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Adenosine A_1 receptor blockade reverses experimental postoperative ileus in rat colon

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Abstract

Inhibition of colonic propulsive motility is the main contributor to postoperative ileus in humans. Experimental models for investigating colonic propulsion in surgically induced postoperative ileus have not previously been available. This study was designed to assess whether adenosine A_1 receptor antagonists (*R*)-1-[(*E*)-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl) acryloyl]-piperidin-2-yl acetic acid (FK352) and 8-cyclopentyl-1, 3-dipropylxanthine (DPCPX) reverse the colonic motility disorder in newly developed rat experimental ileus models. When rats underwent anesthesia (pentobarbital) or surgical traumas (partial gastrectomy, cecectomy or gentle touching of the colon with fingers), colonic propulsive motility was evaluated by migration of intracolonically injected dye in awake unrestrained rats. Propulsive motility resulted in significant decrease after the treatment of the anesthesia or partial gastrectomy. Intravenous administration of either adenosine A_1 receptor antagonist reversed the slowed colonic propulsion in these experimental ileus models. The present study suggests that the blockade of adenosine A_1 receptors has therapeutic potential for postoperative ileus.

Keywords: Postoperative ileus; Colon; (Rat); Adenosine A_1 receptor, antagonist

1. Introduction

''Adynamic ileus'' is a common complication after abdominal surgery (see reviews by Furness and Costa, 1974; Livingston and Passaro, 1990). Patients with this disorder accumulate gas and fluids leading to abdominal distension, anorexia, nausea, vomiting and visceral pain. In humans, immobility of the colon is the major determinant of the extent and duration of postoperative ileus. Rather than mere gastrointestinal contractility, propulsive activity is lacking in postoperative ileus. Despite the importance of colonic propulsive motility for postoperative ileus, investigation of colonic propulsion has never been performed in rat models of postoperative ileus. The pathophysiology of postoperative ileus is therefore still being debated and so it follows that no therapy to specifically eliminate motility disorder underlying postoperative ileus has been forthcoming.

Recently, we have found that adenosine can act at presynaptic adenosine A_1 receptors to suppress synaptic neurotransmission in the guinea pig colon (Kadowaki et al., 2000a) and mRNA of adenosine A_1 receptor is abundantly expressed in the colon of guinea pigs (Kadowaki et al., 2000a) and rats (Kadowaki et al., 2000b). We have also demonstrated that in the isolated guinea pig colon 8-cyclopentyl-1, 3-dipropylxanthine (DPCPX) is a selective and potent adenosine A_1 receptor antagonist (pA2 value of 8.8 for adenosine A_1 receptor versus 6.6 for adenosine A_{2B} receptor), whereas 8-phenyltheophylline (8-PT) is a weak adenosine A_1 receptor antagonist and nonselective adenosine A_1 and A_{2B} receptor antagonist (pA2 value of 6.5 for adenosine A_1 receptor versus 5.7 for adenosine A_{2B} receptor) (Kadowaki et al., 2000a). In addition, we have revealed that adenosine A_1 receptor antagonist is a new prokinetic drug for the colon that accelerates basal colonic propulsive motility and has therapeutic potential for the treatment of ischemia-reperfusion-induced motility disorder in the colon (Kadowaki et al., 2000b).

In the present study, we focused on colonic propulsive motility and designed experiments to determine whether selective adenosine A_1 receptor antagonists [(*R*)-1-[(*E*)-3-(2-

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phenylpyrazolo[1,5-*a*]pyridin-3-yl) acryloyl]-piperidin-2-yl acetic acid (FK352; Maemoto et al., 1997) and DPCPX and nonselective adenosine receptor antagonist 8-PT restore colonic propulsion in rat models of experimental postoperative ileus.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (250–330 g) were used. The use and treatment of animals followed the European Community guidelines as accepted principles for the use of experimental animals and the Guide to Animal Use and Care of Fujisawa Pharmaceutical.

2.2. Evaluation of colonic propulsive motility

After the rat was anesthetized with pentobarbital (50 mg/kg, i.p.), a chronic indwelling polyethylene cannula was implanted into the proximal colon about 1.5 cm from the ileocecal junction to infuse a red dye (carmine) as a non-absorbable marker. The cannula was led subcutaneously to the interscapular region of the animal's neck and the rat was allowed to recover from surgery for 4–5 days. For the evaluation of colonic propulsion in awake and unrestrained rats, the gentle infusion of carmine and intravenous injection of adenosine receptor antagonists were simultaneously carried out and then 2 h later, the animals were stunned by a blow to the head and exsanguinated. The entire colon was carefully and quickly removed and the length of the colon colored by carmine was measured and expressed as a percentage of the total length of the colon.

