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The effects of three different prokinetic drugs on the motility hormones level in lambs with postoperative ileus

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Abstract: The aim of this study was to evaluate the effects of the three different prokinetic agents, namely erythromycin, lidocaine, and metoclopramide in lambs with postoperative ileus (POI) using motilin and ghrelin, which are known as motility markers. Twenty lambs were allocated to 4 groups of 5 lambs each. An enterotomy was performed on the ileum of each lamb. Erythromycin (8.8 mg/kg), metoclopramide (0.2 mg/kg), and saline (2 mL) were administered as single dose IM before surgery in the ERT, MET, and CNTR groups, respectively. Lidocaine was administered to the LID group, both as a bolus before (1.3 mg/kg IV) and as a continuous rate infusion during surgery (0.05 mg/kg/h IV). Serum motilin and ghrelin concentrations as motility hormones were measured with ELISA. The serum motilin concentration was significantly higher at 0, 1, 3, 5, 10, 24, and 48 h in the ERT group and at 1 and 10 h in the LID group. The serum ghrelin concentration was significantly higher at 0, 1, and 48 h in the ERT group and at 3, 5, 10, 24, and 48 h in the LID group. As a result, erythromycin and lidocaine was thought to be useful in lambs with POI according to the stimulatory effects on the motility hormones.

Key words: Ghrelin, motilin, postoperative ileus, lamb

1. Introduction

Postoperative ileus (POI) occurs as an inevitable outcome of abdominal surgery caused by a combination of factors such as the use of anesthetics and opioid agents in the perioperative period, neural mechanisms, and intestinal inflammation (1,2). It can be seen during the first 48 h after many abdominal surgeries, particularly for intestinal disorders, which are a crucial problem likely to require surgical consideration in ruminants. This condition causes a risk of leakage of ingesta at the surgery site if anastomosis was performed. The use of prokinetic drugs can prevent this risk (3).

Prokinetic drugs increase the smooth muscle contractions in the gastrointestinal tract, accelerating the movement of the ingesta through the digestive organs. Prokinetic drugs have been reported to contribute to treatment when gastric, pyloric, and intestinal motility are disrupted (4,5).

Motilin and ghrelin (the homology of motilin) are gastrointestinal peptide hormones that are involved in the regulation of the migrating motor complex (MMC) in many animals (6,7). These hormones are synthesized in the upper gastrointestinal tract and are structurally similar. They have a prokinetic effect on gastrointestinal motility (8). Motilin is a hormone released by the endocrine cells of the duodenal mucosa during fasting to stimulate gastrointestinal motility (9). Ghrelin is a peptide synthesized in the endocrine cells of the gastric mucosa with orexigenic, gastroprokinetic, and antiinflammatory effects (10). Ghrelin has also been reported to stimulate motility in the small and large intestines and to shorten colonic transit time in many species (11). The decrease of ghrelin after surgery, in particular, could be a key driver of POI (10).

Erythromycin is prokinetic agent and a motilin agonist; its effect on gastrointestinal motility has been observed in many animal species (12), and it has been used as a positive control in studies. Lidocaine infusion has also been successfully used in POI treatment, especially in horses (13,14), but there is not enough literature related to its use in ruminants. Furthermore, there is little information on the effects of metoclopramide on POI in ruminants (12,15).

Gastrointestinal motility is generally evaluated via several methods such as clinical tests, various imaging

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techniques, absorption tests, and the activity of the MMC (16–20). However, little work has been done on motility hormones in gastrointestinal disorders such as POI in ruminants. For this reason, the aim of this study was to evaluate the effects of three different prokinetic agents, namely erythromycin, lidocaine, and metoclopramide in lambs with POI by using motilin and ghrelin, which are known as motility markers.

2. Materials and methods

2.1. Animals

Twenty 30–45 day-old male lambs (weighing 8.7–15.6 kg) were used. Healthy lambs were included in the study after a routine clinical examination (appetite, rectal temperature, heart rate, respiratory rate, and defecation). Standard lamb diet and water were provided ad libitum. The study was performed with the approval of the Animal Ethical Committee of the University of Dicle (No: 2014/059).

2.2. Study design

This study was designed as a randomized, controlled, and blinded experimental trial. Lambs were allocated to 4 groups of 5 lambs each. The individuals performing the surgery and laboratory measurements and observing the lambs during the postoperative period were unaware of the group design.

