FULL PAPER *Pharmacology*

Anti-emetic drug maropitant induces intestinal motility disorder but not anti-inflammatory action in mice

Shoma MIKAWA[1)#], Shohei YAMAMOTO[1)#], Md Shafiqul ISLAM[1)], Noriyuki KAJI[1)], Takahisa MURATA[2)], Risuke MIZUNO[1)], Hiroshi OZAKI[1)] and Masatoshi HORI[1)]*

[1)]*Department of Veterinary Pharmacology, Graduate School of Agriculture and Life Sciences, The University of Tokyo, Tokyo 113–8657, Japan*
[2)]*Department of Animal Radiology, Graduate School of Agriculture and Life Sciences, The University of Tokyo, Tokyo 113–8657, Japan*

(Received 26 March 2015/Accepted 21 April 2015/Published online in J-STAGE 3 May 2015)

ABSTRACT. Maropitant is a neurokinin 1 receptor (NK1R) antagonist that is clinically used as a new anti-emetic drug for dogs. Substance P (SP) and its receptor NK1R are considered to modulate gastrointestinal peristalsis. In addition, SP works as an inflammatory mediator in gastrointestinal diseases. Aim of this study is to clarify the effects of maropitant on intestinal motility and inflammation in mice. *Ex vivo* examination of luminal pressure-induced intestinal motility of whole intestine revealed that maropitant (0.1–10 μM) increased frequency of contraction, decreased amplitude of contraction and totally inhibited motility index in a concentration-dependent manner. We measured intestinal transit *in vivo* by measuring transportation of orally administered luminal content labeled with phenol red. Our results demonstrated that maropitant (10 mg/kg, SC) delayed intestinal transit. Geometric center value was significantly decreased in maropitant-treated mice. Anti-inflammatory effects of maropitant against leukocytes infiltration into the intestinal smooth muscle layer in post-operative ileus (POI) model mice were measured by immunohistochemistry. In POI model mice, a great number of CD68-positive macrophages or MPO-stained neutrophils infiltrated into the inflamed muscle region of the intestine. However, in the maropitant treated mice, the infiltration of leukocytes was not inhibited. The results indicated that maropitant has ability to induce disorder of intestinal motility in mice, but has no anti-inflammatory action in the mouse of a POI model. In conclusion, in mice, maropitant induces disorder of intestinal motility *in vivo*.
KEY WORDS: inflammation, maropitant, neurokinin receptor 1, peristalsis, substance P

doi: 10.1292/jvms.15-0182; *J. Vet. Med. Sci.* 77(10): 1195–1199, 2015

Maropitant is a selective neurokinin 1 receptor (NK1R) antagonist that is clinically used as anti-emetic drugs (Cerenia® by Pfizer Animal Health, Madison, NJ, U.S.A.) for dog [12]. NK1R is expressed in emetic center and chemoreceptor trigger zone (CTZ). Maropitant blocks the binding of substance P (SP), a ligand of NK1R, to the receptor in emetic center and CTZ, resulting in the prevention of emesis.

NK1R is also expressed in many types of cells in intestine. NK1R has been identified on smooth muscle cells, ascending and descending neurons, interstitial cells of Cajal (ICC) at deep muscular plexus and myeloid cells of the villi [14, 19]. In mice, it has been reported that SP / NK1R signaling is involved in non-adrenergic and non-cholinergic neuronally mediated contractions in circular smooth muscle in intestine [4]. On the other hand, SP also stimulates ICC pacemaker activity via NK1R [2, 19]. It is possible that pharmacological substrates for NK1R can modulate gastrointestinal motility.

Ligands for NKRs are tachykinin peptides, including SP, neruokinin A-B, hemokinin-1 and endokinins A-D. SP is primarily found in enteric neuron, but SP is also secreted by inflammatory stimuli from many kinds of immune reactive cells, such as lymphocytes, eosinophils, macrophages and dendritic cells [8]. Secreted SP from neuronal cells and immune cells is considered to accelerate inflammation in an autocrine and/or paracrine manner [6, 15]. So, tachykinin peptides are considered as important inflammatory mediators in gastrointestinal and inflammatory diseases.

