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Short communication

Adenosine A_1 receptor blockade reverses dysmotility induced by ischemia–reperfusion in rat colon

Makoto Kadowaki *, Kenichi Tokita, Yasunori Nagakura, Masahiro Takeda, Kaori Hanaoka, Masaaki Tomoi

Pharmacological Research Laboratories, Fujisawa Pharmaceutical Co. Ltd., Osaka 532-0031, Japan

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Abstract

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 dysmotility induced by ischemia–reperfusion in the rat colon. The gene of adenosine A_1 receptor was expressed in the colon. Clamping (30 min) of the colonic marginal vessels was followed by reperfusion, and the propulsive colonic motility was evaluated. Propulsion was significantly slowed by ischemia–reperfusion, while FK352 and DPCPX abolished this delay. In contrast, the non-selective adenosine receptor antagonist, 8-phenyltheophylline, failed to affect the dysmotility. Thus, adenosine A_1 receptor antagonists have potent therapeutic potential against ischemia–reperfusion-induced dysmotility in the colon.

Keywords: Dysmotility; Ischemia–reperfusion; Colon; (Rat); Adenosine A_1 receptor antagonist; RT-PCR (reverse transcription-polymerase chain reaction)

1. Introduction

Transient abdominal ischemia, such as that caused by surgery of abdominal aortic aneurysm, organ transplantation and spontaneous ischemia, lead to profound functional and structural alterations of the gastrointestinal tract, which contribute to disorders of intestinal motility (Hierholzer et al., 1999; Greenwald and Brandt, 1998) and affect nutrient absorption or the intestinal barrier function against bacterial translocation. Spontaneous ischemic disease of the intestine, which is a frequent disease among elderly people and predominantly affects the left and sigmoid colon (Greenwald and Brandt, 1998), may be the result of a variety of clinical conditions associated with a decrease or redistribution of blood flow, such as abdominal angina, mesenteric embolus, and chronic mesenteric arterial insufficiency caused by hyperglycemia, hyperlipemia and/or aging. However, little is known about the effect of transient ischemia on intestinal motor function. It has been demonstrated that transient occlusion of the mesenteric artery significantly delays small intestinal transit in vivo (Alican et al., 1998) and that hypoxia–reoxygenation significantly reduces intestinal motility in vitro (Bielefeldt and Conklin, 1997; Hierholzer et al., 1999).

It has been reported that adenosine A_1 receptor antagonists increase defecation in rats (Suzuki et al., 1995). However, the physiological role of adenosine in the gastrointestinal tract is still poorly understood, particularly with regard to colonic motor function (Bailey and Hourani, 1992). Recently, we have demonstrated that adenosine receptor agonists mediate relaxation through presynaptic adenosine A_1 receptors on the enteric neurons of the guinea pig distal colon (Kadowaki et al., 2000). This finding greatly motivated us to define the physiological role of adenosine A_1 receptors and the therapeutic potential of adenosine A_1 receptor antagonists to correct alterations in colonic dysmotility.

The present study was designed to determine whether new prokinetic drugs, adenosine A_1 receptor antagonists, affect basal colonic propulsion and restore colonic propulsion in the model of the experimental ischemia–reperfusion-induced motility disorder.

* Corresponding author. Department of Neuroscience, University of Pennsylvania School of Medicine, 234 Stemmler Hall, Philadelphia, PA 19104-6074, USA. Tel.: +1-215-898-2441; fax: +1-215-573-2015.

E-mail address: makotok@mail.med.upenn.edu (M. Kadowaki).

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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. Reverse transcription-polymerase chain reaction for gene expression of adenosine A_1 receptors

Tissue samples (479–1088 mg) were obtained from the rat gastrointestinal tract and kidney (a positive control). The reverse transcription-polymerase chain reaction (RT-PCR) protocol for messenger RNA (mRNA) of adenosine A_1 receptors was previously reported in detail (Kadowaki et al., 2000). Briefly, total RNAs were reverse-transcribed by Moloney Murine Leukemia Virus reverse transcriptase (GIBCO BRL, Grand Island, NY, USA) and single-stranded cDNA products were denatured and amplified by PCR with Taq DNA polymerase (Toyobo, Osaka, Japan).

Adenosine A_1 receptor genes were amplified for 40 cycles with sense primer nucleotide sequences of 37–60 and antisense primer of 490–513 of the rat adenosine A_1 receptor (Reppert et al., 1991). The predicted size of the PCR products was 477 base pairs (bp). The total RNAs were also subjected to RT-PCR for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which was used as a housekeeping gene (an internal control). The expected product size was 311 bp.

