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Anti-inflammatory drugs ameliorate opposite enzymatic changes in ileal 5-hydroxytryptamine metabolism in the delayed phase after cisplatin administration to rats

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ABSTRACT

The effects of anti-inflammatory drugs on ileal 5-hydroxytryptamine (5-HT) metabolic dynamics at 72 h after a single administration of cisplatin were investigated in rats. Cisplatin 5 mg/kg i.p. caused pathological changes, with an inflammatory response occurring 72 h after its administration. The inflammatory response was associated with the induction of cyclooxygenase-2, but not cyclooxygenase-1, in the ileal mucosa at 72 h after the cisplatin administration. Daily treatment with meloxicam 3 mg/kg s.c. ameliorated the cisplatin-induced mucosal damage, whereas dexamethasone 1 mg/kg s.c. did not. Cisplatin administration also caused a significant increase in cyclooxygenase-2 mRNA expression at 72 h after administration, which was blunted by dexamethasone, but not by meloxicam. Cisplatin increased the content of 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), but had no effect on 5-HT turnover (5-HIAA/5-HT ratio). Meloxicam and dexamethasone did not significantly decrease 5-HT and 5-HIAA content. Cisplatin significantly decreased monoamine oxidase activity but increased tryptophan hydroxylase (TPH) activity and TPH_1 mRNA expression in ileal tissue. Meloxicam and dexamethasone significantly restored the decreased monoamine oxidase activity and inhibited the cisplatin-induced increase in tryptophan hydroxylase activity toward the control levels. These drugs also decreased the cisplatin-induced increase in TPH_1 mRNA expression. Neither cisplatin nor the anti-inflammatory drugs had significant effect on mRNA expression of the serotonin re-uptake transporter. These results suggest that the inflammatory response associated with cyclooxygenase-2 induction is involved in the opposite change in ileal tryptophan hydroxylase and monoamine oxidase activities in the delayed phase after single administration of cisplatin to rats.

1. Introduction

Cisplatin-based chemotherapy is associated with the severe side effects of nausea and vomiting. The emetic response is classified into acute emesis, evoked within the first 24 h after cisplatin administration, and delayed emesis, occurring 1 day to 1 week after cisplatin administration (Grunberg and Hesketh, 1993). The main mechanism of acute emesis is thought to be the stimulation of the vomiting center of the medulla oblongata via stimulation of 5-hydroxytryptamine (5-HT) 5-HT_3 receptors on the abdominal vagus nerve fibers by 5-HT, which is released from intestinal enterochromaffin cells following cisplatin administration (Andrews et al., 1998; Minami et al., 2003). The acute emesis, therefore, is well controlled by the use of selective 5-HT_3 receptor antagonists, such as granisetron and ondansetron, in animal experimental models and human studies (Andrews et al., 1998; Minami et al., 2003).

However, the delayed nausea and vomiting still remains a significant problem, because 5-HT_3 receptor antagonists alone usually have little therapeutic effect on cisplatin-induced delayed emesis in either humans or experimental animals (Grunberg and Hesketh, 1993; Andrews et al., 1998; Minami et al., 2003). Several reports have shown that improved control of delayed emesis is achieved by a combination of a 5-HT_3 receptor antagonist and dexamethasone in both human patients (Kris et al., 2005) and ferrets (Rudd and Naylor, 1996; Fukunaka et al., 1998; Sam et al., 2001), or by dexamethasone alone (Ioannidis et al., 2000). Further, non-steroidal anti-inflammatory drugs such as indomethacin and meloxicam have exhibited anti-emetic activity against cisplatin-induced delayed emesis in piglets (Girod et al., 2002). These studies suggest that 5-HT and 5-HT_3 receptors also play a role in the elicitation of delayed emesis and that there is an interaction between the 5-HT/5-HT_3 receptor system and the inflammatory response involving chemical mediators, such as

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arachidonate metabolites, in the delayed emetic response. However, the mechanism underlying these interactions in delayed emesis still remains uncertain.

Over 90% of the 5-HT in the human body is localized in the intestinal tract (Erspamer and Testinini, 1959). Intestinal 5-HT is synthesized by tryptophan hydroxylase, the rate-limiting enzyme of 5-HT formation, and stored mainly in the enterochromaffin cells of the mucosal epithelium (Yu et al., 1999). 5-HT is released from enterochromaffin cells into the interstitial space by a variety of stimuli (Racké and Schwörer, 1991). It is taken up via the serotonin re-uptake transporter (SERT) mainly into mucosal epithelial cells in the rat intestine (Wada et al., 1996), and catabolized into 5-hydroxyindoleacetic acid (5-HIAA) by monoamine oxidase, an intracellular enzyme, which is widely localized in mucosal and submucosal cells (Saura et al., 1992). Our previous studies showed that cisplatin induced a significant increase in the activity of tryptophan hydroxylase, but a significant decrease in the activity of monoamine oxidase, in the ferret ileum (Minami et al., 1995). However, there is little biochemical data showing such changes in the delayed phase of cisplatin administration. Cisplatin has been shown to cause an increase in the 5-HT content in both the acute and delayed phases of administration in duodenal and ileal mucosa of the piglet (Grélot et al., 1995). Therefore, it is possible that the precise mechanism by which cisplatin causes changes in 5-HT metabolism is different in the acute and delayed phases. In an attempt to clarify the mechanism by which drugs suppress cisplatin-induced delayed emesis, we investigated the effects of two anti-inflammatory drugs, meloxicam, a selective cyclooxygenase-2 inhibitor (Engelhardt et al., 1995), and dexamethasone, on cisplatin-induced ileal 5-HT metabolic dynamics in the delayed phase after cisplatin administration to rats.