Taken together, it is hypothesized that maropitant may inhibit gastrointestinal motility and inflammatory responses. Based on these backgrounds, we investigated the effects of maropitant on intestinal motility in *ex vivo* and *in vivo* assays and leukocytes infiltration into the inflamed muscle layer in post-operative ileus (POI) model mice.

MATERIALS AND METHODS

Measurement of luminal pressure-induced neurogenic contraction of whole intestine in ex vivo: Luminal pressure-induced neurogenic contraction of whole intestine was measured as described previously [10]. Briefly, the mid colon and terminal ileum (about 3 cm) were isolated from C57BL/6 mice and flushed of luminal contents. The samples were cannulated and placed in water-jacked organ bath in modified physiological salt solution (PSS; containing NaCl 136.9 mM, KCl 5.4 mM, $MgCl_2$ 1.0 mM, $CaCl_2$ 1.5 mM, $NaHCO_3$ 23.8 mM, glucose 5.5 mM and EDTA 0.01 mM at PH 7.4) at 37°C in a 95% O_2–5% CO_2 atmosphere. The contraction was measured using a MDL 1401 magnetic blood

*CORRESPONDENCE TO: HORI, M., Department of Veterinary Pharmacology, Graduate School of Agriculture and Life Sciences, The University of Tokyo, Tokyo 113–8657, Japan.
e-mail: ahori@mail.ecc.u-tokyo.ac.jp
#These authors contributed equally to this work.

flow meter (SKALAR, Breda, The Netherlands) equipped with computerized PowerLab system (AD Instruments, Colorado Springs, CO, U.S.A.). Frequency of peristalsis like neurogenic motility, maximum amplitude of peristalsis like neurogenic motility and total motility index (total luminal flow) for 15 min were analyzed. Total motility index was estimated by calculating the area under the curve (AUC) of one neurogenic motility by luminal pressure.

Measurement of intestinal transit in vivo: Intestinal transit *in vivo* was measured by luminal content labeled with phenol red [13, 17]. Briefly, after 18 hr of fasting, the mice (C57BL/6, male, 8–12 weeks age, 12 light-dark cycles) were orally administered 0.25% (w/v) phenol red in 5% (w/v) glucose via a gastric tube. One hour after administration, the gastrointestinal tract was isolated and divided into 15 (stomach; Sto, small intestine; Si1-Si10, cecum; Sec and colon; Co1-Co3) segments at equal intervals. Maropitant was given 30 min before administration of phenol red. The volume of phenol red in each segment and the geometric center of distribution were calculated as previously described [13, 17].

Model mice of POI: POI is one of major diseases of gastroenterological surgery with gastrointestinal motility disorder [9, 16, 20]. Recent works revealed that inflammation of intestinal smooth muscle layer is pathogenesis of POI [13, 17]. Recruited monocyte derived macrophages, neutrophils and activated resident macrophages are considered important causal agents for generating POI. In the present study, we used model mice of POI to analyze anti-inflammatory action of maropitant. The mice were anesthetized with pentobarbital sodium 40 mg/kg i.p. (Somnopentyl; Kyoritsu Seiyaku Corp., Tokyo, Japan). After median section, the distal ileum was exteriorized and then gently scraped three times with a sterile moist cotton applicator (intestinal manipulation; IM). The mice were sacrificed, and the ilea were isolated 24 hr after IM. Maropitant (1, 3, 5 or 10 mg/kg) was administered subcutaneously 1 hr before IM.

Immunohistochemistry and histochemistry: Whole mount ileal muscularis preparations were prepared as described previously [13]. For immunohistochemistry analysis, samples were washed twice with TBS for 30 min and then permeabilized with 0.2% Triton-X-100 at room temperature for 90 min followed by blocking with 2% BSA in TBS at room temperature for 30 min. The samples were then incubated with primary antibody (rabbit anti-human PGP9.5 polyclonal antibody, 1:1,000; Cosmo Bio Co., Ltd., Tokyo, Japan; and rat anti-mouse CD68 antibody, 1:500; Serotec, Dusseldorf, Germany) overnight at 4°C. After washing, the samples were incubated with secondary antibody (Alexa568 goat anti-rabbit IgG, 1:500; Invitrogen, Carlsbad, CA, U.S.A.; and Alexa488 donkey anti-rat IgG, 1:500; Molecular Probes Inc., Eugene, OR, U.S.A.) at room temperature for 1.5 hr. After washing secondary antibody, the samples were examined with a confocal microscope (ECLIPSE Ti; Nikon, Tokyo, Japan). In histochemistry analysis, myeloperoxidase (MPO) histochemical staining was performed as described previously [13]. Observation was performed with an ACT-1C for DXM1200C microscope (Nikon). The number of CD68 positive cells and MPO positive cells were counted in four randomly selected areas of each preparation, and the average number of these cells was calculated.