2.3. Ileus model by anesthesia

Experimental ileus was induced by intraperitoneal administration of pentobarbital sodium (50 mg/kg) in the cannula-implanted rats; control rats received saline instead of pentobarbital. One hour later, carmine was infused into the proximal colon and then the colonic transit was evaluated.

2.4. Ileus model by surgical trauma

The cannula-implanted rats were fasted for 24 h before the following operations with free access to water and were divided into four groups in a randomized way. The following operations were done under pentobarbital anesthesia. The first group (sham operation group) underwent only a laparotomy consisting of a 5-cm incision from the processus xiphoideus. The second group underwent a cecectomy (Fondacaro et al., 1990). The cecum was ligatured without compromising ileocolonic patency and then was resected. The third group underwent a partial gastrectomy similar to the procedure for preparation of chronic denervated gastric pouches in rat (Alphin and Lin, 1959). A clamp was applied along a line 1–2 cm apart from the greater curvature of the stomach, and the blood vessels supplying the "squamous" pouch were ligatured and severed. After a lot of care was taken so as not to injure the blood vessels supplying the main stomach and to not prevent transit of the luminal contents in the main stomach, an incision was made along the line and the pouch was resected. The stomach wall was then finely sutured. In the fourth group, the experiment was performed basically according to the procedure in the upper gastrointestinal tract described by De Winter et al. (1998). Colonic transit was measured in rats that underwent laparotomy and following "surgical manipulation". The colon was gently touched with the fingers, starting from the ileocecal junction up to the distal end of the distal colon. This procedure was repeated three times over 5 min.

Colonic transit was evaluated in all of the operated rats from these four groups 24 h later to avoid the influence of pentobarbital anesthesia.

2.5. Statistics

Values for the experiments represent mean ± S.E.M. The data from the ileus models were compared by analysis of variance (ANOVA) followed by Dunnett's multiple range test. Probability values of 0.05 or less were considered statistically significant.

2.6. Drugs

(*R*)-1-[(*E*)-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl) acryloyl]-piperidin-2-yl acetic acid (FK352) was synthesized by Fujisawa Pharmaceutical (Osaka, Japan). 8-Cyclopentyl-1, 3-dipropylxanthine (DPCPX) and 8-phenyltheophylline (8-PT) were purchased from Research Biochemicals International (Natick, MA, USA). Carmine was from Sigma (St. Louis, MO, USA) and pentobarbital sodium was from Dainippon Pharmaceutical (Osaka, Japan). FK352, DPCPX and 8-PT were initially dissolved in dimethyl sulphoxide, and then diluted in physiological saline (final concentration of dimethyl sulphoxide was 0.1%) for intravenous administration. The sham-operated and control animals were injected with the vehicle.

3. Results

3.1. Ileus model by anesthesia

Rats all developed a decrease in colonic propulsion in response to intraperitoneal administration of pentobarbital (50 mg/kg), the colonic transit being significantly ($P < 0.01$) slowed from 86.3 ± 3.2% (saline, $n = 12$) to 56.0 ± 2.4% (pentobarbital, $n = 11$, Fig. 1). Administration of FK352 (0.1 and 1.0 mg/kg) dose-dependently and significantly improved

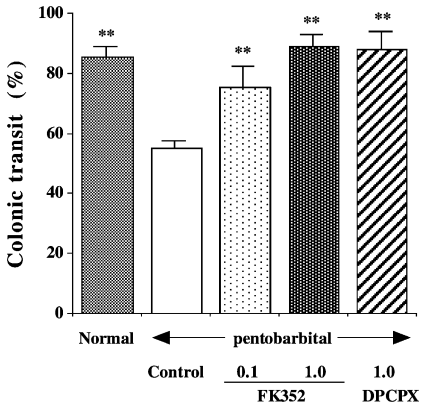

Fig. 1. Adenosine A_1 receptor antagonists, FK352 (0.1 and 1.0 mg/kg, i.v.) and DPCPX (1.0 mg/kg, i.v.), significantly improved reduced colonic transit induced by intraperitoneal injection of pentobarbital (50 mg/kg) in rats. The nonabsorbable marker carmine was infused 1 h after pentobarbital injection and test drugs or vehicle were intravenously administered at the same time. Distance traveled by carmine for 2 h was measured. The ratio of the length of the colon marked by carmine to the total length of the colon was recorded, and data are expressed as the mean ± S.E.M. for 10 to 12 animals. $^{**}P<0.01$ compared with the vehicle-injected control group.

