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Methotrexate causes a change in intestinal 5-hydroxytryptamine metabolism in rats

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ABSTRACT

The effects of methotrexate on 5-hydroxytryptamine (5-HT) metabolism in the intestinal tissue of rats were investigated during the delayed phase after a single administration. Rats were i.p. injected with methotrexate or with saline as a control, and kaolin and food intakes were measured by an automatic monitoring apparatus. At 96 h after administration, dissected-out ileal tissue was frozen rapidly in liquid nitrogen for further analysis or fixed for immunohistochemical staining. Methotrexate at a dose of 50 mg/kg caused a time-dependent increase in kaolin intake lasting up to 72 h after administration, which returned to the control level at 96 h after administration. This dose of methotrexate caused a gradual decrease in body weight, food intake, and water intake lasting up to 72 h, which approached the control level at 96 h. Methotrexate caused pathologic changes, including a moderate inflammatory response in the ileal tissue and an increase in the number of L-tryptophan hydroxylase (TPH)-expressing cells in the ileal mucosa. Methotrexate also caused a significant increase in 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) content and in TPH1 mRNA expression in the ileal tissues. It had no significant effects on mRNA expression of serotonin transporter, COX-1, or COX-2 or on myeloperoxidase activity. This study demonstrated, for the first time, that methotrexate caused a change in the ileal 5-HT metabolism associated with hyperplasia of mucosal enterochromaffin cells.

1. Introduction

Methotrexate, a structural analog of folic acid that inhibits dihydrofolate reductase and thus DNA synthesis, is widely used as a chemotherapeutic agent for leukemia and other malignancies such as malignant lymphoma or sarcoma. However, one of the major side effects of methotrexate in clinical settings is gastrointestinal mucosal damage, which results in various symptoms including malabsorption, diarrhea, nausea, and vomiting (Altmann, 1974; Jolivet et al., 1983; Hesketh et al., 1997). These side effects are a significant clinical problem because they decrease quality of life and hamper the achievement of proper chemotherapeutic effect. It is important, therefore, to clarify the mechanism by which methotrexate causes gastrointestinal damage so as to prevent these side effects and achieve effective cancer chemotherapy.

Cancer chemotherapy-induced nausea and vomiting is classified into acute emesis, evoked within the first 24 h after anticancer drug administration, and delayed emesis, occurring 1 day to 1 week after such administration (Hesketh, 2008). The main mechanism of acute emesis is thought to involve the stimulation of the vomiting center of the medulla oblongata via stimulation of serotonin 5-HT_3 receptors on the abdominal vagus nerve fibers by 5-hydroxytryptamine (5-HT) released from intestinal enterochromaffin cells after anticancer drug administration (Minami et al., 2003; Hesketh, 2008). However, the precise mechanism involved in delayed emesis remains unclear. Several studies have shown that control of delayed emesis can be improved by a combination of a serotonin 5-HT_3-receptor antagonist and dexamethasone in human patients (Kris et al., 2005) and animals (Rudd and Naylor, 1996; Fukunaka et al., 1998). These studies suggest that 5-HT and serotonin 5-HT_3 receptors play a role in the elicitation of delayed emesis as well as in acute emesis, by interacting with certain inflammatory mediators (Chu et al., 2014).

Intestinal 5-HT is synthesized by the rate-limiting enzyme L-tryptophan hydroxylase (TPH) and is stored mainly in enterochromaffin cells sparsely localized in the mucosal epithelium and

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crypts. 5-HT released from the enterochromaffin cells is taken up via the serotonin transporter (SERT) into mucosal epithelial cells or enteric neurons and catabolized to 5-hydroxyindoleacetic acids (5-HIAA) by monoamine oxidase (see Gershon and Tack (2007) for a review). It is unclear whether alteration of the 5-HT metabolism in the intestinal tract is a common feature of all emetogenic anticancer drugs. Several studies have also reported that methotrexate induces gastrointestinal injury and enterocolitis in rodents (Margolis et al., 1971; Carneiro-Filho et al., 2004; Kolli et al., 2008). Changes in enterochromaffin cells and 5-HT production occur in several gastrointestinal diseases including enteritis and inflammatory bowel disease (Wheatcroft et al., 2005; Spiller, 2008). However, to our knowledge, it is not known whether methotrexate has any influence on intestinal 5-HT metabolism in the delayed phase of emesis. Therefore, here we investigated such influence in rats.

2. Materials and methods

2.1. Drugs and reagents

Methotrexate prepared for intravenous injection was obtained from Pfizer Co., Ltd., (Tokyo, Japan). Other reagents used in this study were of special grade, purchased from local suppliers, unless otherwise described.