Statistical analysis: Data were statistically evaluated with an unpaired Student's *t*-test for comparisons between two groups and with one-way ANOVA followed by Tukey's test for comparisons among three or more groups. Values of $P<0.05$ were considered statistically significant.

RESULTS

Effect of maropitant on luminal pressure-induced neurogenic motility of whole intestine in ex vivo: As shown in Fig. 1A, luminal pressure (5 cm H_2O) elicited rhythmic luminal flow in a reflection of contraction and relaxation of mid colon. This rhythmic motility was completely abolished by tetrodotoxin (100 *n*g/m*l*; n=4, data not shown), suggesting that the luminal pressure-induced intestinal motility is neurogenic motility via submucosal / myenteric plexus neural system as previously reported [10]. In the mid colon of control mice, slow and big waves of luminal flow were observed based on fast and small waves of luminal flow. Maropitant (100 nM–10 μM) modulated the neurogenic motility in a concentration-dependent manner (Fig. 1B–1D). Maropitant decreased the big waves of luminal flow and also decreased amplitude, resulting in increased frequency of the neurogenic motility (Fig. 1B and 1C). To reflect these changes, total motility index assessed by AUC was inhibited (Fig. 1D). In the terminal ileum, luminal pressure induced rhythmic luminal flow in a reflection of contraction and relaxation. Maropitant also decreased the rhythmic luminal flow, resulting in the decreased total motility index as shown in Fig. 1E.

Effect of maropitant on intestinal transit in mice: We measured transportation of luminal contents labeled with phenol red within 1 hr after oral administration. In control healthy mice, approximately 5% of luminal content remained inside the stomach, whereas, approximately 75% was transported down the distal ileal part (Si7-Si10) as shown in Fig. 2A. On the other hand, in maropitant (10 mg/kg, SC)-treated mice, approximately 15% of luminal content remained inside the stomach, and the 85% was transported down the intestine. However, the transported luminal content inside the small intestine showed a wide distribution with several peaks (Si4, Si8 and Si10). Maropitant significantly decreased geometric center value, indicating average distribution of luminal content as shown in Fig. 2B (Control; 8.29 ± 0.55, Maropitant; 5.78 ± 0.80, $P<0.05$). These results suggest that maropitant delays intestinal transit *in vivo*.

Effect of maropitant on leukocytes infiltration in POI model mice: In model mice of POI, muscularis inflammation with motility disorder is main symptom [18]. CD68-positive macrophages and MPO-stained neutrophils infiltrated into myenteric plexus and serosal regions in the inflamed intestinal smooth muscle layer [13]. In this experiment, we could confirm the leukocytes infiltration at 24 hr after IM (Neutrophils: control; 0 ± 0, IM; 803.04 ± 58.76, $P<0.01$. Macrophages: control; 533.51 ± 55.53, IM; 3173.72 ± 446.30, $P<0.01$). Maropitant (1 mg/kg–10 mg/kg) had no effect on neutrophils infiltration by IM (Fig. 3A). Maropitant (1 or 3

