Effects of nafamostat mesilate on 5-hydroxytryptamine release from isolated ileal tissues induced by anti-cancer drugs in rats

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ABSTRACT
Administration of cisplatin and methotrexate significantly increased 5-hydroxytryptamine (5-HT) release from intestinal tissues isolated at 72 h after administration in rats. Daily administration with nafamostat mesilate, a potent serine protease inhibitor, significantly inhibited the release of 5-HT induced by methotrexate, but not by cisplatin, in a dose-dependent manner. When applied to isolated ileal tissues in vitro, nafamostat mesilate also significantly inhibited the release of 5-HT induced by methotrexate, but not by cisplatin, in a concentration-dependent manner. These results suggest that serine proteases are involved in the mechanism of the methotrexate-induced release of 5-HT from the rat small intestine.

Anti-cancer chemotherapy is associated with severe side effects. One of the major side effects is mucosal injury in the gastrointestinal tract, which results in a variety of symptoms, including malabsorption, diarrhea, nausea, and vomiting (Hesketh 2008). The important gastrointestinal signaling molecule 5-hydroxytryptamine (5-HT) is critically involved in intestinal injury due to its role as a paracrine messenger, neurotransmitter, and inflammatory mediator (Margolis and Gershon 2016). Intestinal 5-HT is mainly stored in and released from enterochromaffin cells, which are sparsely localized in the intestinal mucosa (Margolis and Gershon 2016).

A previous study indicated that the administration of cisplatin, a cytotoxic anti-cancer drug, to rats induces 5-HT release from isolated small intestinal tissues (Kudo et al. 2001). Although the precise mechanism by which anti-cancer drugs induce 5-HT release from enterochromaffin cells is unknown, it may involve not only direct injury to enterochromaffin cells by cytotoxicity but also stimulation by mediators derived from the inflammatory response induced by these drugs (Minami et al. 2003). Indeed, we previously demonstrated that the administration of cisplatin and methotrexate, widely used for anti-cancer chemotherapy, to rats induces a severe and moderate, respectively, inflammatory response in the small intestinal mucosa (Ju et al. 2008; Takano et al. 2014). On the other hand, recent studies have revealed that endogenous and exogenous proteases such as serine proteases are widely distributed in the gastrointestinal tract and crucially involved in the pathogenesis of various gastrointestinal diseases including intestinal inflammation (Vergnolle 2016). Nafamostat mesilate (nafamostat), a potent and specific serine protease inhibitor (Aoyama et al. 1984), exhibits a therapeutic effect on experimental colitis in rats (Isozaki et al. 2006; Cho et al. 2011). Therefore, in the present study, we investigated the in vivo and in vitro effects of nafamostat on 5-HT release from isolated intestinal tissues induced by anti-cancer drug administration to rats, in an attempt to clarify whether serine proteases are involved in the release of 5-HT induced by anti-cancer drugs.

Cisplatin was obtained from Nippon Kayaku Co., Ltd. (Tokyo, Japan). Methotrexate and nafamostat were a generous gift from Chugai Pharmaceutical.
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administration of cisplatin or methotrexate was investigated first. Cisplatin administration significantly increased 5-HT release from ileal tissues in a time-dependent manner as compared with control (Fig. 1A). Daily administration of nafamostat tended to inhibit the cisplatin-induced release of 5-HT from isolated ileal tissues. However, when the cumulative release of 5-HT was evaluated over 180 min, nafamostat administration had no significant inhibitory effect on cisplatin-induced 5-HT release, although

The animal experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals by the Animal Research Committee of Health Sciences University of Hokkaido. Male Wistar rats weighing 180–200 g were purchased from Sankyo Laboratory Service Co., Ltd. (Shizuoka, Japan). They were housed under constant conditions at a room temperature of 22 ± 2°C and humidity of 50 ± 10% with a 12-h light-dark cycle, and had access to water and food ad libitum. Rats were injected intraperitoneally (i.p.) with 5 mg/kg cisplatin, 10 mg/kg methotrexate, or physiological saline (control), and left for 72 h. When the in vivo effect of nafamostat on 5-HT release was evaluated, nafamostat at doses of 1 and 3 mg/kg nafamostat or vehicle (physiological saline) was administered subcutaneously to the rats at 10 min before the administration of the anticancer drugs, and subsequently two times every 24 h. At the 72 h, ileal tissues were dissected out at 20 cm from the pylorus in approximately 3-cm-long segments under anesthesia, and used for the 5-HT release experiments.

5-HT release from isolated ileal tissues was evaluated as described previously (Kudo et al. 2001). In brief, rat ileal segments were placed in jacketed organ baths and perfused with modified Krebs solution containing (in mM) NaCl 120, KCl 5.0, CaCl₂ 2.5, MgSO₄ 1.0, NaHPO₄ 1.0, and glucose 11.0 (pH 7.4), which was aerated with 95% O₂ and 5% CO₂. After a 15-min equilibration period, the buffer solutions were collected every 20 min for up to 180 min. When the in vitro effect of nafamostat on 5-HT release was evaluated, ileal tissues were isolated at 72 h after a single i.p. administration of cisplatin or methotrexate. Nafamostat was added to the perfusion solution at various concentrations after the equilibration period. 5-HT concentration in the perfusates was measured using high-performance liquid chromatography with an electrochemical detector (Kudo et al. 2001). 5-HT release during a 180-min period was cumulatively calculated as nanograms per gram wet tissue (ng/g tissue).

