facts that the ghrelin-producing cells are stimulated by low ambient D-glucose (36), and the intra-gastric infusion of nutrients provoke the increase in dopamine in the brain (37) may confer an indirect evidence for the interaction between dopamine

and ghrelin in the gastrointestinal system. A simpler explanation of our results may depend on the ability of D_2 stimulation to inhibit acetylcholine release (38). The antagonism of D_2 receptors may result in the potentiation of cholinergic transmission, which leads to the increment of ghrelin secretion. Interestingly, Sudakov and Bashkatova (39) showed that domperidone increases the feeding behavior, although it cannot penetrate the blood-brain barrier. Their finding may be connected with the induction of ghrelin secretion by this antidopaminergic prokinetic. Conclusively, from this point, one can speculate that the inhibition of dopamine type 2 receptors may relieve the basal inhibitory effect of dopamine or may provoke the activity of dopamine type 1 receptors, and this subsequently leads to the increase in ghrelin. From a much more mechanistic view, the increase in ghrelin may be generated from stimulation of ghrelin-producing cells by smooth muscle contractions. To our results, it is not possible to make a deduction about whether the increase of ghrelin is a result of a direct effect on ghrelin-producing cells or an indirect effect arisen by dopamine antagonism. Nevertheless, this seminal study warrants further investigation of the relation between prokinetic dopamine antagonists and ghrelin.

Finally, the present study demonstrated that the levels of circulating ghrelin increase by the administration of antidopaminergic and cholinergic prokinetics. Hence, this effect on ghrelin may partly be responsible for the motility stimulating actions of domperidone, metoclopramide, and erythromycin.

Learning points

- The antidopaminergic prokinetics metoclopramide and domperidone increase the circulating levels of ghrelin.
- The stimulating effect of antidopaminergic prokinetics on ghrelin secretion is comparable to that of erythromycin.

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