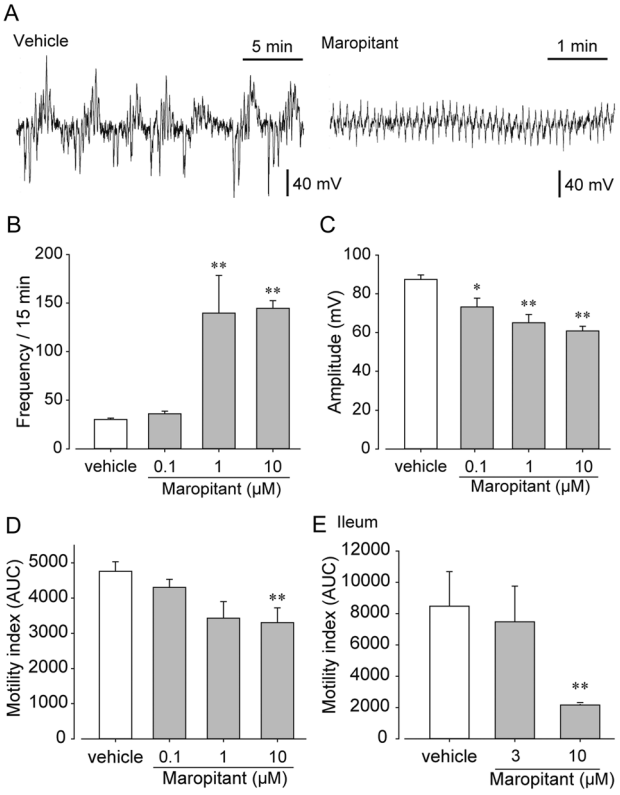

Fig. 1. Effect of maropitant on the luminal pressure-induced neurogenic motility of whole intestine in *ex vivo*. A: Typical result out of 5 independent experiments in mid colon. Left or right panel showed a typical result in the absence or presence of maropitant (1 μM), respectively. Whole colon tissue was treated with maropitant for 15 min before measurement of the motility. B: Frequency of peristalsis like neurogenic motility for 15 min in mid colon. C: Maximum amplitude of peristalsis like neurogenic motility for 15 min in mid colon. D: Total motility index calculated from AUC for 15 min. E: Total motility index in terminal ileum. Each column showed mean ± SEM. *and **showed $P<0.05$ and $P<0.01$ vs vehicle, respectively.

mg/kg) tended to decrease cell number of infiltrated macrophages, but these effects were not significantly different (Fig. 3B). Maropitant (5 or 10 mg/kg) had no effect on the macrophages infiltration by IM.

DISCUSSION

Maropitant is a selective NK1R antagonist, which is a new anti-emetic drug for dogs. Recommended dosage of maropitant is 1 mg/kg SC or 2 mg/kg orally once daily for up to 5 consecutive days for acute emesis and 8 mg/kg orally once daily for up to 2 consecutive days for motion sickness of dogs [12]. Another pharmacokinetics study by using gerbil estimated that plasma concentration ranges of maropitant at 2 or 4 hr after the administration (1 mg/kg SC) are 10.0–25.5 *n*g/m*l* [3], that are calculated to 14.7 μM−37.6 μM. In this study, dosages of maropitant we used are 1 to 10 mg/kg SC in *in vivo* and 1 to 10 μM in *ex vivo*, indicating that concentrations used in this study are dosages within clinical use.

NK1R is expressed in various kinds of cells in murine intestine [4, 19]. NK1R is located on cholinergic neurons, nitrergic neurons, ICC and smooth muscle cells. In general, all NKRs, especially NK1R, are involved in non-adrenergic and non-cholinergic neuronally mediated contractions of circular muscle layer of the intestine. In contrast, it was reported that SP inhibited intestinal peristalsis and gastric activity through nitrergic inhibitory myenteric neuron by stimulating NK1R [7]. In this study, the luminal pressure-induced neurogenic motility in *ex vivo* was measured by using isolated mid colon and terminal ileum. Submucosal / myenteric nervous system is important to detect luminal pressure, which in turn induced neurogenic peristalsis like motility [10]. Maropitant inhibited the neurogenic motility elicited by luminal pressure, suggesting that maropitant induced motility disorder in whole intestine in *ex vivo*. We further investigated the effect of maropitant on intestinal transit in *in vivo* by measuring transportation of luminal content labeled with phenol red. Results indicated that maropitant delayed geometric center value. In the *ex vivo* experiment to measure the luminal pressure-induced neurogenic motility, maropitant increased frequency of motility whereas decreased the amplitude, indicating hyperactivity of the neurogenic motility. The segmented luminal content labeled with phenol red in maropitant-treated mice in *in vivo* may be caused as the results of hyperactivity. Taken together, maropitant caused motility disorder relatively at high concentration range in mice ileum and colon, although detailed mechanism of maropitant to induce intestinal dysmotility in *in vivo* was not identified in this study.

