의학박사 학위논문

비글견에서 전정부-십이지장 운동에 대한 Azithromycin의 효과

Effect of Azithromycin for Antroduodenal Motor Activity in Beagle Dogs

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노 진 희

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이 논문을 의학박사 학위 논문으로 제출함

2021년 8월

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Abstract

Background/Aims: A few studies have reported the effects of azithromycin (AZM) as a prokinetic drug. The present non-clinical study aimed to clarify the effect of AZM on antroduodenal motility and investigate the contractility pattern from the gastric antrum, through the pylorus to the duodenum.

Methods: We conducted our experiments on three male beagles, three times at one-week intervals. The beagles underwent percutaneous endoscopic gastrostomy (PEG), and the antroduodenal contractility was measured using a high-resolution manometry catheter, inserted through a PEG tube, after receiving intravenous AZM infusions.

Results: Nine experiments were conducted, where each beagle received 146.3±5.8 mg of AZM. The median amplitude and duration after AZM infusion in the antrum, pylorus, and duodenum were higher and longer than those at baseline. The median area under the curve of AZM infusion were significantly higher than baseline in the antrum (3055.5 *vs*. 26.7, $p < 0.001$), pylorus (692.3 *vs*. 17.0, $p < 0.001$), and duodenum (555.6 *vs*. 14.0, $p < 0.001$). The motility index was also significantly increased with administration of AZM on antrum (4.41 *vs*. 3.65, $p < 0.001$), pylorus (4.55 *vs*. 3.63, $p < 0.001$), and duodenum (4.76 *vs*. 3.62, $p < 0.001$).

Conclusions: AZM enhanced the antroduodenal motility in beagles. It amplified the contractile activity of the gastric antrum, and the contraction was propagated from the antrum to the duodenum. It may remarkably shorten the gastric emptying time, which is expected to be particularly useful for the treatment of patients with delayed gastric emptying.

Keywords: Antroduodenal; Area under the curve; Azithromycin; Beagle; High resolution manometry

차 례

표 및 그림 목차

INTRODUCTION

Gastroparesis is a chronic gastrointestinal motility disorder defined by delayed gastric emptying without evidence of mechanical obstruction. The symptoms including post-prandial fullness/early satiety, nausea, vomiting, abdominal pain, and bloating, are associated with great impairment in patients' quality of life.[1] The treatments are selected according to symptom severity that include dietary modifications, medical therapy with prokinetics, antiemetics, and non-opiate analgesics, and endoscopic interventions including botulinum toxin injection to the pylorus and surgery.

Multiple prokinetic agents including dopamine receptor antagonists, serotonin receptor agonists, cholinesterase inhibitors, motilin-like agents, and ghrelin-like agents are used for the treatment of gastroparesis.[2] Erythromycin (ERY), a macrolide antibiotic, induces a marked acceleration of gastric emptying by stimulating motilin receptors in the intestinal smooth muscle.[1] Because ERY has a higher gastric-emptying effect than other prokinetics, it has been widely used in patients with gastroparesis.[3] However, it has side effects including nausea, vomiting, abdominal pain, and sudden cardiac death due to the prolongation of QTc causing torsade de pointes, in addition to the inhibition of CYP3A causing drug interactions.[4, 5] Moreover, tachyphylaxis caused by motilin receptor downregulation occurs when used for more than four weeks.[6] These critical side effects and the suspension of domestic imports have made it no longer available in Korea. Azithromycin (AZM) is a semisynthetic macrolide antibiotic with similar activity to that erythromycin; however, it does not interact with the cytochrome P450 pathway of hepatic metabolism and has fewer drug interactions and cardiac side effects.[7] Thus far, a few studies with a small number of patients have reported the effects of AZM regarding increased antrum and small bowel motility.[8]

The present study aimed to clarify the effect of AZM on antroduodenal motility during drug infusion. In these non-clinical experiments, we tried to demonstrate and investigate not only the gastroprokinetic activity of AZM but also the contractility pattern from the gastric antrum, through the pylorus to the duodenum.

METHODS

Animals

A total of 3 male beagles were purchased from Orient Bio (Seongnam, Korea) (Table 1). All dogs were fed regularly and were kept in different cages at 22±2˚C. Beagles were kept under fasting from midnight and received cefazolin (25 mg/kg) intravenously before the experiment. They received mild anesthesia using alfaxalone (2 mg/kg) and xylazine (1 mg/kg). Isoflurane (3-4%) was introduced for the induction of general anesthesia, followed by endotracheal intubation, and isoflurane (2.0-2.3%) was given as a maintenance anesthetic during the experiment. An intravenous line using a 20-gauge needle was peripherally inserted for drug infusion.

This study was approved by the Institutional Animal Care and Use Committee of Asan Medical Center (approval no. 2020-13-179).

Drugs

AZM (Zithromax® Injection 500 mg, Pfizer) was used for this experiment. Body surface area (BSA) was calculated by measuring the height and weight of dogs in each experiment, and 290 mg of AZM per BSA was mixed with 150 mg of normal saline

and administered by an intravenous infusion for 30 minutes.