2. Materials and methods

2.1. Drugs and reagents

Cisplatin was obtained from Nippon Kayaku Co., Ltd., (Tokyo, Japan). Dexamethasone was purchased from Banyu Pharmaceutical Co. Ltd., (Tokyo, Japan). Meloxicam was a generous gift from Nippon Boehringer Ingelheim, Co., Ltd., (Hyogo, Japan). Other reagents used in this study were of special grade, purchased from local suppliers, unless otherwise described.

2.2. Treatment of animals

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 free access to water and food.

Rats were injected with a single dose of either 5 mg/kg cisplatin i.p. or physiological saline i.p., for the control. This low dose of cisplatin was based on our previous study showing that cisplatin elicited a behavior known as 'pica' during the delayed phase (24–72 h after the injection) in rats (Liu et al., 2003). Pica, eating of non-nutritive substances such as kaolin, is considered to be analogous to emesis and, thus, an experimental model of emesis (Takeda et al., 1993; Saeki et al., 2001). Additionally, 3 mg/kg meloxicam, 1 mg/kg dexamethasone, or physiological saline was administered s.c. to the rats 10 min before the administration of cisplatin and subsequently injected every 24 h for 3 days.

At the end of the 72 h, the rats were anesthetized with urethane 500 mg/kg i.p. and α-chloralose 50 mg/kg i.p. Ileal tissues were dissected out at 20 cm from the pylorus in approximately 3-cm-long segments and frozen rapidly in liquid nitrogen and stored until further analysis.

2.3. Measurement of 5-HT and 5-HIAA

Frozen segments of ileal tissues were weighed and homogenized in 2 ml of 0.1 mM perchloric acid and 0.7 mM EDTA, and centrifuged at 9000 ×g for 5 min. The supernatant was then filtered through a 0.45 μm membrane filter (Kurabo, Centricut W-MO, Japan). 5-HT and 5-HIAA in the filtrates were measured by high-performance liquid chromatography (HPLC) (Eicom, EP-10, Japan) with a 5-μm ODS column (Eicom, Eicompack SC-5ODS, Japan) and an electrochemical detector (Eicom, ECD-100, Japan), as described by Endo et al. (1993). The mobile phase consisted of 5 mg/l EDTA disodium, 190 mg/l sodium 1-octanesulfonate, and 17% methanol. The oxidation potential of the electrochemical detector was set at +750 mV.

2.4. Tryptophan hydroxylase and monoamine oxidase activities

Frozen ileal segments were weighed and homogenized in 10 volumes of 0.25 M sucrose. Monoamine oxidase activity was determined by the method of Kraml (1965), using kynuramine (Sigma, USA) as substrate, as described by Endo et al. (1993). 4-Hydroxyquinolin, the reaction product, was quantified fluorometrically (Hitachi, F-4000, Japan) at an emission wavelength of 380 nm with an excitation wavelength of 315 nm. Protein assays were performed by the method of Lowry et al. (1951) with bovine serum albumin (Roche Diagnostics GmbH, Germany) as standard.

Tryptophan hydroxylase activity in the homogenates was measured by the method of Kuhn et al. (1978) with a slight modification. The reaction mixture consisted of tissue homogenate, 50 mM Tris–HCl (pH 7.4), 4 mM dithiothreitol, 2 mM 6-methyltetrahydropterine, 8000 U/ml catalase, and 8 mM L-tryptophan and was incubated for 30 min at 37 °C. The reaction was stopped by adding 6 M perchloric acid. The reaction product, 5-hydroxytryptophan, was determined with HPLC, as described above. The mobile phase consisted of 4 mg/l EDTA disodium, 0.65 mM sodium-1-octanesulfonate and 8% methanol. The oxidation potential of the electrochemical detector was set at +700 mV.