Another function of SP / NK1R signaling is pro-inflammatory effects on various inflammatory diseases [15, 21]. SP produced from T cells, macrophages, dendritic cells and eosinophils can activate NK1R on T cells, which in turn produce IFN-γ to lead to mucosal inflammation in the gastrointestinal tract. NK1R antagonist ameliorates the inflammation in trinitrobenzene sulfonic acid-induced colitis [5] and the non-steroidal anti-inflammatory medication-induced intestinal inflammation in IL-10 null mice [1, 22]. These reports lead to a hypothesis that maropitant may have an anti-inflammatory action to prevent gastrointestinal inflammatory diseases. However, in the murine model of POI, maropitant did not prevent leukocytes infiltration by IM, concluding that maropitant does not have an anti-inflammatory action in mice.

Leffler and colleague reported that NKRs differ to a large degree among animal species with respect to their affinities for antagonists [11]. They cloned NKRs from gerbil, mice, rat, dog and human and transfected those receptors into CHO cells to compare affinity of NKRs antagonists. They concluded that dog NK1R had similar pharmacological characteristics for NK1R antagonists with NK1R of human and gerbil, but different characteristics compared with NK1R of mice and rat. In this report, a selective NK1R antagonist, an aprepitant, that is an anti-emetic drug for human, is clearly less potent at rat NK1R than at NK1R from dog, human and gerbil. As maropitant is a potent anti-emetic drug for dogs, it will be possible that maropitant may have weak affinity for

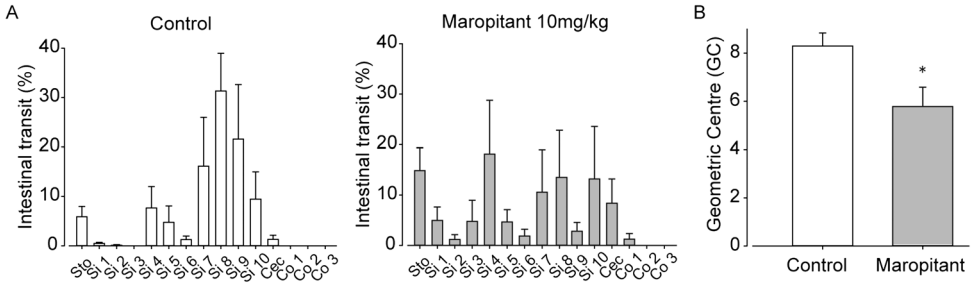

Fig. 2. Effect of maropitant on intestinal transit in *in vivo*. Intestinal transit 1 hr after administration of phenol red was measured *in vivo*. A: Distribution of luminal content labeled with phenol red in segmented gastrointestine with or without maropitant. Maropitant (10 mg/kg) was subcutaneously given 30 min before administration of phenol red. B: Geometric center values were calculated from A. Each column showed mean ± SEM (n=4 each). *showed $P<0.05$ vs Control.

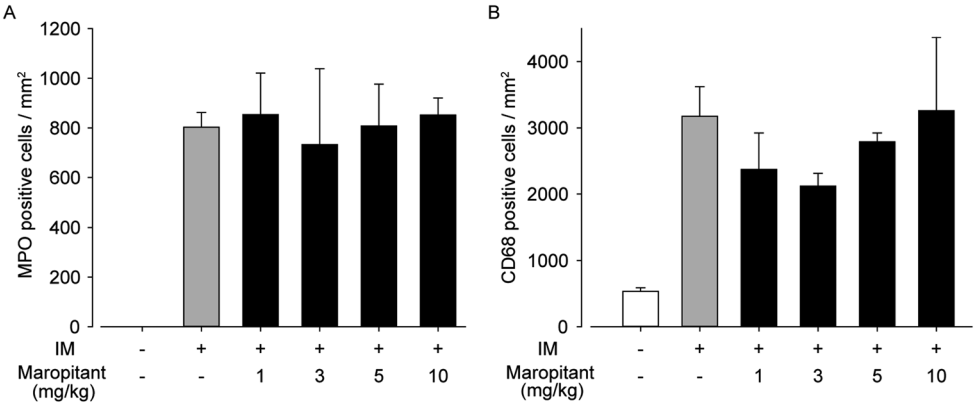

Fig. 3. Effects of maropitant on leukocytes infiltration into the intestinal smooth muscle layer in mice model of POI. Effect of maropitant on MPO-positive neutrophils infiltration (A) and CD68-positive macrophages infiltration (B) by IM. Maropitant (1, 3, 5 or 10 mg/kg) was subcutaneously administered 30 min before IM. Detailed method for quantification of cells number was shown in the Materials and Methods. Each column showed mean ± SEM (n=4–12).