Endoscopic procedure

The Beagles were positioned in the left decubitus and underwent percutaneous endoscopic gastrostomy (PEG) through the pull-type technique using a 24 Fr catheter (US Endoscopy, Mentor, OH, US) (Figure 1E). The PEG tube was inserted through the mouth and esophagus. An endoscope (CF-H260AI; Olympus Inc., Tokyo, Japan) was inserted into the stomach, and transillumination was performed to determine where to insert the PEG tube. The guidewire was punctured through this site and removed through the oral cavity using a forcep. The guidewire was connected with the gastrostomy tube, and the other side of the guidewire was retracted from the abdominal wall to place the PEG tube in the stomach. The endoscope was inserted again to confirm the tube position (Figure 1A). After PEG insertion, the manometry catheter was inserted through the PEG tube (Figure 1B) and placed in the second portion of the duodenum through the gastric antrum and pylorus (Figure 1C). The location of the catheter and each channel was confirmed by X-ray imaging (Figure 1D).

After measurement of antroduodenal contractility using manometry, the PEG tube was removed, and the abdominal wall was sutured. The beagles could eat after recovery from anesthesia, and they received prophylactic antibiotics (cefazoline 25 mg/kg) for three days. This experiment was repeated three times every week for each beagle.

Antroduodenal motor activity during drug infusion

A high-resolution impedance manometry (HRM) catheter composed of 32 circumferential sensors and 16 impedance sensors (InSight Ultima, Diversatek^{TM},

Highlands Ranch, CO, USA) was used for this experiment. It was positioned from the gastric antrum, through the pylorus, and into the duodenum. The antroduodenal contractile activity was recorded for a total of 90 minutes, 30 minutes for each period as follows: at baseline, during AZM continuous infusion, and after finishing the drug infusion.

Manometry profiles were analyzed using the BioVIEW® Analysis 5.7.1.0 software system (Diversatek™, Highlands Ranch, CO, USA). The measured parameters included median amplitude (mmHg) of contraction, duration (seconds) of antroduodenal activity, area under the curve (AUC; mmHg·s), and the motility index defined as log [Σ (amplitude X number of contractions) + 1] of each selected section. Manometry tracing was analyzed after approximately 5 minutes from baseline and at peak for 1 min during AZM infusion to compare the changes in contractility before and after drug infusion.

Statistical analysis

Descriptive variables were summarized as median (interquartile range [IQR]) and mean ± standard deviation (SD) values. Differences in AUC between the baseline and AZM infusion ere compared using independent t-tests. A value of p <0.05 was considered statistically significant. Statistical analyses were performed using SPSS, version 24 (IBM Corporation, Somers, NY, USA).

RESULTS

Characteristics

Table 1 shows the baseline characteristics of the three beagles. All of them were male, and aged 6 to 8 months old. Each beagle underwent a total of three experiments, and the body weight and height were checked every time before the experiments. The mean body weight was 9.6±0.5 kg, and the mean body height was 93.3±2.5 cm. BSA was calculated to decide the administration dose of AZM, and the mean BSA was 0.507±0.021.

Increased contractility with azithromycin infusion

Three experiments were performed on each beagle with a total of nine experiments on the three beagles. Each beagle received 146.3±5.8 mg of AZM during each experiment. Changes in the median amplitude and duration in the antrum, pylorus, and duodenum according to the AZM infusion are shown in Table 2 and Figure 2. In the antrum, the median amplitude was 10.3 mmHg (4.2–18.4) at baseline and was 328.5 mmHg (243.3–400.6) during the AZM infusion. In the pylorus, the median amplitude was 6.5 mmHg (3.8–14.8) at baseline and 145.4 mmHg (114.0–228.0) during the AZM infusion. In the duodenum, the median amplitude was 8.3 mmHg (4.6–10.4) at baseline and 141.1 mmHg (116.1–177.9) during the AZM infusion. The antroduodenal contractility increased after the AZM infusion, especially the antral activity, which increased more than the activity of the pylorus and duodenum. The median duration at baseline and during the AZM infusion in the antrum was 2.3 seconds (1.9–3.7) and 9.5 seconds (5.5–12.2), respectively. In the pylorus, the median duration was 2.2 seconds (1.9–3.4) at baseline and 4.6 seconds (3.9–7.9) during the AZM infusion. In the duodenum, the median duration was 2.1 seconds (1.8–2.5) at baseline and 4.0 seconds (3.6–4.5) during the AZM infusion.

Area under the curve and motility index of the antroduodenum

The median AUC was significantly increased in the antrum, pylorus, and duodenum with the administration of AZM (Figure 3). In the antrum, the median AUC during the AZM infusion was significantly higher than that at baseline (3055.5 *vs*. 26.7, $p < 0.001$). The median AUC significantly increased with the AZM infusion in the pylorus (692.3 *vs*. 17.0, $p < 0.001$) and duodenum (555.6 *vs*. 14.0, $p < 0.001$). The motility index also significantly increased with the administration of AZM (Figure 4). In the antrum, the motility index during the AZM infusion was significantly higher than that at baseline (4.41 *vs*. 3.65, $p < 0.001$) and was also significantly increased with the AZM infusion in the pylorus (4.55 *vs*. 3.63, $p < 0.001$) and duodenum (4.76 *vs*. 3.62, $p < 0.001$).