2.5. Western blot analysis

Protein expression was analyzed by Western blot analysis as described by Machida et al. (2005). Briefly, frozen segments of ileal tissues were weighed and homogenized in lysis buffer, and centrifuged at 13,000 ×g for 10 min. Lysate proteins (30–50 μg) were separated on a 7.5% (w/v) sodium dodecyl sulfate-polyacrylamide gel by electrophoresis and transferred to a polyvinylidene difluoride transfer membrane. The blot was incubated with anti-cyclooxygenase-1 mouse polyclonal antibody (1:500; Takara Biomedicals, Japan), anti-cyclooxygenase-2 mouse monoclonal antibody (1:1000; Santa Cruz Biotechnology, USA), or anti-β-actin mouse monoclonal antibody (1:1000; Sigma-Aldrich, USA) for 2 h. The immunoblot was incubated with a 1:5000 dilution of horseradish peroxidase-conjugated secondary antibody (Zymed Laboratories, USA) and visualized using an enhanced chemiluminescence kit (PerkinElmer, USA). The densitometric analysis of each protein was performed using Image J (NIH, USA) and calculated relative to β-actin.

2.6. Expression of cyclooxygenase-2 and TPH_1 mRNA

The expression of cyclooxygenases and TPH_1 mRNA was determined using reverse transcription-polymerase chain reaction (RT-PCR) with total RNA. Total RNA was isolated from ileal tissues using Trizol reagent (Sigma-Aldrich, USA). cDNA was synthesized from 2 μg of each RNA sample using oligo$(dT)_{12-18}$ (0.5 μg, Invitrogen) and

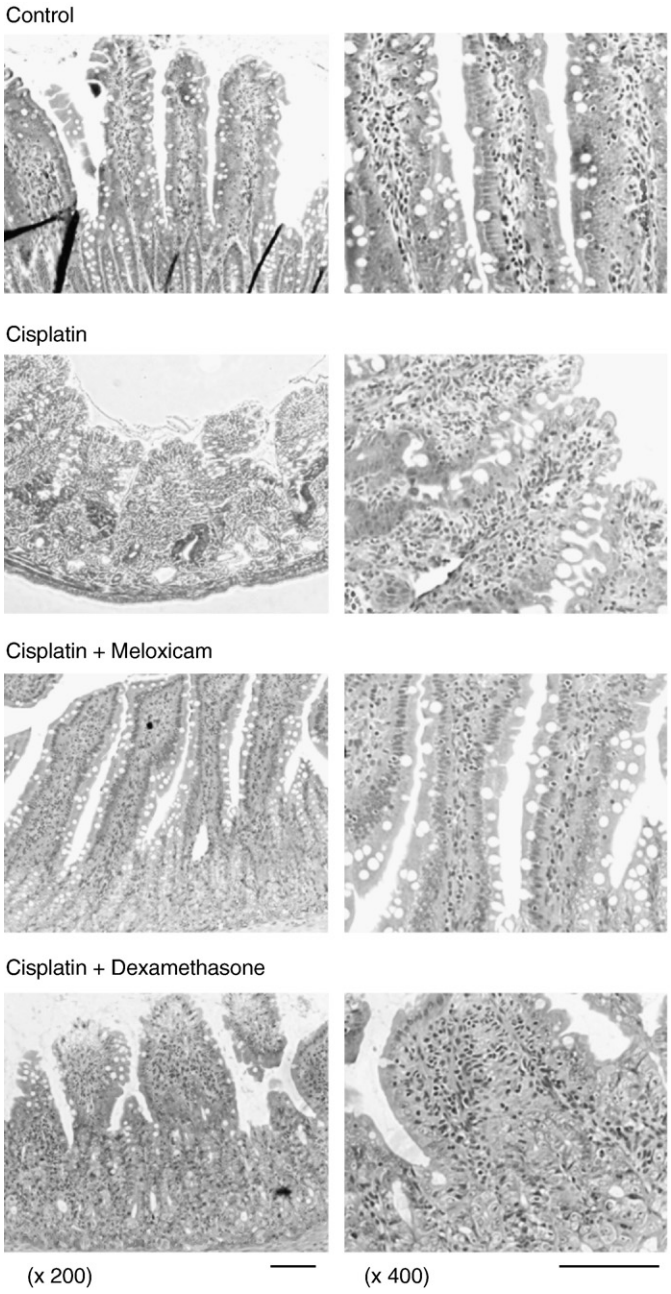

Fig. 1. Hematoxylin–eosin stained sections from rat ileal mucosa. At 72 h after administration of physiological saline (control), cisplatin 5 mg/kg i.p., cisplatin 5 mg/kg i.p. once + meloxicam 3 mg/kg s.c. every 24 h, or cisplatin 5 mg/kg i.p. once + dexamethasone 1 mg/kg s.c. every 24 h, ileal tissue was dissected out and fixed with 4% paraformaldehyde and embedded in paraffin. Original magnifications are indicated in parenthesis. Scale bars = 100 μm.