mice NK1R rather than dog, human and gerbil NK1Rs. As emesis has not been observed in common laboratory animals including mice, we could not evaluate anti-emetic efficacy of maropitant to mice by using substitute methods. So, it still remained as unsolved issues whether motility disorder mediated by maropitant is adverse effect or not in mice. Further detailed investigation will be necessary to clarify effects of maropitant on gastrointestinal motility and inflammation by using another laboratory animals and/or dogs.

In conclusion, maropitant induced motility disorder in ileum and colon *ex vivo* and *in vivo* in mice. Maropitant did not show significant anti-inflammatory action assessed by using model mice of POI.

ACKNOWLEDGMENTS. We thank Pfizer Inc. for supplying maropitant. This work was supported by Grant-in-Aid for Scientific Research from the Japanese Ministry of Education to MH and HO. No conflicts of interest, financial or otherwise, are declared by the authors. SY, SM, HO and MH designed the experiments and performed the research; SY, MH, SM, MS, NK, TM and HO discussed the research study; SM, MS, NK, RM and MH wrote the paper.

REFERENCES

1. Berg, D. J., Zhang, J., Weinstock, J. V., Ismail, H. F., Earle, K. A., Alila, H., Pamukcu, R., Moore, S. and Lynch, R. G. 2002. Rapid development of colitis in NSAID-treated IL-10-deficient mice. *Gastroenterology* **123**: 1527–1542. [Medline] [CrossRef]
2. d'antonio, C., Wang, B., McKay, C. and Huizinga, J. D. 2009. Substance P activates a non-selective cation channel in murine pacemaker ICC. 2009. *Neurogastroenterol. Motil.* **21:** 985-e79. [Medline]
3. de la Puente-Redondo, V., Tingley, F. D. 3rd., Schneider, R. P. and Hickman, M. A. 2007. The neurokinin-1 antagonist activity of maropitant, an antiemetic drug for dogs, in a gerbil model. *J. Vet. Pharmacol. Ther.* **30**: 281–287. [Medline] [CrossRef]
4. De Schepper, H. U., De Winter, B. Y., Seerden, T. C., Herman, A. G., Pelckmans, P. A. and De Man, J. G. 2006. The role of tachykinins in circular muscle contractility of the murine ileum: a functional investigation. *Auton. Neurosci.* **126–127**: 273–276.

[Medline] [CrossRef]