Antroduodenal contractility pattern

Changes in the contractile activity in the antroduodenum after intravenous administration of AZM in the beagles are shown in Figure 5. The pressure wave was initiated in the distal antrum approximately 10 minutes after administering AZM, and it propagated to the duodenum (Figure 5A). There was a marked increase in the amplitude in the antrum, followed by the pylorus and duodenum. The contractile activity continued during the 30 minutes' observation period after discontinuation of the AZM injection (Figure 5B).

DISCUSSION

In this study, the intravenous administration of AZM in beagles enhanced the motility of the gastric antrum, pylorus, and duodenum. AZM amplified the contractile activity of the gastric antrum and enhanced the contractility propagating from the

antrum to the duodenum. This suggests that AZM could be used as an alternative prokinetic agent to ERY for the treatment of gastroparesis.

ERY, a macrolide antibiotic and motilin receptor agonist, causes marked acceleration of gastric emptying by inducing gastric antral contractions, and it has been used as a therapeutic agent for gastrointestinal dysmotility such as diabetic gastroparesis.[9, 10] However, the serious side effects of ERY related to cardiac toxicity have made it no longer available as a prokinetic.[4] AZM, which is also a macrolide antibiotic, began to draw attention because it showed fewer drug interactions and cardiac side effects while having similar activity. However, AZM has not yet been approved for use as a prokinetic, and only few studies have been reported about it. Our non-clinical experiment proved the prokinetic effect of AZM and demonstrated the contractility pattern from the stomach to the duodenum.

According to a previous study, the intensity, duration, and frequency of gastric antral contractions were significantly increased with intravenous administration of 500 mg ERY. In addition, this effect was initiated approximately 6 minutes after administering an injection of ERY on an empty stomach and lasted for 53 minutes. Furthermore, it began after approximately 9 minutes in the postprandial state and lasted for approximately 38 minutes.[11] In another study, a strong gastric antral contraction occurred 30 minutes after a 200-mg ERY injection and lasted for approximately 2 hours.[12] Our experiment showed similar results to those of previous studies. The antroduodenal contractile activity was initiated 10 minutes after starting the intravenous administration of AZM to the beagle. This enhanced motility continued during a 30-minute observation period even after discontinuing the drug infusion. This may be due to the time interval it takes for the drug to be injected and reach the plasma concentration for causing a contractility effect.

ERY activates motilin receptors located in the cholinergic neurons in the nerves and smooth muscles of the duodenum to induce the migrating motor complex (MMC) of

the upper gastrointestinal tract. It induces antral contractility, and the contractions migrate to the duodenum, jejunum, and terminal ileum in sequence.[13] AZM also induced MMCs in both the stomach and small intestine.[14] In our study, after the administration of AZM, the contractile activity was transmitted from the gastric antrum to the duodenum, and this finding is similar to that of a previous study.[8] In some experiments, the paradoxical contraction seemed to propagate from the distal antrum to the proximal antrum, which is thought to be caused by the air inflation during the PEG insertion procedure. The contraction of the distal antrum is traced immediately, because it is in direct contact with the manometry catheter from the beginning. However, because the gastric wall of the upper side would have not been able to contact the catheter until the air deflation, the proximal antrum might be not tracing contractility promptly.

AZM has a much longer half-life (up to 68 hours) in comparison with ERY (1.5 hours).[15] It has an increased efficacy and duration of action by reaching higher intracellular concentrations. It provides prolonged tissue concentration and AZM can be administered only once a day.[14] In this study, the antroduodenal contraction tended to persist for 30 minutes even after the discontinuation of the AZM infusion. In one beagle, the baseline amplitude was slightly different between the first and third experiments. The baseline amplitude of the third experiment was slightly elevated compared with the first one, and it might be the residual effect of the AZM administered in the previous experiment considering the long half-life of AZM. The concentration of the drug producing a prokinetic effect and the duration of action are important to produce a therapeutic response. Further studies are required to demonstrate the appropriate dose and duration of action of AZM.

CONCLUSION

The intravenous administration of AZM enhanced the antroduodenal motility in beagles. AZM amplified the contractile activity of the gastric antrum, and the contraction was propagated from the antrum to the duodenum. It may remarkably shorten the gastric emptying time, which is expected to be particularly useful for the treatment of patients with delayed gastric emptying. Further studies are needed to decide the dose and duration of AZM administration.