2.3. Evaluation of colonic propulsive motility

The operation was carried out as previously reported (Nagakura et al., 1996). After rats were anesthetized with pentobarbital (50 mg/kg i.p.), a chronic indwelling polyethylene cannula (PE-50; Becton, Dickinson and Co., Parsippany, NJ, USA) was implanted into the proximal colon about 1.5 cm from the ileocecal junction to infuse carmine as a non-absorbable marker. The cannula was led subcutaneously to the interscapular region of the animal's neck. The abdominal incision was closed with a suture. Rats were allowed to recover from surgery for 5 days and almost all rats survived in good health. For the evaluation of basal colonic propulsion in awake and unrestrained rats, the animals were killed 40 min after gentle infusion of carmine and the entire colons were carefully and quickly removed. The length of the colon colored by carmine was measured and expressed as a percentage of the total length of the colon. Adenosine receptor antagonists were intravenously administered 30 min before the infusion of carmine.

2.4. Ischemia–reperfusion

The cannula-implanted rats were assigned to three groups: sham operation (laparotomy), ischemia and ischemia–reperfusion groups. Thirty minutes after pentobarbital administration, in the rats in the ischemia group ligatures with silk suture were applied to the marginal vessels at two points on the splenic flexure and 2 cm distal to it. In the ischemia–reperfusion group, ischemia was performed by clamping the marginal vessels at the same points as in the ischemia group with atraumatic microvessel clamps, which were released 30 min later. In both groups, carmine was infused 1 h after ligation or clamping and colonic propulsion was evaluated 5 h later. Adenosine receptor antagonists were intravenously injected 3 h after the release of clamping.

Blood flow in the colon was measured with a laser-Doppler blood flow meter (FLO-C1; OMEGA WAVE, Tokyo, Japan). A blood flow probe was gently positioned upon the serosal surface at the middle of the two clamping points.

2.5. Statistics

Values for the experiments represent means ± S.E.M. The data for colonic motility were compared by analysis of variance (ANOVA) followed by Dunnett's multiple range test. Paired *t*-test was used for comparisons of colonic blood flow. 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 (Maemoto et al., 1997). 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX) and 8-phenyltheophylline were purchased from Research Biochemicals International (Natick, MA, USA). All drugs were initially dissolved in dimethyl sulphoxide (500 mg/ml) and then diluted in physiological saline. The normal, sham-operated and control animals were injected with the vehicle (0.2% dimethyl sulphoxide).

3. Results

3.1. RT-PCR

RT-PCR analysis for adenosine A_1 receptor showed the expression of a single product of approximately 477 bp throughout the gastrointestinal tract and in the kidney (Fig. 1). Furthermore, in comparison with the expression of