2.3. Preparation of lambs for the surgery and anesthesia protocol

Food was withheld 12 h prior to surgery for each lamb and only water was provided. The area between the umbilicus and pelvic region was shaved for surgery. Surgeries were carried out under general anesthesia performed by injecting 6 mg/kg of ketamine hydrochloride (Ketasol, İnterhas, İstanbul, Turkey) and 0.2 mg/kg of xylazine hydrochloride (Rompun, Bayer, İstanbul, Turkey) intramuscularly.

2.4. Surgical procedure for POI model

The lambs were restrained in the dorsal recumbency position. Each lamb underwent a laparotomy consisting of a paramedian incision of 10 cm through the abdominal skin and muscle layers, followed by evisceration and a 5 cm-long longitudinal incision performed at the antimesenteric side of the ileum, followed by closure with suture. The sutured line was then sanitized using saline moistened gauze at room temperature and checked for any leakages, followed by an abdominal skin and muscle closure with sutures. Gastrointestinal motility for the POI model was also evaluated in terms of abdominal auscultation of the intestines and clinical symptoms of the POI (loss of appetite, pain in abdominal palpation, tenderness, and absence of feces upon digital rectal examination).

2.5. Applied drugs

Erythromycin (Apirocin-f, Teknovet Drug Industry, İstanbul, Turkey) 8.8 mg/kg was administered intramuscularly as single dose to the lambs in the ERT group before surgery.

Metoclopramide (Metpamid, Recordati Drug Industry, İstanbul, Turkey) 0.2 mg/kg was administered intramuscularly before the surgery to the lambs in the MET group.

Lidocaine HCl (Vilcain, Vilsan Drug Industry, Ankara, Turkey) 1.3 mg/kg as a bolus was administered intravenously before the surgery and 0.05 mg/kg/h as a continuous rate infusion (CRI) during the surgery to the lambs in the LID group.

Two mL of saline was administered intramuscularly as a placebo before the surgery to the lambs in the control (CNTR) group.

Throughout the course of the operation, the lambs in all groups were administered 0.9% isotonic saline intravenously. Postoperative analgesia and antibiotics were performed with a single dose of meloxicam (0.2 mg/kg SC, Maxicam, Sanovel, Turkey) and a 5-day dose of penicillin G (22.000 IU/kg IM, Devapen, Deva, İstanbul, Turkey) in all groups, respectively.

2.6. Blood sampling

Blood samples via an intravenous catheter placed in the jugular vein of the lambs were collected into silicone gel-coated tubes before (preop values) and following surgery (0 h) and at post-operative 1, 3, 5, 10 and 48 h. Following clotting, collected blood samples were centrifuged at 2500 g for 10 min and serum samples were obtained.

2.7. Laboratory measurements

Concentrations of the serum motilin and ghrelin were analyzed by a commercial sheep-specific assay kit (Sunred Biological Technology ELISA Kit CO, LTD, China) according to the manufacturer's instructions in an ELISA/spectrophotometer reader (Multiskan Go Spectrophotometer, Thermo Scientific, NH, USA) in a blind fashion.

2.8. Data analysis

Statistical analysis of the data was performed using the Statistical Package for Social Sciences (SPSS) v20.0 software (IBM Corp., Armonk, NY, USA). First, the Shapiro-Wilkes test was used for evaluating the normal distribution of the variables. They were then evaluated by using a one-way analysis of variance (ANOVA), and the Tukey post hoc test. $P < 0.05$ was considered as a statistically significant difference.

3. Results

3.1. Clinical findings

Clinical data of the study are presented in Table 1. In all lambs, gastrointestinal motility was not detected during abdominal auscultation; the suckling reflex was negative, and there was pain and tenderness in abdominal

Table 1. Clinical findings in the lambs.

Clinical data	Groups	zero h			24 h			48 h		
		–	±	+	–	±	+	–	±	+
Anorexia	CNTR			5		5		3	2	
	ERT			5	3	2		5		
	LID			5	1	3	1	4	1	
	MET			5		5		3	2	
Pain in abdominal palpation	CNTR			5			5	4		1
	ERT			5	2	2	1	4	1	
	LID			5	1	1	3	3	2	
	MET			5		1	4	4		1
Motility in abdominal auscultation	CNTR	5			3	2			3	2
	ERT	5			1	1	3		1	4
	LID	5			1	3	1		1	4
	MET	5			3	2			2	3
Defecation	CNTR	5			4		1	1	2	2
	ERT	5			1	1	3		1	4
	LID	5			1	3	1		2	3
	MET	5			3	2			3	2