REFERENCES

1. Parkman HP, Hasler WL, Fisher RS. American Gastroenterological Association technical review on the diagnosis and treatment of gastroparesis. Gastroenterology 2004;127:1592-622.
2. Ma J, Rayner CK, Jones KL, et al. Diabetic gastroparesis: diagnosis and management. Drugs 2009;69:971-86.
3. Luan R, Tang H, Zhai S, et al. [Erythromycin for improving enteral nutrition tolerance in adult critical patients: a systematic review and meta-analysis]. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue 2014;26:425-30.
4. Ray WA, Murray KT, Meredith S, et al. Oral erythromycin and the risk of sudden death from cardiac causes. N Engl J Med 2004;351:1089-96.
5. Shaffer D, Singer S, Korvick J, et al. Concomitant risk factors in reports of torsades de pointes associated with macrolide use: review of the United States Food and Drug Administration Adverse Event Reporting System. Clin Infect Dis 2002;35:197-200.
6. Camilleri M, Bharucha AE, Farrugia G. Epidemiology, mechanisms, and management of diabetic gastroparesis. Clin Gastroenterol Hepatol 2011;9:5-12; quiz e7.
7. Hopkins S. Clinical toleration and safety of azithromycin. Am J Med 1991;91:40s-45s.
8. Chini P, Toskes PP, Waseem S, et al. Effect of azithromycin on small bowel motility in patients with gastrointestinal dysmotility. Scand J Gastroenterol 2012;47:422-7.
9. Cucchiara S, Minella R, Scoppa A, et al. Antroduodenal motor effects of intravenous erythromycin in children with abnormalities of gastrointestinal motility. J Pediatr Gastroenterol Nutr 1997;24:411-8.
10. Weber FH, Jr., Richards RD, McCallum RW. Erythromycin: a motilin agonist and gastrointestinal prokinetic agent. Am J Gastroenterol 1993;88:485-90.

11. Sarna SK, Soergel KH, Koch TR, et al. Gastrointestinal motor effects of erythromycin in humans. Gastroenterology 1991;101:1488-96.
12. Annese V, Janssens J, Vantrappen G, et al. Erythromycin accelerates gastric emptying by inducing antral contractions and improved gastroduodenal coordination. Gastroenterology 1992;102:823-8.
13. Kendall BJ, Chakravarti A, Kendall E, et al. The effect of intravenous erythromycin on solid meal gastric emptying in patients with chronic symptomatic post-vagotomy-antrectomy gastroparesis. Aliment Pharmacol Ther 1997;11:381-5.
14. Shakir AK, Altaf MA. Azithromycin Induces Migrating Motor Complexes in Pediatric Patients Undergoing Antroduodenal Motility Studies. J Pediatr Pharmacol Ther 2018;23:390-394.
15. Amsden GW. Erythromycin, clarithromycin, and azithromycin: are the differences real? Clin Ther 1996;18:56-72; discussion 55.

TABLES

Table 1. Baseline characteristics of beagles

	Beagle A	Beagle B	Beagle C
Age, months	8	8	6
Sex	Male	Male	Male
Body weight, kg (mean±SD)	9.5±0.0	10.1±0.1	9.2±0.6
Body height, cm	95	95	90
BSA (mean±SD)	0.519±0.00	0.522±0.00	0.481±0.01

BSA, body surface area; SD, standard deviation

Table 2. Median amplitude (mmHg) and duration (seconds) in the antrum, pylorus, and duodenum on azithromycin (AZM) administration

	Amplitude (mmHg)			Duration (seconds)		
Beagle number	A	B	C	A	B	C
Antrum						
Baseline (IQR)	4.0 (3.4–21.3)	18.3 (4.2–20.3)	10.3 (5.0–11.1)	2.0 (1.9–2.7)	2.9 (2.1–5.4)	2.1 (1.9–3.6)
AZM infusion (IQR)	292.7 (215.8–479.1)	351.8 (319.8–418.9)	248.5 (195.8–350.0)	10.2 (9.9–10.7)	11.8 (9.5–13.5)	5.5 (5.3–6.3)
Pylorus						
Baseline (IQR)	7.0 (5.1–31.5)	3.6 (3.2–14.8)	6.8 (6.4–7.4)	2.1 (1.9–2.2)	5.0 (2.9–5.3)	1.9 (1.8–2.3)
AZM infusion (IQR)	163.5 (86.5–352.8)	141.0 (131.9–215.5)	145.4 (103.5–208.5)	8.5 (5.1–12.6)	4.7 (4.6–8.1)	3.9 (3.5–4.2)
Duodenum						
Baseline (IQR)	5.2 (4.6–10.2)	8.3 (2.8–13.0)	8.7 (4.9–14.5)	2.2 (2.1–2.5)	2.3 (1.8–6.0)	1.8 (1.6–1.9)
AZM infusion (IQR)	117.3 (88.2–153.5)	174.1 (144.5–191.8)	131.7 (112.0–162.9)	4.0 (2.9–4.6)	4.6 (3.5–5.8)	3.9 (3.7–4.1)

IQR, interquartile rage; SD, standard deviation

Table 3. Median area under the curve (mmHg·s) on azithromycin (AZM) administration

	Beagle A	Beagle B	Beagle C
Antrum			
Baseline (IQR)	32.9 (7.0–40.0)	38.8 (23.5–44.7)	23.5 (9.9–36.5)
AZM infusion (IQR)	4185.6 (2309.1–4886.8)	4415.9 (3586.3–5030.5)	1352.0 (1106.1–2187.9)
Pylorus			
Baseline (IQR)	14.0 (10.7–53.7)	18.5 (17.1–38.7)	13.9 (12.6–19.2)
AZM infusion (IQR)	2141.9 (512.9–3262.1)	765.0 (571.5–1639.4)	517.9 (361.4–838.1)
Duodenum			
Baseline (IQR)	11.5 (9.9–19.7)	36.8 (4.8–49.3)	14.6 (9.7–18.5)
AZM infusion (IQR)	434.1 (334.8–660.1)	767.8 (506.6–959.0)	511.2 (429.8–611.0)