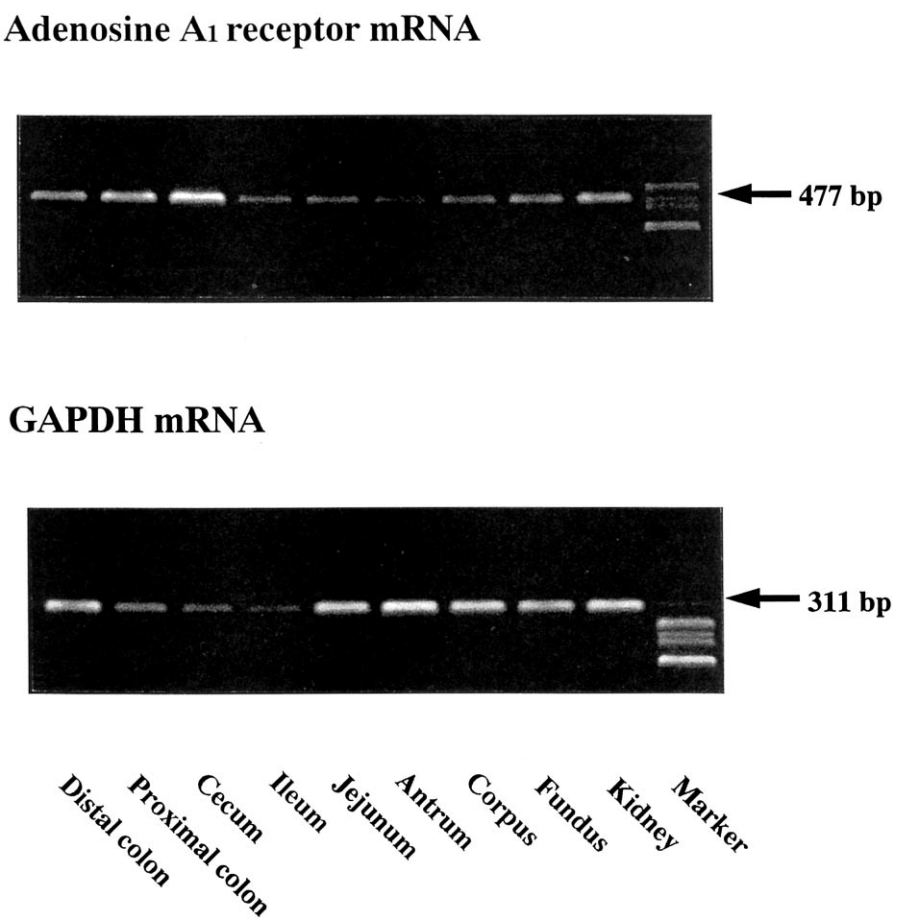

Fig. 1. Tissue distribution of adenosine A_1 receptor mRNA in the rat gastrointestinal tract as determined by RT-PCR. The kidney was used as a positive control and GAPDH mRNA served as a housekeeping gene. The PCR products were analyzed electrophoretically on 2% agarose gels and visualized with ethidium bromide.

GAPDH in the same tissue, the greatest amplification was found in the stomach followed by the colon, and the lowest in the jejunum and ileum. Moreover, no detectable expression was observed without reverse transcriptase; the specificity of RT-PCR products was verified by digestion with specific endonucleases (*Alu*I, Toyobo).

3.2. Basal colonic propulsion

Intravenous administration of FK352 (0.01 to 1.0 mg/kg) dose-dependently and significantly accelerated colonic propulsion in normal rats from $56.7 \pm 5.1\%$ (vehicle, $n = 10$) to $62.0 \pm 6.5\%$ (0.01 mg/kg, $n = 10$), $82.0 \pm 8.3\%$ (0.1 mg/kg, $P < 0.01$, $n = 10$) and $85.9 \pm 6.9\%$ (1.0 mg/kg, $P < 0.01$, $n = 10$). Likewise, DPCPX also significantly enhanced colonic propulsion at 1.0 mg/kg ($83.5 \pm 5.2\%$, $P < 0.01$, $n = 10$). In contrast, the non-selective adenosine receptor antagonist, 8-phenyltheophylline, had no effect on basal colonic propulsion (1.0 mg/kg, $61.7 \pm 7.2\%$, $n = 10$).

3.3. Dysmotility induced by ischemia–reperfusion

The steady-state baseline microcirculatory blood flow in the colon was 44.8 ± 6.7 ml/min per 100 g ($n = 5$). The blood flow significantly decreased to $62.4 \pm 3.6\%$ of baseline ($P < 0.05$, $n = 5$) during clamping of the marginal vessels and quickly returned to $83.2 \pm 14.2\%$ of baseline ($n = 5$) after the release of clamping.

The propulsive motility was significantly reduced from $87.1 \pm 3.0\%$ (laparotomy, $n = 15$) to $74.4 \pm 4.7\%$ ($P < 0.05$, $n = 15$) in the ischemia group, and ischemia–reperfusion further significantly slowed propulsion ($56.5 \pm 4.0\%$, $n = 15$) as compared to laparotomy ($P < 0.01$) and ischemia ($P < 0.01$).

FK352 dose-dependently and significantly reversed the slowed colonic propulsion from $60.1 \pm 4.8\%$ (vehicle, $n = 15$) to $73.1 \pm 3.2\%$ (0.1 mg/kg, $P < 0.05$, $n = 15$) and $92.0 \pm 2.9\%$ (1.0 mg/kg, $P < 0.01$, $n = 17$), respectively (Fig. 2A). DPCPX (1.0 mg/kg) also accelerated the slowed propulsion from $60.5 \pm 5.0\%$ (vehicle, $n = 11$) to $91.7 \pm 3.9\%$ ($n = 15$, $P < 0.01$, Fig. 2B). 8-Phenyltheophylline