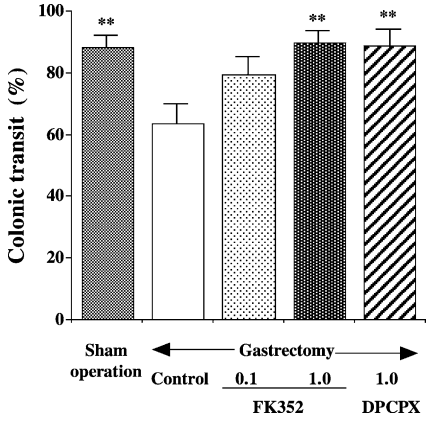

Fig. 3. Adenosine A_1 receptor antagonist, FK352 (0.1, 1.0 mg/kg, i.v.) and DPCPX (1.0 mg/kg, i.v.), significantly reversed slowed colonic transit after partial gastrectomy in rats. The nonabsorbable marker carmine was infused 24 h after the gastrectomy and test drugs or vehicle were intravenously administered at the same time. Distance traveled by carmine for 2 h was measured. The ratio of the length of the colon marked by carmine to the total length of the colon was recorded, and data are expressed as the mean ± S.E.M. for 10 to 12 animals. $^{**}P<0.01$ compared with the vehicle-injected control group.

the slowed colonic transit, abolishing the effect of pentobarbital at 1.0 mg/kg (90.3 ± 3.8%, $n=10$, $P<0.01$ compared with control, Fig. 1). Likewise, a decrease in colonic propulsion was not observed after pretreatment with DPCPX at 1.0 mg/kg (89.3 ± 5.3%, $n=10$, $P<0.01$ compared with control, Fig. 1), whereas 8-PT (0.1 mg/kg, i.v.) failed to affect the slowed colonic transit (60.4 ± 7.2%, $n=5$).

3.2. Ileus model by surgical trauma

In the sham operation group, carmine migrated over a distance of 97.5 ± 1.2% of total length of the colon ($n=5$, Fig. 2). Neither the cecectomy nor the "surgical manipulation" significantly altered colonic transit (99.7 ± 0.3%, $n=5$ and 91.1 ± 5.5%, $n=5$, Fig. 2). In contrast, partial gastrectomy resulted in slowed colonic transit (71.8 ± 8.9%, $P<0.05$ compared with sham operation, $n=5$, Fig. 2). Consequently, the partial gastrectomy was chosen as the surgical trauma for the experimental ileus model. FK352 (0.1 and 1.0 mg/kg) dose-dependently reversed the reduced propulsive motility in gastrectomized rats [80.0 ± 5.7%, $n=10$ and 89.9 ± 3.8%, $n=10$ ($P<0.01$ compared with control; 63.9 ± 6.1%), respectively, Fig. 3] and thus completely restored the motor function at a dose of 1.0 mg/kg [compared with sham operation (88.6 ± 3.9%, $n=10$), Fig. 3]. Likewise, administration of DPCPX completely improved the slowed colonic transit (1.0 mg/kg; 89.3 ± 5.1%, $n=10$, $P<0.01$ compared with control, Fig. 3), whereas 8-PT (0.1 mg/kg, i.v.) failed to affect the slowed colonic transit (64.2 ± 6.0%, $n=5$).

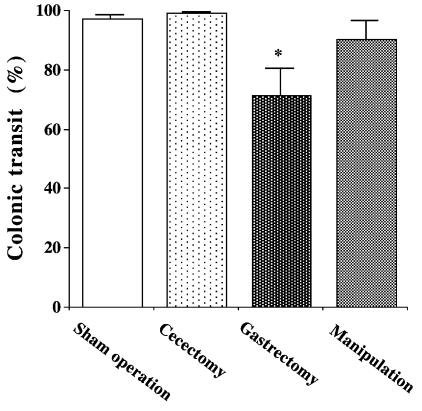

Fig. 2. Colonic transit was largely slowed only after partial gastrectomy in rats. Surgical traumas took place 24 h before infusion of the nonabsorbable marker carmine. Distance traveled by carmine for 2 h was measured. The ratio of the length of the colon marked by carmine to the total length of the colon was recorded, and data are expressed as the mean ± S.E.M. for five animals. $^{*}P<0.05$ compared with sham operation group.