IQR, interquartile rage

Table 4. Median area under the curve (mmHg·s) on azithromycin (AZM) administration

	Beagle A	Beagle B	Beagle C
Antrum			
Baseline	3.440	3.777	3.719
AZM infusion	3.810	4.897	4.512
Pylorus			
Baseline	3.787	3.497	3.617
AZM infusion	4.293	4.642	3.700
Duodenum			
Baseline	3.567	3.690	3.600
AZM infusion	4.493	4.887	4.897

FIGURES

Figure 1. Method of antroduodenal motor activity measurement through percutaneous endoscopic gastrostomy (PEG). (A) A 24-Fr-PEG tube was inserted into the abdominal wall by the pull-type insertion method. (B) The transducer probe was introduced through the PEG tube, and (C) positioned at the gastric antrum, pylorus, and second part of the duodenum; (D) X-ray imaging was performed to confirm the location of each channel of the inserted probe. (E) The contractile activity was measured during intravenous administration of drugs.

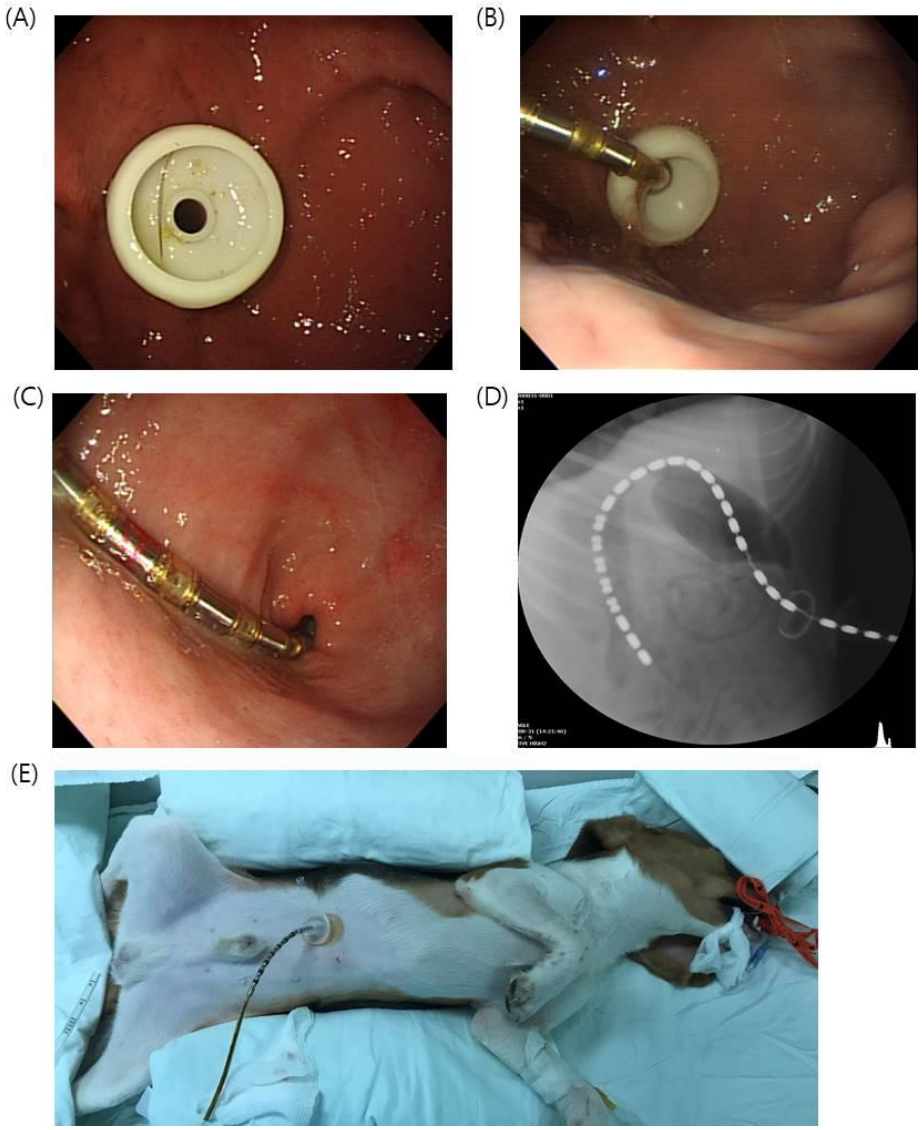
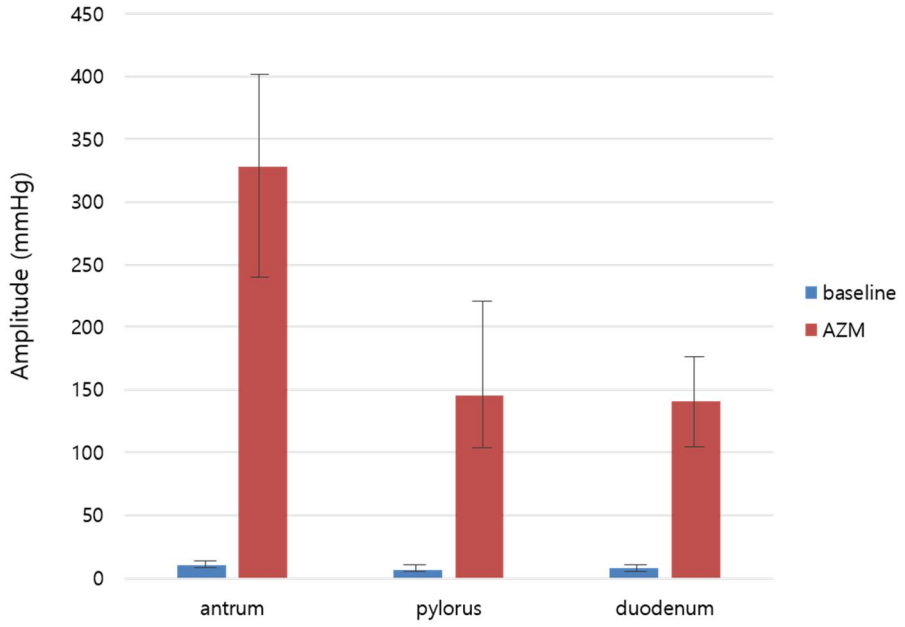