2.2. Animals

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 $50 \pm 10\%$ with a regular 12-h light – dark cycle and free access to water and food. 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.

Rats were i.p. injected with methotrexate or with saline as a control. At 96 h after injection, the rats were killed by exsanguination under light anesthesia with diethyl ether. Ileal tissues at 20 cm from the pylorus were dissected out in approximately 3-cm-long segments, frozen rapidly in liquid nitrogen, and stored until further analysis.

2.3. Measurement of kaolin and food intake

Intake of kaolin and food was measured with an automatic feeding monitoring apparatus (FDM700SW; Melquest Ltd.,Toyama, Japan). This apparatus consists of a housing cage, two containers for kaolin pellets (PMI Nutrition International, Richmond, IN, USA) and normal chow pellets (MF; Oriental Yeast, Tokyo, Japan), and a controller equipped with two weight sensors. The kaolin and food pellets were provided in their respective containers facing the housing cage. Kaolin and food intake was monitored hourly to the nearest 0.01 g, and the data were analyzed on a personal computer. Results are reported as cumulative daily amounts (g) during 24-h periods up to 96 h after administration.

2.4. Measurement of 5-HT and 5-HIAA

5-HT and 5-HIAA were quantified as described previously (Ju et al., 2008). Frozen segments of ileal tissue were weighed and homogenized in 2 ml of 0.1 mM perchloric acid and 0.7 mM ethylenediaminetetraacetic acid disodium salt (EDTA), and centrifuged at 9,000*g* for 5 min. The supernatant was then filtered through a 0.45-μm membrane filter (Centricut W-MO; Kurabo, Osaka, Japan). 5-HT and 5-HIAA in the filtrates were measured by high-performance liquid chromatography (EP-10; Eicom, Kyoto, Japan) with a 5-μm ODS column (Eicompack SC-5ODS; Eicom) and an electrochemical detector (ECD-100; Eicom). The mobile phase consisted of 5 mg/l EDTA, 190 mg/l sodium 1-octanesulfonate, and 17% methanol.

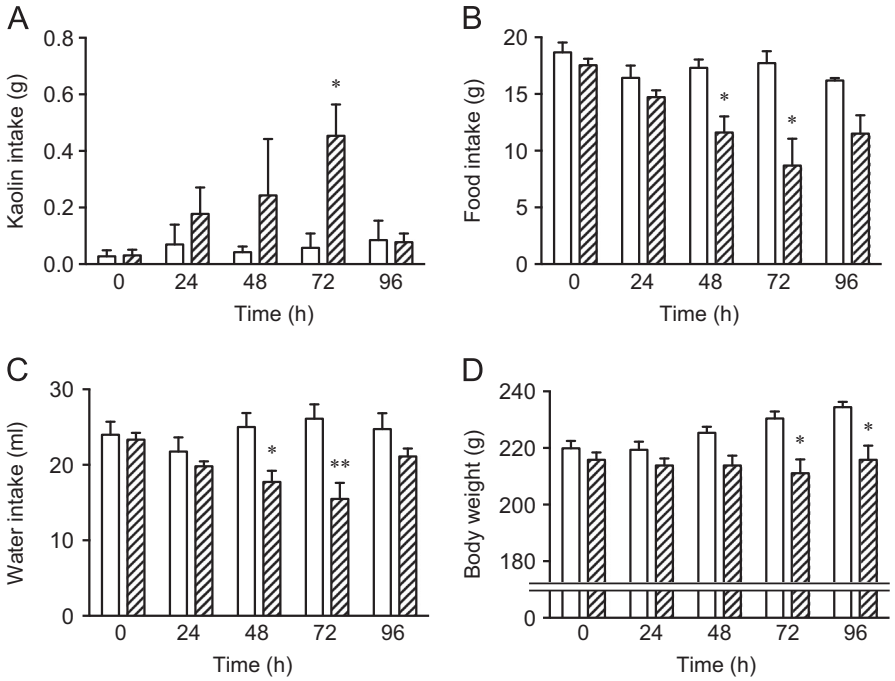

Fig. 1. Effects of methotrexate on kaolin (A), food (B), and water (C) intake and body weight (D) in rats after a single administration (50 mg/kg. i.p.). Kaolin and food intake were measured by an automatic monitoring apparatus. Results are shown as cumulative daily amounts (g) during 24-h periods up to 96 h. Each column represents the mean ± S.E.M. of 4 and 8 animals for the control (open column) and methotrexate (hatched column) groups, respectively. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus control group.