Numerical data: number of lambs showing clinical findings belongs to POI in the groups. (–): absent; (±): moderate; (+): present.

palpation and an absence of feces on rectal examination at 10 h postoperative. Some of these symptoms were still sustained in the group CNTR at 24 and 48 h. Dysmotility, however, was detected in only one of the lambs in the ERT group, in two lambs in the LID group, and in three lambs in the MET group at 24 h. All POI symptoms improved except in one of the lambs in the ERT group at 48 h. In addition, it was observed that the anorexia, appetite, pain at abdominal palpation, and defecation improved in the three lambs; however, improvement in all symptoms occurred in the LID group at 48 h.

3.2. Serum motilin and ghrelin Concentration

The mean serum motilin and ghrelin concentrations are presented in Table 2. Serum motilin concentration was significantly higher at 0, 3, 5, 24, and 48 h in the ERT group, at 1 and 10 h in the ERT and LID groups ($P < 0.05$). The mean serum ghrelin concentration was significantly higher at 0 and 1 h in the ERT, at 3, 5, 10, and 24 h in the LID group, and at 48 h in the ERT and LID groups ($P < 0.05$).

4. Discussion

Due to their anatomic structure, ruminants are often predisposed to gastrointestinal motility disorders associated with many diseases such as paralytic ileus, invagination, and intestinal problem (17,20). These disorders can lead to yield loss and to many other complications that result in mortality unless treated. Use of prokinetics in gastrointestinal motility disorders is very important. However, the presence of several controversies (animal species, dose, etc.) regarding prokinetic effects restrict the use of these drugs. There is little information showing the use and clinical effects of motility modifiers in ruminants. In this study, three different prokinetic agents, namely erythromycin, lidocaine, and metoclopramide were used. The effect of these drugs was attempted to be identified using motilin and its homologue ghrelin.

Ghrelin and motilin participate in initiating the MMC in the stomach, stimulating gastrointestinal motility and accelerating gastric emptying in many species (21–23). Similarly, activation of these hormones occurs to enhance the gastric emptying of ruminants with impaired abomasal motility and/or outlet occlusion in the displaced abomasum (8). According to our results, ghrelin and motilin stimulate gastrointestinal motility more and accelerate gastric emptying time, especially in the ERT and LID groups compared to the CNRT and MET groups.

Decreasing the motilin concentration might lead to gastrointestinal dysmotility. In addition, gastrointestinal motility is further decreased after surgical correction of

Table 2. Motilin and ghrelin concentrations in the lambs with postoperative ileus (mean ± SD).

	Groups	Preop	zero h	1 h	3h	5 h	10 h	24 h	48th h
MOT	CNTR	285.93 ± 5.66	259.95 ± 40.46^{AB}	268.05 ± 36.03^{B}	277.26 ± 1.06^{B}	267.02 ± 19.92^{B}	232.03 ± 6.36^{C}	212.12 ± 18.56^{C}	274.73 ± 15.84^{BC}
	ERT	285.93 ± 5.66	308.30 ± 19.67^{A}	330.89 ± 21.19^{A}	422.27 ± 15.40^{A}	374.59 ± 8.67^{A}	405.02 ± 26.16^{A}	359.25 ± 8.61^{A}	332.21 ± 4.66^{A}
	LID	285.93 ± 5.66	265.68 ± 32.36^{AB}	336.97 ± 25.55^{A}	299.02 ± 40.01^{B}	247.27 ± 22.64^{B}	379.47 ± 11.65^{A}	271.12 ± 34.32^{B}	300.92 ± 38.36^{AB}
	MET	285.93 ± 5.66	248.15 ± 10.16^{C}	260.93 ± 20.03^{B}	284.33 ± 14.39^{B}	254.45 ± 7.44^{B}	266.65 ± 18.89^{B}	283.94 ± 1.29^{B}	249.59 ± 13.05^{C}
GHR	CNTR	64.48 ± 3.19	34.27 ± 3.71^{D}	60.66 ± 5.32^{C}	65.17 ± 9.45^{C}	60.86 ± 6.48^{C}	79.80 ± 4.04^{C}	69.30 ± 4.87^{C}	46.10 ± 5.60^{B}
	ERT	64.48 ± 3.19	75.81 ± 5.24^{A}	90.31 ± 4.29^{A}	94.46 ± 4.47^{B}	89.94 ± 0.64^{B}	104.05 ± 4.36^{B}	112.42 ± 2.15^{B}	84.14 ± 5.52^{A}
	LID	64.48 ± 3.19	59.51 ± 3.43^{B}	79.26 ± 2.62^{B}	137.54 ± 0.07^{A}	142.55 ± 0.61^{A}	206.67 ± 13.72^{A}	163.73 ± 12.26^{A}	79.08 ± 2.34^{A}
	MET	64.48 ± 3.19	47.93 ± 4.89^{C}	66.60 ± 2.30^{C}	74.49 ± 7.70^{C}	65.04 ± 5.73C	68.15 ± 9.52^{C}	45.15 ± 2.23^{D}	35.45 ± 3.65^{C}