Figure 2. Changes in (A) median amplitude (mmHg) and (B) median duration (seconds) according to azithromycin (AZM) administration

(A)



(B)

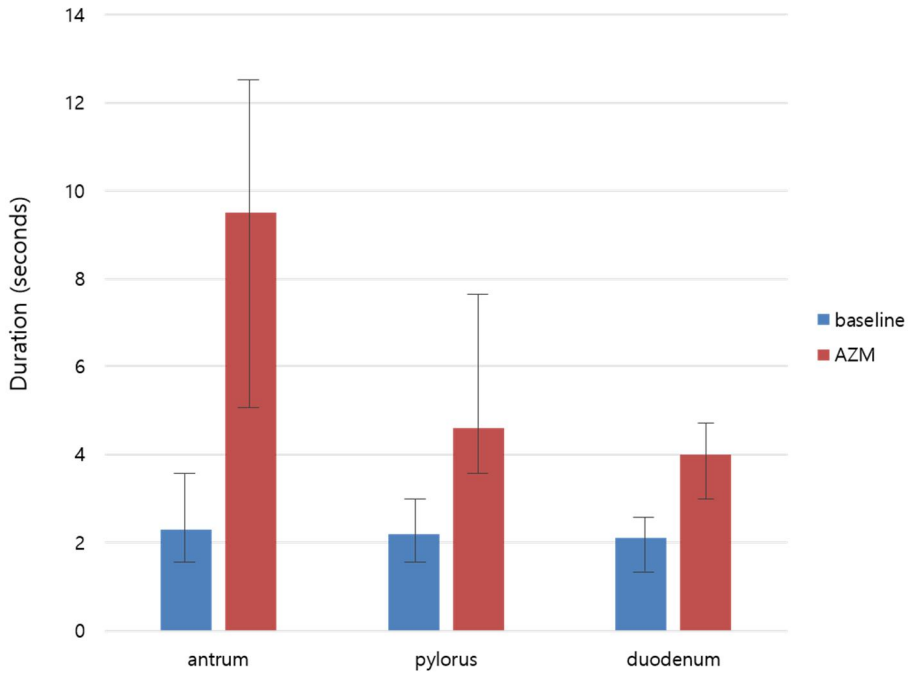


Figure 3. Changes in the median area under the curve (AUC; mmHg·s) according to azithromycin (AZM) administration