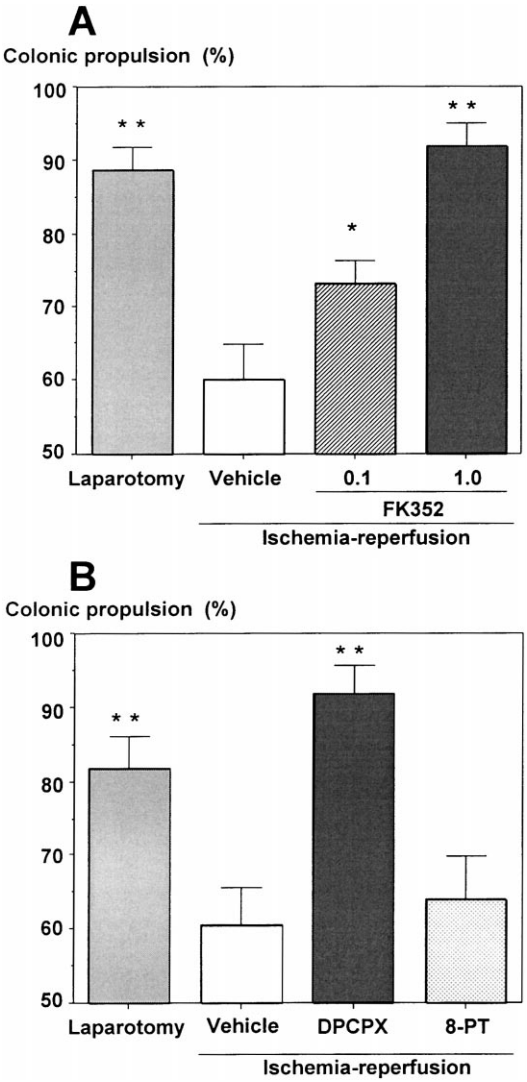

Fig. 2. The selective adenosine A_1 receptor antagonists FK352 (0.1 and 1.0 mg/kg, i.v.) and DPCPX (1.0 mg/kg, i.v.) significantly reversed the slowed colonic motility seen after ischemia–reperfusion in rats. In contrast, the non-selective adenosine antagonist 8-phenyltheophylline (8-PT; 1.0 mg/kg, i.v.) failed to affect the dysmotility. The data are expressed as the means ± S.E.M. for 11 to 17 animals. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the vehicle-treated control group.

failed to affect propulsion (1.0 mg/kg, 63.9 ± 6.0%, $n = 10$, Fig. 2B).

4. Discussion

Ischemia–reperfusion very often induces critical disorders of gastrointestinal motility. In the present study, ischemia by itself slowed colonic propulsion. Furthermore, ischemia–reperfusion exerted a greater inhibitory effect than ischemia, suggesting that, in addition to hypoxia, the enhanced generation of oxygen-derived free radicals may play a critical role in the dysmotility (Bielefeldt and Conklin, 1997). Moreover, we observed that propulsive motility was reversibly restored by adenosine A_1 receptor antagonists, indicating that the dysmotility could not have been due to cessation of neuronal function or neuronal cell death in the enteric nervous system. A possible explanation for the mechanism of the dysmotility is that an increase in the intracellular calcium concentration, as seen after exposure to free radicals, secondarily alters cellular physiology (Bielefeldt and Conklin, 1997), which may lead to functional disturbances in neuroeffector transmission (Corbett and Lees, 1997). This, in turn, may lead to disruption of the endogenous rhythm and coordination of propulsive movements in the enteric nervous system of the colon.

It has been demonstrated that adenosine can act at presynaptic adenosine A_1 receptors to suppress synaptic neurotransmission in the guinea pig small intestine (Christofi and Wood, 1993) and colon (Kadowaki et al., 2000) and the release of acetylcholine, substance P and neurokinin A in the myenteric plexus (Nitahara et al., 1995). Taking the present findings together with previous results, it can be postulated that endogenous adenosine continuously interferes with the excitatory neuronal pathway for peristalsis through presynaptic adenosine A_1 receptors in the enteric nervous system. Furthermore, FK352 and DPCPX completely restored propulsive motility in the ischemia–reperfusion model, suggesting that endogenous adenosine has a sustained and potent inhibitory effect on colonic propulsion not only under normal conditions but also under pathophysiological conditions.

However, the problem still remains to be resolved. To date, adenosine A_1 receptors have been only functionally characterized in the rat colon (Bailey and Hourani, 1992; Suzuki et al., 1995) and have never been demonstrated to be distributed in the rat colon (Dixon et al., 1996). The results of RT-PCR presented here for the first time show the expression of mRNA of adenosine A_1 receptors in the rat small intestine and colon. This finding can resolve the discrepancy between functional data and molecular biological data on adenosine A_1 receptors in the rat colon. Furthermore, adenosine A_1 receptors are more abundant in the colon than in the small intestine, indicating that adenosine A_1 receptor antagonists may exert a prokinetic effect mainly in the colon rather than in the small intestine.

Finally, it seems appropriate to note that adenosine A_1 receptor antagonists have therapeutic potential for the treatment of ischemic colitis in the colon.

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