reverse transcriptase (15 U, Invitrogen) at 42 °C for 60 min. For PCR, 12 μl of 1×PCR buffer containing 0.42 U of *Taq* polymerase (Roche Diagnostics GmbH, Germany) and 0.24 μM of primer were added. Primers for rat cyclooxygenase-1 were sense, 5′-CAT GGA TCC GGA TTG GTG GGG GTA G-3′ and anti-sense, 5′-ATC TCG AGG GGC AGG TCT TGG TGT TG-3′, to give a product of 447 bp (Beiche et al., 1998). Primers for cyclooxygenase-2 were sense, 5′-ACA CTC TAT CAC TGG CAT CC-3′ and anti-sense, 5′-GAA GGG ACA CCC TTT CAC AT-3′, to give a product of 584 bp (Machida et al., 2005). Primers for rat TPH_1 were sense, 5′-CAA GGA GAA CAA AGA CCA TTC-3′ and anti-sense, 5′-ATT CAG CTG TTC TCG GTT GAT G-3′, to give a product of 208 bp. Primers for TPH_2 were sense, 5′-TAA ATA CTG TTC TCG GTT G-3′ and anti-sense, 5′-GAA GTG TCT TTG CCG CTT CTC-3′, to give a product of 132 bp (Sugden, 2003), and primers for SERT were sense, 5′-GGA TCC CTG CTC ACA CTG-3′ and anti-sense, 5′-TTA CAC AGC ATT CAT GCG-3′, to give a product of 495 bp (Li et al., 2006). Concurrent RT-PCR amplification of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was performed as an internal control for variation in the efficiency of RNA isolation and reverse transcription, using the sense primer, 5′-CGG AGT CAA CGG ATT TGG TCG TAT-3′ and anti-sense primer, 5′-AGC CTT CTC CAT GGT GGT GAA GAC-3′, to give a product of 306 bp. These primers were obtained from Invitrogen (Tokyo, Japan). The thermocycling program included 3 min at 95 °C, 1 min at 94 °C, 1 min at 54 °C, and 1 min at 72 °C, and a final extension for 7 min at 72 °C in a thermal cycler (Takara PCR thermal cycler Dice, Takara, Japan). The PCR amplification was optimized to remain within the linear portion of the reaction.

The RT-PCR products were separated and visualized on an ethidium bromide-stained 1.0% agarose gel. The semi-quantitative analysis of each product was performed by densitometry using Image J and calculated relative to GAPDH.

2.7. Statistical analysis

All values are expressed as means ± S.E.M. Data were subjected to one-way analysis of variance (ANOVA), followed by a post-hoc test to compare more than two groups. A value of $p<0.05$ was considered statistically significant.

3. Results

3.1. Cisplatin-induced inflammation

Single administration of cisplatin 5 mg/kg i.p. to the rats caused pathological changes, with an inflammatory response in the ileal mucosa. Histological examination of the ileal tissue at 72 h after the administration of cisplatin revealed regional inflammation characterized by edema, hyperemia, leukocyte infiltration, and disturbances of epithelial architecture in the ileal mucosa (Fig. 1). Daily co-administration of meloxicam 3 mg/kg s.c. with a single administration of cisplatin ameliorated these pathological mucosal damages, whereas dexamethasone 1 mg/kg s.c. did not. Administration of these anti-

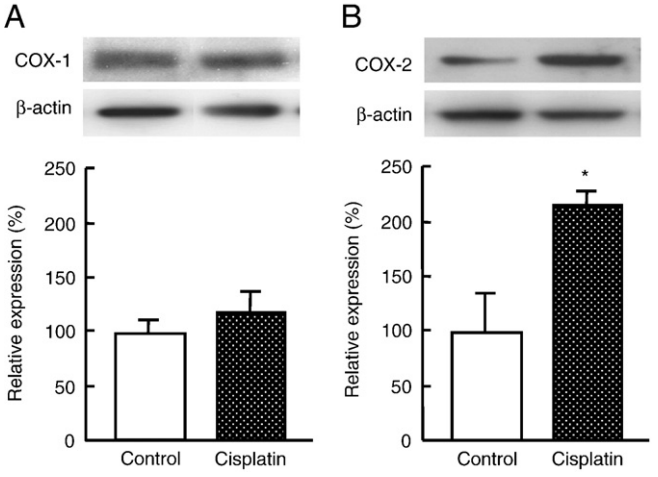

Fig. 2. Effects of cisplatin administration on cyclooxygenase-1 (A) and cyclooxygenase-2 (B) expression in rat ileal tissue. The ileal tissue was dissected out 72 h after a single administration of cisplatin. Cyclooxygenase protein expression was determined by Western blotting. Upper: a representative blot image of cyclooxygenase and β-actin expression; lower: summary of densitometric analysis expressed relative to β-actin. Each column represents the mean ± S.E.M. for 3 and 4 animals for the control and cisplatin groups, respectively. *$p<0.05$ versus control group.

inflammatory drugs alone did not induce histological changes compared with the control (data not shown).

3.2. Cyclooxygenase-1 and cyclooxygenase-2 expression

To examine whether the inflammatory response in the ileal mucosa is associated with cyclooxygenase expression, cyclooxygenase-1 and cyclooxygenase-2 protein expression relative to β-actin was determined by Western blot analysis. Both of the cyclooxygenase proteins were detected in normal ileal tissue isolated from control rats. Cisplatin caused a significant 2.2-fold increase in cyclooxygenase-2 expression (Fig. 2B) but had no effect on cyclooxygenase-1 expression in the ileal tissue at 72 h after administration (Fig. 2A).