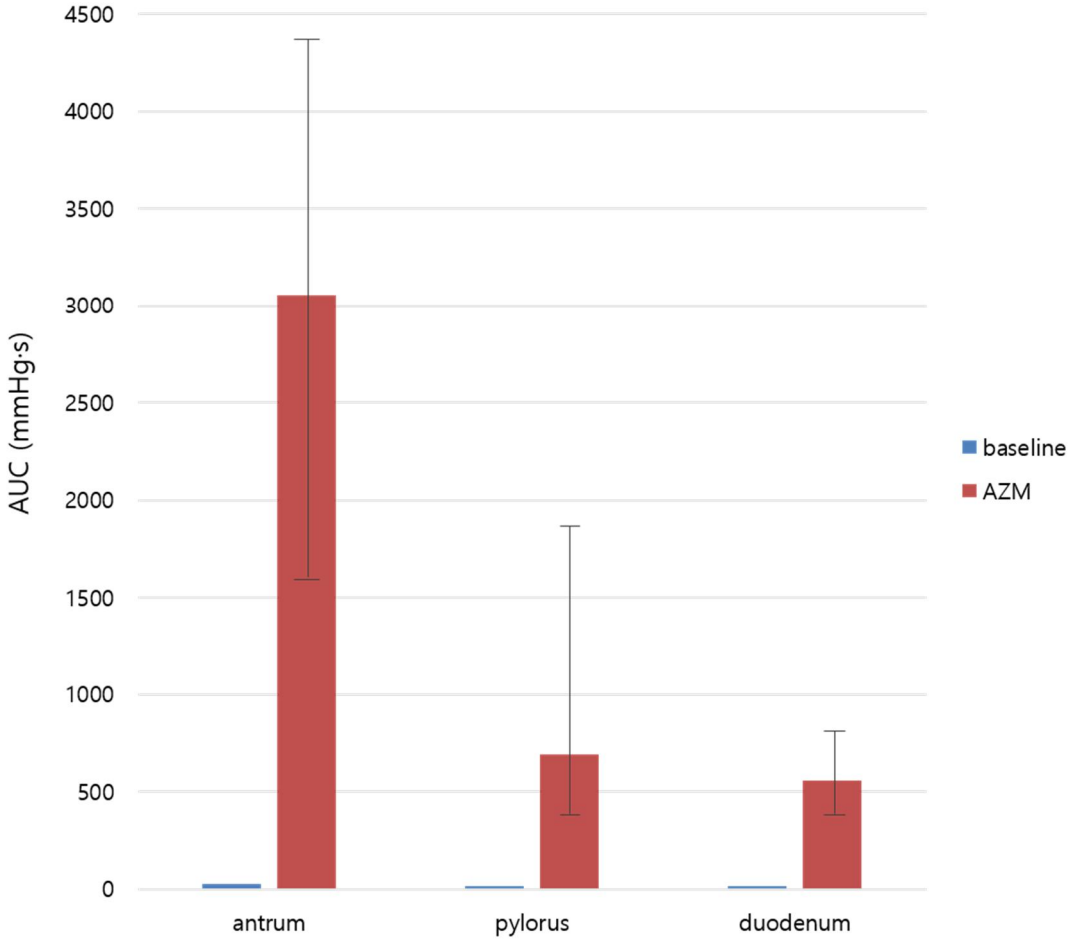


Figure 4. Changes in the motility index according to azithromycin (AZM) administration

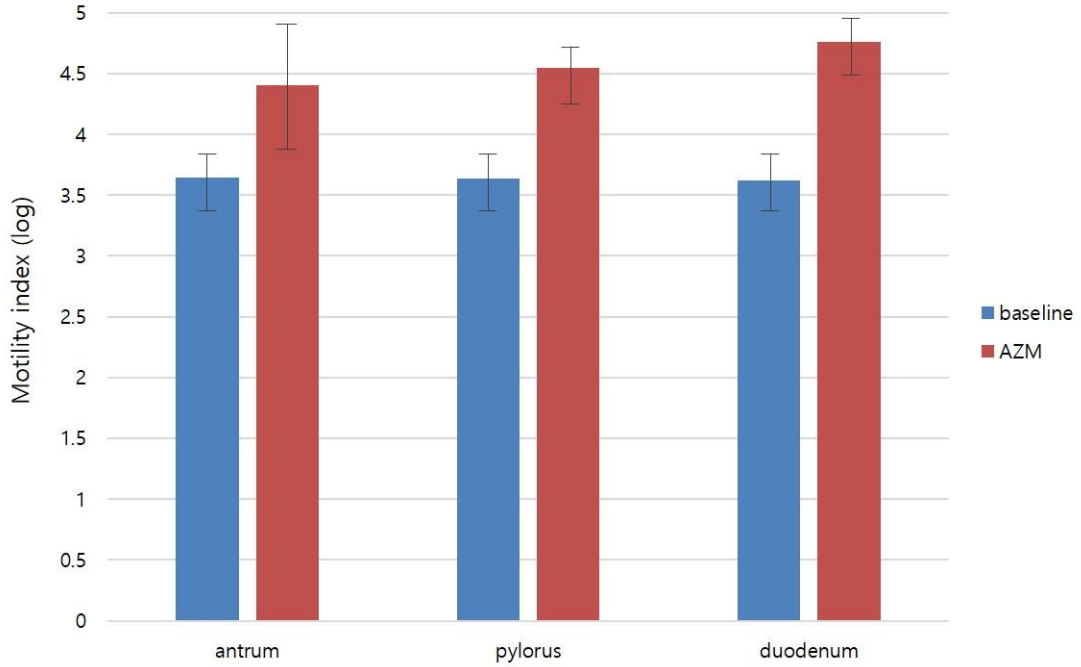
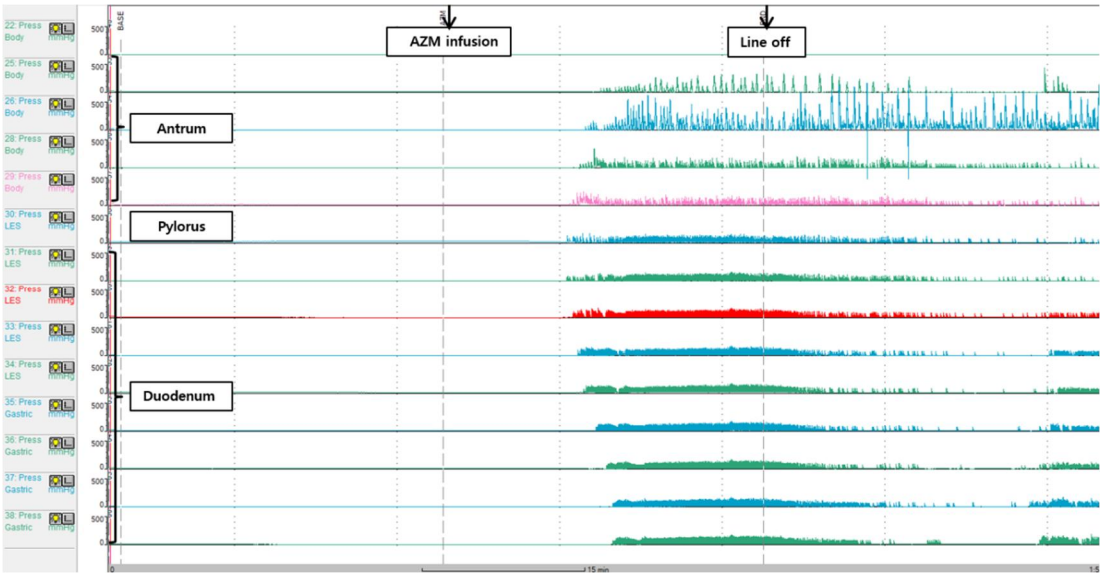
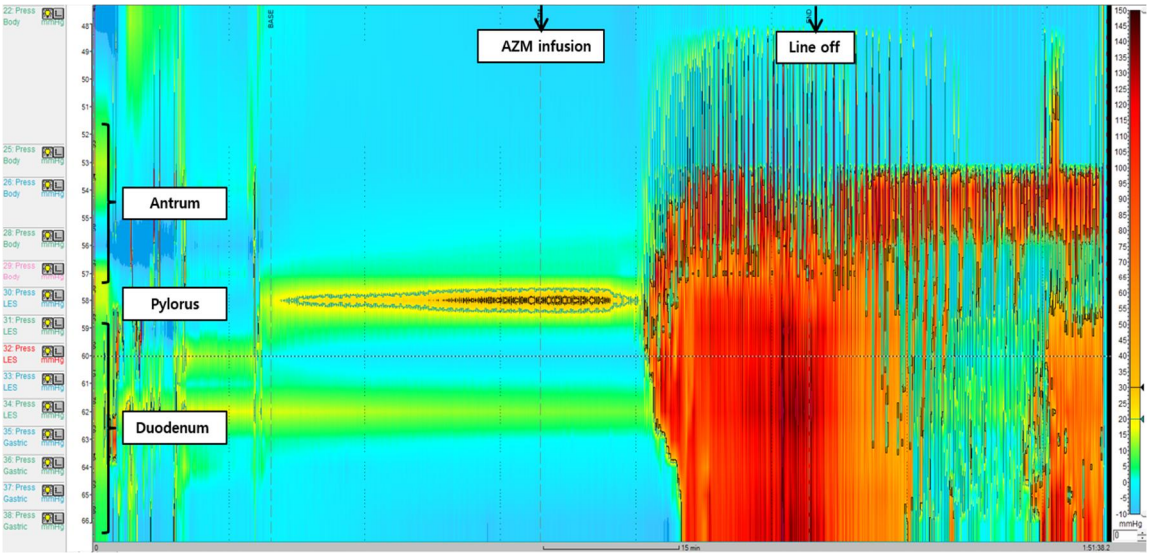


