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ORIGINAL ARTICLE

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Gastric mucosal blood flow and gastric secretion following intravenous administration of 5-fluorouracil in anesthetized rats

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Abstract Acute gastric mucosal lesions are often observed after the intravenous administration of high doses of anticancer drugs. To investigate the acute toxic effects of such anticancer therapy on the gastric mucosa, 5-fluorouracil (5-FU) was administered intravenously to anesthetized rats. Gastric mucosal blood flow (GMBF) was measured continuously using laser Doppler velocimetry. Acid secretion was measured using a perfusion method for 1 h after the administration of 5-FU. No significant change was observed with a low dose of 5-FU (50 mg/kg), but a high doses of 5-FU (100 or 200 mg/kg) caused a significant decrease in GMBF in a dose-dependent manner. The selective antagonist of the muscarinic acetylcholine receptor, pirenzepine, prevented the decrease in GMBF with high doses of 5-FU. Acid secretion decreased after the administration of 5-FU, but not significantly. This study indicates that a decrease in GMBF may be an important factor in gastric mucosal injury induced by chemotherapy. Pirenzepine may prevent the gastric mucosal lesions which are induced by the administration of 5-FU.

Key words Gastric mucosal blood flow · Gastric secretion · 5-Fluorouracil

Introduction

Nausea and vomiting, and mucositis are common symptoms of gastrointestinal toxicity in patients receiving cancer chemotherapy. Acute gastric mucosal lesions have been observed after the intravenous administration of high doses of anticancer agents [8, 10, 11]. The mechanism of such gastric toxicity induced by cancer chemotherapy is not completely understood.

We conducted a study to investigate whether the administration of 5-fluorouracil (5-FU) has a direct toxic effect on the gastric mucosa. The protective effect of pirenzepine, a selective antagonist of the muscarinic acetylcholine receptor, was also investigated.

Materials and methods

Animal preparation

Male Wistar rats weighing between 250 and 350 g were obtained from Nippon Rat Co. (Hamamatsu, Japan). Animals were maintained in a room at 24 °C, at a humidity of 40% and under a lighting cycle of 14 h of light and 10 h of darkness. Animals were observed for 1 week prior to the study to exclude preexisting diseases. Animals had free acess to MF rat chow (Oriental Yeast Co., Chiba, Japan) and tap water, except 24 h prior to the experimentals when they were deprived of food but allowed access to water. All animals were maintained in accordance with the guidelines described in the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (DHEW Publication No. (NIH) 85-23, revised 1985, Office of Science and Health Reports).

The rats were anesthetized with intraperitoneal pentobarbital sodium (35 mg/kg). Supplementary doses were administered as required. They were fixed in the supine position on a surgical board throughout the experiments. A 24-gauge polyethylene tube was inserted into the left jugular vein for drug infusion. Saline solution was infused intravenously at a rate of 10 ml/kg per h throughout the experiments.

Measurement of gastric mucosal blood flow

Gastric mucosal blood flow (GMBF) was measured by laser Doppler velocimetry (PeriFlux PF3, Perimed KB Co., Sweden). The technique was similar to that described by Saita et al. [9]. Following a laparotomy, the forestomach was incised, and the gastric contents was gently lavaged using saline at 37 °C. The velocimetry probe was inserted into the glandular stomach through an incision in the

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forestomach, and was placed lightly on the mucosal surface at the posterior wall of the corpus. GMBF was recorded continuously. Following GMBF at steady-state for 10 min, drugs were administered intravenously for 5 min. GMBF was recorded for 60 min after the administration of the drugs. The GMBF data were expressed as percentage change from basal values.

Measurement of acid secretion

Acid secretion was measured in rats prepared according to the method of Ghosh and Schild [3] with modification. The pyloroduodenal junction was exposed following a laparotomy. Polyethylene tubes with side holes were inserted into the stomach through the duodenum and the right lateral abdominal wall for drainage. The tubes were secured firmly by tying a ligature around the pyloric ring. The tip of a 20-gauge tube was placed in the forestomach, and a 16-gauge tube was positioned in the antrum. The gastric lumen was washed out with saline at 37 °C infused through a 20-gauge tube at the rate of 0.5 ml/min. No regurgitation to the esophagus was observed. Finally, the abdominal wound was closed. Pentagastrin dissolved in a saline solution was administered intravenously as a stimulant at a continuous dose of 1.5 μg/kg per h throughout the experiments.