Values on the same line (A, B, C, D) with different superscripts are statistically significant ($P < 0.05$). MOT: motilin; GHR: ghrelin; h: hour.

gastrointestinal disorders such as displacement of the abomasum (8), whereas erythromycin, which is a motilin agonist, increases the abomasal emptying rate in cows that have undergone surgical correction of displacement of the abomasum (24). In this study, the serum motilin concentration was at the highest level immediately after surgery and during the postoperative 48 h in the erythromycin administered group. It was expected to remain at a high level in the ERT group. Furthermore, it was higher in the lidocaine-administered group at 1 h and 10 h postoperation.

Ghrelin is one of the primary hormones involved in the regulation of appetite as well as multiple physiological functions such as the regulation of growth hormone secretion, gastroprokinetic effects, and gut motility (10,21,25). In this study, the serum ghrelin levels were higher at 48 h in the ERT and LID groups. These data are also supported with clinical observations at 24 h (Table 1), particularly with reference to improving appetites in these two groups (ERT and LID).

In a study carried out on cows with left and right displaced abomasum, it was revealed that serum motilin and ghrelin concentrations increased. This is also supported by results from the cows undergoing surgical correction of a left displaced abomasum (8). In our study, however, no remarkable alteration in motilin and ghrelin levels was observed in the nonmedicated surgical group (CNTR). In this respect, it was different from the aforementioned study.

Active ghrelin levels decrease during abdominal surgery conditions (10,26) and gradually normalize over 24 h (10). Ozturk et al. (8) revealed that increased concentration of motilin and ghrelin can induce the acceleration of abomasal motility. In this study, serum ghrelin concentration significantly decreased immediately after surgery in the MET and CNTR groups ($P < 0.05$). However, its increase in the ERT group compared to other groups during this period was insignificant. In addition, serum ghrelin concentrations gradually normalized within 48 h in all groups according to differences in the drug's efficacy. In the study, serum motilin levels significantly decreased immediately after surgery in the MET group and insignificantly decreased in the CTRL and LID groups, whereas the ghrelin levels insignificantly increased in the ERT group (Table 2). This situation can be explained by the fact that erythromycin is a potent motility modifier which binds to specific motilin receptors.

In POI, sympathetic (adrenergic) hyperactivity or parasympathetic (cholinergic) hypoactivity occurring at the same time leads to a decrease in gastrointestinal motility (1,27,28). Therefore, cholinergic, antidopaminergic (metoclopramide), motilin agonist (erythromycin), and local anesthetic drugs (lidocaine) can be used in ileus treatment (28). Erythromycin induces phase III of the interdigestive MMC, increases the amplitude of antral contractions, and improves antroduodenal coordination in several species. It produces a prokinetic effect primarily by acting as a motilin-receptor agonist via binding to motilin receptors in the pyloric antrum and proximal portion of the small intestine (12). It was found that high doses (8.8 mg/kg IM) of erythromycin in healthy calves increase frequency through abomasal intraluminal pressure and speed up the abomasal emptying time (24,29). In this study, we noted that POI symptoms (anorexia, pain in abdominal palpation, tenderness, defecation rate, etc.) decreased clinically within the first 24 h in the ERT group and that the number of lambs was higher than in the other groups (Table 1). Moreover, a larger increase in motilin and ghrelin, which are motility markers, than in the MET and CNTR groups (Table 2) may suggest that erythromycin and lidocaine are significantly effective in lambs with POI.

As a result, erythromycin can be used in lambs with POI as in other animal species. In addition, lidocaine is also thought to be useful in lambs with POI because of its stimulatory effects on motility hormones such as motilin and ghrelin.

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