5. Di Sebastiano, P., Grossi, L., Di Mola, F. F., Angelucci, D., Friess, H., Marzio, L., Innocenti, P. and Büchler, M. W. 1999. SR140333, a substance P receptor antagonist, influences morphological and motor changes in rat experimental colitis. *Dig. Dis. Sci.* **44**: 439–444. [Medline] [CrossRef]
6. Goode, T., O'Connell, J., Anton, P., Wong, H., Reeve, J., O'Sullivan, G. C., Collins, J. K. and Shanahan, F. 2000. Neurokinin-1 receptor expression in inflammatory bowel disease: molecular quantitation and localisation. *Gut* **47**: 387–396. [Medline] [CrossRef]
7. Holzer, P. 1997. Involvement of nitric oxide in the substance P-induced inhibition of intestinal peristalsis. *Neuroreport* **8**: 2857–2860. [Medline] [CrossRef]
8. Holzer, P. and Holzer-Petsche, U. 2001. Tachykinin receptors in the gut: physiological and pathological implications. *Curr. Opin. Pharmacol.* **1**: 583–590. [Medline] [CrossRef]
9. Kalff, J. C., Schraut, W. H., Billiar, T. R., Simmons, R. L. and Bauer, A. J. 2000. Role of inducible nitric oxide synthase in postoperative intestinal smooth muscle dysfunction in rodents. *Gastroenterology* **118**: 316–327. [Medline] [CrossRef]
10. Kinoshita, K., Horiguchi, K., Fujisawa, M., Kobirumaki, F., Yamato, S., Hori, M. and Ozaki, H. 2007. Possible involvement of muscularis resident macrophages in impairment of interstitial cells of Cajal and myenteric nerve systems in rat models of TNBS-induced colitis. *Histochem. Cell Biol.* **127**: 41–53. [Medline] [CrossRef]
11. Leffler, A., Ahlstedt, I., Engberg, S., Svensson, A., Billger, M., Oberg, L., Bjursell, M. K., Lindström, E. and von Mentzer, B. 2009. Characterization of species-related differences in the pharmacology of tachykinin NK receptors 1, 2 and 3. *Biochem. Pharmacol.* **77**: 1522–1530. [Medline] [CrossRef]
12. Lesman, S. P., Boucher, J. F., Grover, G. S., Cox, S. R. and Bidgood, T. L. 2013. The pharmacokinetics of maropitant citrate dosed orally to dogs at 2 mg/kg and 8 mg/kg once daily for 14 days consecutive days. *J. Vet. Pharmacol. Ther.* **36**: 462–470. [Medline] [CrossRef]
13. Maehara, T., Matsumoto, K., Horiguchi, K., Kondo, M., Iino, S., Horie, S., Murata, T., Tsubone, H., Shimada, S., Ozaki, H. and Hori, M. 2015. Therapeutic action of 5-HT_3 receptor antagonists targeting peritoneal macrophages in post-operative ileus. *Br. J. Pharmacol.* **172**: 1136–1147. [Medline] [CrossRef]
14. Marcello, T., Grakoui, A., Barba-Spaeth, G., Machlin, E. S., Kotenko, S. V., MacDonald, M. R. and Rice, C. M. 2006. Interferons alpha and lambda inhibit hepatitis C virus replication with distinct signal transduction and gene regulation kinetics. *Gastroenterology* **131**: 1887–1898. [Medline] [CrossRef]
15. O'Connor, T. M., O'Connell, J., O'Brien, D. I., Goode, T., Bredin, C. P. and Shanahan, F. 2004. The role of substance P in inflammatory disease. *J. Cell. Physiol.* **201**: 167–180. [Medline] [CrossRef]
16. The, F. O., Boeckxstaens, G. E., Snoek, S. A., Cash, J. L., Bennink, R., Larosa, G. J., van den Wijngaard, R. M., Greaves, D. R. and de Jonge, W. J. 2007. Activation of the cholinergic antiinflammatory pathway ameliorates postoperative ileus in mice. *Gastroenterology* **133**: 1219–1228. [Medline] [CrossRef]
17. Tsuchida, Y., Hatao, F., Fujisawa, M., Murata, T., Kaminishi, M., Seto, Y., Hori, M. and Ozaki, H. 2011. Neuronal stimulation with 5-hydroxytryptamine 4 receptor induces anti-inflammatory actions via α7nACh receptors on muscularis macrophages associated with postoperative ileus. *Gut* **60**: 638–647. [Medline] [CrossRef]
18. van Bree, S. H., Nemethova, A., Cailotto, C., Gomez-Pinilla, P. J., Matteoli, G. and Boeckxstaens, G. E. 2012. New therapeutic strategies for postoperative ileus. *Nat. Rev. Gastroenterol. Hepatol.* **9**: 675–683. [Medline] [CrossRef]
19. Vannucchi, M. G. and Faussone-Pellegrini, M. S. 2000. NK1, NK2 and NK3 tachykinin receptor localization and tachykinin distribution in the ileum of the mouse. *Anat. Embryol. (Berl.)* **202**: 247–255. [Medline] [CrossRef]
20. Wehner, S., Behrendt, F. F., Lyutenski, B. N., Lysson, M., Bauer, A. J., Hirner, A. and Kalff, J. C. 2007. Inhibition of macrophage function prevents intestinal inflammation and postoperative ileus in rodents. *Gut* **56**: 176–185. [Medline] [CrossRef]
21. Weinstock, J. V. 2004. The role of substance P, hemokinin and their receptor in governing mucosal inflammation and granulomatous responses. *Front. Biosci.* **9**: 1936–1943. [Medline] [CrossRef]
22. Weinstock, J. V., Blum, A., Metwali, A., Elliott, D., Bunnett, N. and Arsenescu, R. 2003. Substance P regulates Th1-type colitis in IL-10 knockout mice. *J. Immunol.* **171**: 3762–3767. [Medline] [CrossRef]