The expression of cyclooxygenase-1 and -2 mRNA was evaluated by semi-quantitative RT-PCR. Cisplatin alone or in combination with meloxicam or dexamethasone had no significant effects on cyclooxygenase-1 mRNA expression (Fig. 3A). However, cisplatin caused an 11-fold increase in cyclooxygenase-2 mRNA expression, which was faintly detectable in control ileal tissue. Dexamethasone tended to decrease cyclooxygenase-2 mRNA expression toward the control level, while meloxicam had no effect (Fig. 3B).

3.3. 5-HT and 5-HIAA content

The content of 5-HT and 5-HIAA, a monoamine oxidase metabolite of 5-HT, in rat ileal tissue and the effects of anti-inflammatory drugs were determined at 72 h after cisplatin administration. Cisplatin caused increases in the content of 5-HT and its metabolite, 5-HIAA, but had no effect on 5-HT turnover (5-HIAA/5-HT ratio), an index of 5-HT dynamics (Table 1).

Table 1
5-HT and 5-HIAA contents in rat ileal tissue 72 h after cisplatin administration

Group	(*n*)	5-HT (ng/mg protein)	5-HIAA (ng/mg protein)	5-HIAA/5-HT
Control	(5)	19.55±0.79	0.44±0.05	0.023±0.003
Cisplatin	(6)	44.19±8.28[a]	0.94±0.20	0.021±0.001
+Meloxicam	(6)	42.78±9.17[a]	1.07±0.24[a]	0.026±0.003
+Dexamethasone	(7)	37.91±8.60	0.81±0.14	0.026±0.006

Rat ileal tissue was dissected out 72 h after a single administration of cisplatin 5 mg/kg without or with daily treatment of meloxicam 3 mg/kg and dexamethasone 1 mg/kg. 5-HT and 5-HIAA in ileal tissue homogenates were measured by high-performance liquid chromatography. Each value represents the mean±S.E.M. for (*n*) animals. [a]$p<0.05$ versus control group.

The daily treatments with meloxicam and dexamethasone plus cisplatin did not significantly reduce the cisplatin-induced increase in the ileal content of 5-HT and 5-HIAA. There were no statistical differences in 5-HT turnover compared with the cisplatin group.

3.4. Monoamine oxidase activity

Monoamine oxidase catalyzes the degradation of 5-HT into 5-HIAA. The enzyme activity of monoamine oxidase in rat ileal tissue was measured at 72 h after cisplatin administration in order to elucidate whether the increase in ileal 5-HT content is due to a change in monoamine oxidase activity. Cisplatin administration caused a significant 30% decrease in monoamine oxidase activity (Fig. 4). When rats were treated daily with anti-inflammatory drugs, the cisplatin-induced decrease in monoamine oxidase activity was significantly reversed back to the control level by meloxicam and dexamethasone.

3.5. Tryptophan hydroxylase activity

The enzyme activity of tryptophan hydroxylase in the rat ileum was measured at 72 h after cisplatin administration in order to elucidate whether the increase in ileal 5-HT content is due to the change in tryptophan hydroxylase activity, the rate-limiting enzyme of 5-HT formation. Cisplatin administration caused a significant 120%

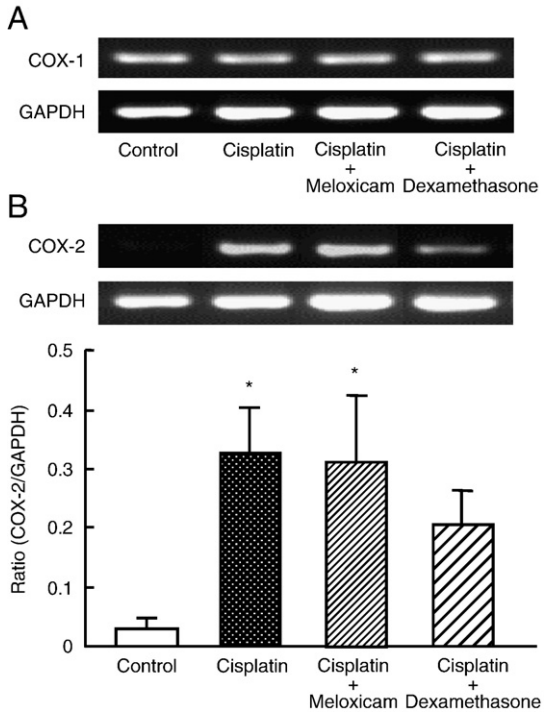

Fig. 3. Effects of meloxicam and dexamethasone on cisplatin-induced cyclooxygenase mRNA expression in rat ileal tissue. Ileal tissue was dissected out at 72 h after a single administration of cisplatin with or without daily treatment of anti-inflammatory drugs. Cyclooxygenase mRNA expression was determined by RT-PCR. A: a representative image of cyclooxygenase-1 (COX-1) and GAPDH mRNA expression. B: upper, a representative fluorescence image of cyclooxygenase-2 (COX-2) and GAPDH mRNA expression; lower, summary of densitometric analysis expressed as a ratio of COX-2/GAPDH. Each column represents the mean±S.E.M. of 5 experiments. *$p<0.05$ versus control group.