All values were expressed as the mean ± standard error (SE). Data were subjected to one-way or two-way analysis of variance, followed by Tukey’s post hoc test to compare more than two groups. *P < 0.05 was considered statistically significant.

The in vivo effect of nafamostat on 5-HT release from ileal tissues isolated at 72 h after a single i.p.
Nafamostat and 5-HT release

from ileal tissues isolated at 72 h after a single i.p. administration of cisplatin or methotrexate to the rats was then evaluated. When applied in vitro to ileal tissues, nafamostat had no significant inhibitory effect on the cisplatin-induced release of 5-HT (Fig. 3A). On the other hand, it significantly inhibited the methotrexate-induced release of 5-HT in a concentration-dependent manner, when nafamostat was applied in vitro to ileal tissues (Fig. 3B).

In agreement with a previous study (Kudo et al. 2001), cisplatin caused a significant increase in 5-HT release.
release from isolated ileal tissues at 72 h after a single administration to rats. The present study first demonstrated that methotrexate administration had a similar effect to cisplatin administration. When administered daily, nafamostat dose-dependently inhibited the methotrexate-induced release of 5-HT, although it hardly inhibited the cisplatin-induced release of 5-HT. When applied in vitro to ileal tissues isolated after cisplatin or methotrexate administration, nafamostat potently inhibited the release of 5-HT induced by methotrexate, but not by cisplatin, in a concentration-dependent manner. Methotrexate administration causes a moderate intestinal inflammatory response in rats (Takano et al. 2014), and the number of tryptase-positive mast cells in the lamina propria and submucosa is increased during intestinal inflammation (Isozaki et al. 2006). Nafamostat is a broad-spectrum serine protease inhibitor that, in particular, inhibits potently mast cell-derived tryptase activity at a low dose (Mori et al. 2003). Therefore, our results suggest that certain serine proteases including mast cell-derived tryptase are involved in the mechanism of methotrexate-induced 5-HT release from ileal tissues in rats. This is the first report demonstrating the interaction of serine proteases with the release of 5-HT induced by anticancer drugs.

Although further studies are required to clarify how serine proteases are involved in the mechanism of 5-HT release induced by methotrexate, one possibility is the involvement of protease-activated receptors (PARs) belonging to the G protein-coupled receptor family (Kawabata et al. 2008). Among the four members of the PAR family (PAR-1, -2, -3, and -4), PAR-2, in particular, is distributed throughout the intestinal tract and modulates various functions in physiological and pathological conditions, including inflammatory diseases (Sébert et al. 2019). PAR-2 stimulation by an agonist enhances various gastrointestinal functions in a manner dependent on intracellular Ca\(^{2+}\) mobilization (Kawabata et al. 2008). PAR-2 is expressed in intestinal epithelial cells including enterochromaffin cells, and the intracolonical administration of PAR-2 agonists induces an increase in 5-HT content and enterochromaffin cell proliferation in the rat colon (Li et al. 2009). 5-HT release from enterochromaffin cells is triggered or modulated by multiple receptors, including G protein-coupled receptors, triggering intracellular Ca\(^{2+}\) mobilization (Hirafuji et al. 2000). Therefore, taking these findings into account, it is conceivable that the activation of PAR-2, at least, by certain serine proteases is involved in the mechanism of 5-HT release from enterochromaffin cells following methotrexate administration. However, rat mast cells in the intestinal mucosa also contain 5-HT and tryptophan hydroxylase (Wingren et al. 1983; Yu et al. 1999), and express PARs (Dugina et al. 2003). Therefore, the possibility cannot be excluded that 5-HT is released from mast cells following methotrexate administration.

Cisplatin used at the same dose as in this study causes severe inflammatory injury and increases 5-HT content, tryptophan hydroxylase activity and enterochromaffin cell hyperplasia in rat ileal mucosa (Ju et al. 2008; Obara et al. 2018). Methotrexate causes a more moderate intestinal inflammatory response than cisplatin, but produces a comparable increase in ileal 5-HT content and enterochromaffin cell hyperplasia in rats (Takano et al. 2014). Nafamostat administration attenuates colonic inflammation and mast cell infiltration in a rat model of experimental colitis (Isozaki et al. 2006; Cho et al. 2011). However, in our preliminary study, nafamostat administration at the same dose used in the present study had no effect on the increase of ileal 5-HT content and tryptophan hydroxylase activity after cisplatin administration (unpublished data). Therefore, the severity of the inflammatory response may explain the different effectiveness of nafamostat on 5-HT release between cisplatin and methotrexate. The effect of nafamostat on the change in 5-HT production induced by methotrexate, which causes a more moderate inflammatory response than cisplatin, remains to be evaluated.

In conclusion, this study suggests that serine proteases are involved in the mechanism of the methotrexate-induced release of 5-HT from the rat small intestine. Thus, inhibition of serine proteases by nafamostat may be a novel therapeutic strategy for the prevention and treatment of 5-HT-related side effects associated with anti-cancer chemotherapy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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