4. Discussion

Anesthetic agents, which are always used in abdominal surgery, are thought to be one of the pathogeneses for postoperative ileus as most anesthetic agents can stabilize and inactivate cell membranes. Indeed, pentobarbital caused a decrease in rat colonic propulsion in the present study. In contrast, a significant delay has been never observed in the colonic propulsive motility of rat models from the surgical manipulation, which is inconsistent with the previous studies in the rat upper gastrointestinal tract (De Winter et al., 1998). The main reason for this discrepancy is likely due to the site of evaluation and/or recovery time after operation.

Therefore, we designed abdominal surgery-induced ileus models in the rat colon. For cecectomy, normal pellet

outputs were only observed in cecectomized rats and the propulsive colonic motility did not differ from that of sham-operated rats in 24 h following the cecectomy. This result is consistent with a previous report (Fondacaro et al., 1990). Thus, the cecum cannot greatly regulate colonic motility in rats. In contrast, colonic transit was significantly decreased after partial gastrectomy, compared to sham operation, indicating that the stomach has more serious consequences than the cecum in propulsive motility of the colon and that there is close interaction between the stomach and colon probably through some extrinsic neuronal pathway. Therefore, these rat models of experimental postoperative ileus are useful for understanding the pathophysiology of postoperative ileus and evaluation of potential therapeutic agents.

Several therapeutic agents have been proposed for postoperative ileus (Livingston and Passaro, 1990; Furness and Costa, 1974). However, the prokinetics [metoclopramide (Jepsen et al., 1986), motilin agonist (Ruppin et al., 1976) and cisapride (Roberts et al., 1995)] failed to significantly improve postoperative ileus in humans. Almost all prokinetics are drugs of choice for the treatment of functional motility disorders in upper gastrointestinal tract, however, potent prokinetics in the colon are not yet available (Tonini, 1996). Therefore, it follows that a new class of potent prokinetic agents in the colon is needed to improve postoperative ileus. Adenosine A_1 receptor antagonist would be expected to become a candidate because intravenous administration of selective adenosine A_1 receptor antagonist [FK352 (0.1 and 1.0 mg/kg, i.v.) and DPCPX (1.0 mg/kg, i.v.)] significantly enhances the basal colonic propulsion, and reverses the ischemia-reperfusion-induced colonic dysmotility in conscious rats (Kadowaki et al., 2000b). On the other hand, nonselective adenosine receptor antagonist 8-PT which is equi-effective with DPCPX at adenosine A_{2B} receptor in in vivo study (Hancock and Coupar, 1995) has no effect on these basal and slowed colonic motilities at 1.0 mg/kg, i.v. (Kadowaki et al., 2000b). These findings indicate that DPCPX (1.0 mg/kg, i.v.) behaves as selective adenosine A_1 receptor antagonist on the colonic propulsive motility and that adenosine A_1 receptor but not A_{2B} receptor is involved in regulating the colonic motility.

In the present study, FK352 and DPCPX, but not 8-PT completely restored propulsive motility in two different types of experimental dysmotility models in the colon, which is in close agreement with our previous result in the ischemia-reperfusion-induced colonic dysmotility model (Kadowaki et al., 2000b). Nevertheless, the dye propulsive velocity is much faster in the proximal colon than the distal colon and the anesthesia and surgical traumas in our postoperative ileus models made the dye stay the proximal portion of the distal colon for at least 2 h (~ 56–72% of the total length of the colon). Thus, we have never observed the significant difference in the dye propulsion between the ileus model and the control for a propulsion time shorter than 2 h and could not develop the experimental ileus model till we employed 2-h propulsion time. Consequently, the present data do not tell us whether adenosine A_1 receptor antagonists simply stimulate colonic propulsive motility or counteract the inhibitory effect of the anesthesia or surgery.

In addition, we have evaluated FK352 in rat loperamide- and clonidine-induced constipation models, and FK352 partially but significantly reversed the constipation in both models (unpublished observation). The present study further verifies that endogenous adenosine exerts a sustained and potent inhibitory effect on colonic motor function through adenosine A_1 receptor, not only under normal but also in pathophysiological conditions.

In conclusion, adenosine A_1 receptor antagonist has therapeutic potential for various kinds of functional motility disorders in the colon, including postoperative ileus.

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