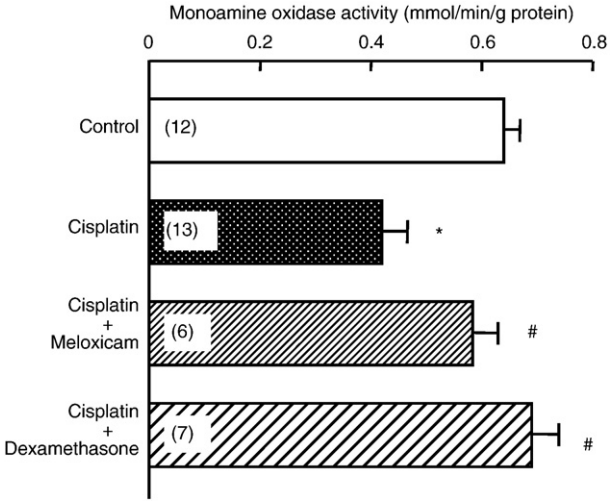

Fig. 4. Effects of meloxicam and dexamethasone on the cisplatin-induced decrease in monoamine oxidase activity in rat ileal tissue. Ileal tissue was dissected out 72 h after a single administration of cisplatin with or without daily treatment of anti-inflammatory drugs. The enzymatic activity of monoamine oxidase was measured by fluorometry, using kynuramine as the substrate. Each column represents the mean±S.E.M. for the number of animals shown in brackets. *$p<0.05$ and #$p<0.05$ versus control group and cisplatin group, respectively.

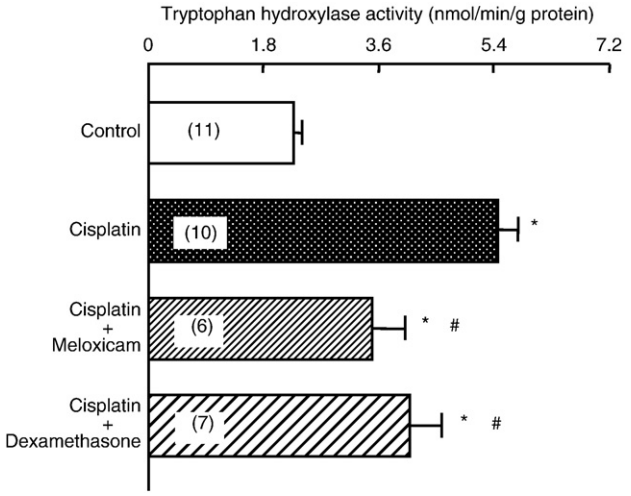

Fig. 5. Effects of meloxicam and dexamethasone on the cisplatin-induced increase in tryptophan hydroxylase activity in rat ileal tissue. Ileal tissue was dissected out 72 h after a single administration of cisplatin with or without daily treatment of anti-inflammatory drugs. The enzymatic activity of tryptophan hydroxylase was measured using tryptophan as the substrate and HPLC for determination of 5-hydroxytryptophan. Each column represents the mean ± S.E.M. for the number of animals shown in brackets. *$p<0.05$ and #$p<0.05$ versus control group and cisplatin group, respectively.

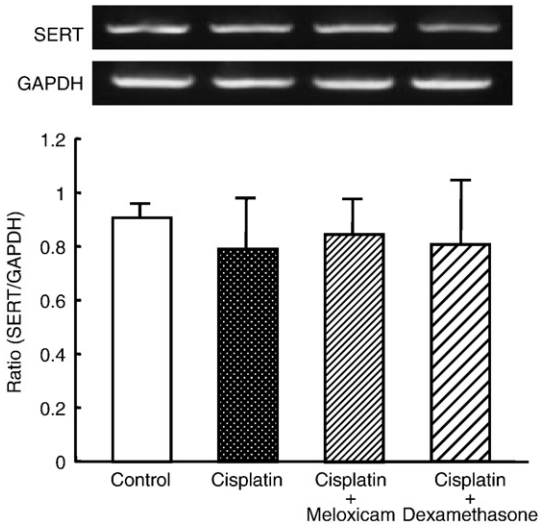

Fig. 7. Effects of meloxicam and dexamethasone on the cisplatin-induced increase in SERT mRNA expression in rat ileal tissue. Ileal tissue was dissected out at 72 h after a single administration of cisplatin with or without daily treatment of anti-inflammatory drugs. SERT mRNA expression was determined by RT-PCR. Upper: a representative fluorescence image of SERT and GAPDH mRNA expression; lower: summary of densitometric analysis expressed as a ratio of SERT/GAPDH. Each column represents the mean ± S.E.M. for 5 animals.

increase in tryptophan hydroxylase activity (Fig. 5). When rats were treated daily with anti-inflammatory drugs, the cisplatin-induced increase in tryptophan hydroxylase activity was significantly inhibited by both meloxicam and dexamethasone. Still, tryptophan hydroxylase activity in these groups was significantly higher than that in the control group.