Figure 5. High-resolution manometry image after azithromycin administration. (A) Changes of antroduodenal manometry tracing, and (B) image of high-resolution manometry in the antrum, pylorus, and duodenum

(A)



(B)



국문요약

연구목적: 위장관 운동 촉진제로써 Erythromycin 의 국내 사용이 불가능해 지면서 이를 대체할 수 있는 약제로 같은 macrolide 계열 항생제인 Azithromycin 에 대한 연구가 필요하나, Azithromycin 의 위 전정부 및 십이지장 운동 항진에 대한 연구는 아직 부족한 실정이다. 이번 비임상 연구를 통하여 AZM 의 위-십이지장 운동 항진에 대한 효과를 명확히 하고, 위 전정부에서 원위부 십이지장까지의 수축 패턴을 분석하고자 하였다.

연구방법: 총 3 마리의 수컷 비글에서 1 주일 간격으로 총 3 번의 실험을 하였다. 모든 비글에게 경피 내시경 위절제술을 시행하였고, 이를 통해 고해상도 내압검사 카테터를 삽입하여 Azithromycin 을 정맥 주사 하는 동안의 위-십이지장의 수축을 기록하였고, 이를 후향적으로 분석하였다.

연구결과: 총 9 번의 실험이 시행되었고 매 실험 마다 평균 146.3±5.8 mg 의 Azithromycin 이 투약 되었다. 약물 주사 전과 비교하여 약물 주사 후에 위 전정부, 유문부, 십이지장의 중앙 진폭 및 중앙 기간 값이 현저히 증가하였다. 곡선 아래 면적의 중앙값과 운동지수는 Azithromycin 을 투약하기 전과 비교하여 투약하고 난 후에 위 전정부, 유문부, 십이지장에서 모두 유의하게 증가하였다 ($p < 0.001$).

결론: Azithromycin 은 비글의 위-십이지장 운동성을 향상시켰다. 특히 위 전정부의 운동을 현저히 항진 시켰고, 수축력이 위 전정부에서 유문부를 지나 십이지장으로 전파되는 양상을 보였다. 이는 위 배출 시간을 단축시킴으로써 위

배출 지연 문제가 있는 환자의 치료에 유용할 것으로 예상된다.

중심단어: 전정부-십이지장, 곡선 아래 면적, Azithromycin, 비글견, 고해상도내압검사