3.6. TPH_1 mRNA expression

There are two 5-HT systems in vertebrates with independent regulation and distinct functions defined by two tryptophan hydroxylase isoforms, TPH_1 and TPH_2 (Walther et al., 2003). Enterochromaffin cells, which produce and release far more 5-HT than serotonergic neurons, contain the TPH_1 isoform, whereas enteric serotonergic neurons contain the TPH_2 isoform (Côté et al., 2003). To examine whether the increase in ileal tryptophan hydroxylase activity after cisplatin administration is associated with the up-regulation of tryptophan hydroxylase, the effect of cisplatin on TPH_1 and TPH_2 mRNA expression was evaluated by semi-quantitative RT-PCR. Cisplatin caused a 1.7-fold increase in the TPH_1/GAPDH mRNA ratio (Fig. 6). In contrast, TPH_2 mRNA expression in ileal tissue was hardly detectable and did not increase 72 h after cisplatin administration (data not shown).

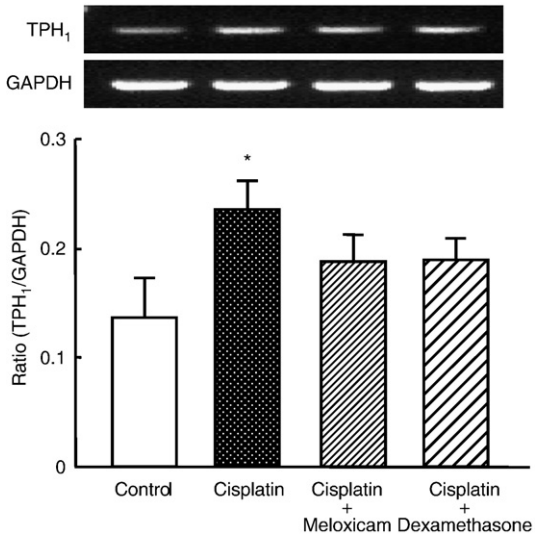

Fig. 6. Effects of meloxicam and dexamethasone on cisplatin-induced increase in TPH_1 mRNA expression in rat ileal tissue. Ileal tissue was dissected out 72 h after a single administration of cisplatin with or without daily treatment of anti-inflammatory drugs. TPH_1 mRNA expression was determined by RT-PCR. Upper: a representative fluorescence image of TPH_1 and GAPDH mRNA expression; lower: summary of densitometric analysis expressed as a ratio of TPH_1/GAPDH. Each column represents the mean ± S.E.M. for 5 animals. *$p<0.05$ versus control group.

When anti-inflammatory drugs were administrated daily, the cisplatin-induced increase in TPH_1 mRNA expression was blunted by meloxicam and dexamethasone, although the effects of these drugs were not statistically significant. However, there were no significant differences in the TPH_1/GAPDH mRNA ratio between the control group and groups treated with these drugs.

3.7. SERT mRNA expression

In the intestinal tract, 5-HT released from enterochromaffin cells or mast cells is largely taken up via SERT into crypt epithelial cells or enteric neurons (Wada et al., 1996). To examine whether the changes in ileal 5-HT content after the administration of cisplatin and anti-inflammatory drugs are associated with a change in SERT activity, the effect of these drugs on SERT mRNA expression was evaluated by semi-quantitative RT-PCR (Fig. 7). These drugs had no significant effect on SERT mRNA expression in ileal tissues.

4. Discussion

The present study first demonstrated that cisplatin induced pathological changes causing an inflammatory response in the ileal mucosa of rats at 72 h after a single administration. Cyclooxygenase-1 is a cyclooxygenase isoform that is constitutively expressed in most cells, whereas cyclooxygenase-2 is induced by inflammatory cytokines such as interleukin-1 in certain cell types, including intestinal neuroendocrine cells (Nakajima et al., 1997). Immunoblot and RT-PCR analysis revealed that cisplatin caused an increase in cyclooxygenase-2 protein

and mRNA expression in rat ileal tissue at 72 h after cisplatin administration, whereas it had no effect on cyclooxygenase-1 expression, suggesting that cisplatin induced ileal inflammation.

The present study demonstrated that a dose of cisplatin (5 mg/kg i.p.) also caused an increase in ileal 5-HT and 5-HIAA content at 72 h after a single administration, suggesting that the availability of 5-HT is increased in the inflamed ileum. There was no change in the ratio of 5-HIAA/5-HT. The elevated ileal 5-HT content after cisplatin administration can be primarily explained by the concomitant increase in tryptophan hydroxylase activity. The elevated 5-HIAA content in spite of the decreased activity of monoamine oxidase may be explained by the dynamic aspect of 5-HT turnover involving a variety of cell types in the intestinal tracts (Gershon and Tack, 2007). Monoamine oxidase, an intracellular enzyme, is widely localized in mucosal and submucosal cells, including epithelial, smooth muscle, and neuronal cells in the intestinal tissue (Saura et al., 1992), while tryptophan hydroxylase is sparsely localized in enterochromaffin, neuronal and mast cells (Yu et al., 1999). Previous studies have shown that 5-HT release from ileal tissues is increased by cisplatin administration (Fukui et al., 1993; Kudo et al., 2001). 5-HT released into the interstitial space can be taken up via SERT, mainly into crypt epithelial cells in the rat intestine (Wada et al., 1996), and is inactivated by monoamine oxidase. The present study indicated that the enzymatic activity of monoamine oxidase was much higher than that of tryptophan hydroxylase, as measured in whole tissue homogenates. Therefore, these results suggest that the capacity of decreased monoamine oxidase activity is still so large that the enzyme is not rate-limiting for the 5-HIAA level, and may account for the discrepancy between the 5-HIAA level and monoamine oxidase activity.

The present study also demonstrated that cisplatin administration caused a significant increase in the expression of TPH_1 mRNA, but not TPH_2 mRNA, in ileal tissue. A preliminary immunohistochemical study using an anti-tryptophan hydroxylase antibody revealed that the number of immunoreactive spindle enterochromaffin cells of the epithelium was increased 72 h after cisplatin administration (data not shown). However, mast cells in the lamina propria and submucosa also contain tryptophan hydroxylase (Yu et al., 1999), and the number of mast cells is increased during intestinal inflammation in rats (Wingren et al., 1983). Therefore, the increase in tryptophan hydroxylase activity in ileal tissue after cisplatin administration may be due to an increase in the number of enterochromaffin and mast cells that express TPH_1 mRNA.

In order to elucidate the involvement of inflammatory processes and mediators in the cisplatin-induced opposite changes in 5-HT metabolism, we investigated the effects of two anti-inflammatory drugs, meloxicam and dexamethasone, on the cisplatin-induced increase in tryptophan hydroxylase activity and decrease in monoamine oxidase activity in rat ileal tissues. Meloxicam, a highly selective cyclooxygenase-2 inhibitor with less gastrointestinal toxicity, was used in the present study at a dose that shows sufficient anti-inflammatory effect (Engelhardt et al., 1995). Dexamethasone is a potent and representative steroidal anti-inflammatory drug that inhibits arachidonate metabolism by multiple mechanisms, involving phospholipase A_2 and cyclooxygenase-2 induction (Vane and Botting, 1995). In fact, the present study showed that dexamethasone blunted the cisplatin-induced cyclooxygenase-2 expression. When meloxicam and dexamethasone were administered daily for 72 h, they significantly reversed the cisplatin-induced decrease in monoamine oxidase activity. Conversely, meloxicam and dexamethasone significantly suppressed the cisplatin-induced increase in tryptophan hydroxylase activity. Furthermore, the cisplatin-induced increase in TPH_1 mRNA expression in ileal tissue was decreased by both meloxicam and dexamethasone. These results suggest that the cisplatin-induced opposite changes in the activity of enzymes are an inflammatory response involving cyclooxygenase-2 products as inflammatory mediators.

Despite the ameliorative effect of meloxicam and dexamethasone on the activity of tryptophan hydroxylase and monoamine oxidase, the 5-HT content of ileal tissue was still higher than the control levels. This may not be due to the decrease in 5-HT inactivating process via SERT, since SERT expression was not significantly affected by these anti-inflammatory drugs. Therefore, although we have no adequate explanation for the discrepancy at present, it is likely that these anti-inflammatory drugs also affected factors or mechanisms involving 5-HT release, resulting in the high 5-HT accumulation in 5-HT-producing cells. Indeed, a previous study demonstrated that 5-HT release induced by cisplatin administration was strongly inhibited by cyclooxygenase-2 inhibitors, although not by dexamethasone (Kudo et al., 2001).

It is noteworthy that the cisplatin-induced histological damage in the ileal mucosa was reversed by meloxicam, but not by dexamethasone. It may not be surprising because, despite the therapeutic potential of anti-inflammatory glucocorticoids, they delay the rate of wound healing by markedly affecting most aspects of the healing process in various tissues, including the intestinal tract (Phillips et al., 1992; Eubanks et al., 1997). Nevertheless, these results may further support the significant role of cyclooxygenase-2 products as inflammatory mediators in the cisplatin-induced enzymatic changes. Further studies are needed to clarify whether the ameliorative effect of cisplatin-induced 5-HT metabolism by dexamethasone has relevance to the efficacy of combination therapy with $5\text{-}HT_3$ receptor antagonist and dexamethasone on the delayed emesis associated with cisplatin chemotherapy.

In conclusion, the present study suggests that inflammatory processes associated with cyclooxygenase-2 induction are involved in the opposite changes in ileal tryptophan hydroxylase and monoamine oxidase activity seen in the delayed phase after a single administration of cisplatin to rats.

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