The perfusate from the 16-gauge tube was collected at 10-min intervals and was titrated with 0.01 *N* NaOH to pH 7.0. Acid output was expressed in microequivalents (μEq) per minute. 5-FU (200 mg/kg, $n = 5$) was administered intravenously after the pH of the collected samples had become stable.

Drugs

5-FU was purchased from Kyowa Hakko Co. (Tokyo, Japan). Pirenzepine was purchased from Nippon Boehringer Ingelheim Co. (Tokyo, Japan). In one experiment, 5-FU diluted with saline was administered at three doses: 50 ($n = 5$), 100 ($n = 6$), and 200 ($n = 5$) mg/kg. Saline solution was administered as a control ($n = 7$). In another experiment, 5-FU (200 mg/kg) and pirenzepine (1 mg/kg) were administered simultaneously for 5 min ($n = 5$).

Morphological study

The rats were sacrificed by dislocation of the neck. The stomach was removed and opened along the greater curvature for macroscopic examination of the mucosal surface. Tissue specimens obtained from the corpus of the stomach were fixed immediately with 10% formalin solution for histologic study. Sections were stained with hematoxylin and eosin.

Statistical analysis

Data are presented as mean ± standard deviation (SD). Statistical analysis was performed using Student's *t*-test. A *P*-value <0.05 was considered statistically significant.

Results

Effect of 5-FU on GMBF

No significant change in GMBF was observed with 50 mg/kg of 5-FU compared with the saline control. With both 100 and 200 mg/kg of 5-FU, GMBF decreased gradually. The decrease was significant 30 to 60 min after the administration of 5-FU compared with the control values. The decrease occurred in a dose-dependent manner (Fig. 1). The GMBF values 60 min after the administration of 5-FU were 60.8 ± 19.1% of the basal values with 100 mg/kg 5-FU and 40.8 ± 16.5% with 200 mg/kg.

Effect of pirenzepine and 5-FU on GMBF

Pirenzepine at a dose of 1 mg/kg was administered simultaneously with 200 mg/kg 5-FU. The values of GMBF 50 and 60 min after the administration of the drugs were 83.8 ± 15.6% and 87.7 ± 16.3% of the basal value, respectively. Pirenzepine prevented the decrease in GMBF. The pirenzepine group showed a significantly ($P < 0.01$) higher value than the group receiving 5-FU alone (Fig. 2).

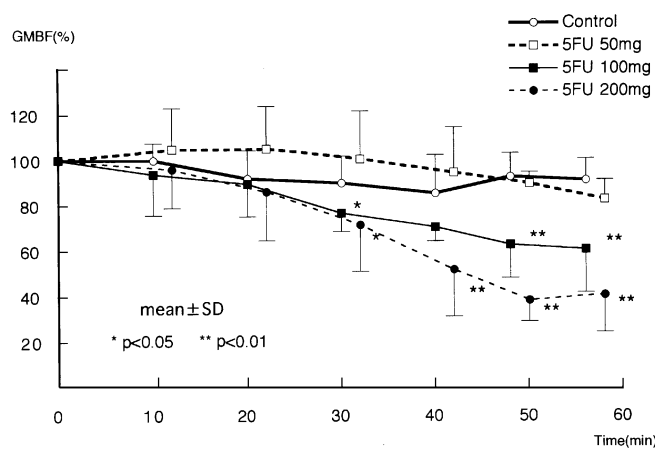

Fig. 1 Gastric mucosal blood flow during administration of 5-FU

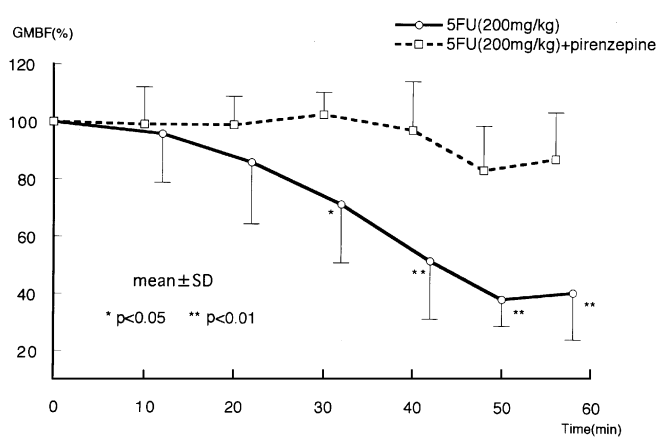

Fig. 2 Effect of pirenzepine (1 mg/kg) and 5-FU (200 mg/kg) on gastric mucosal blood flow

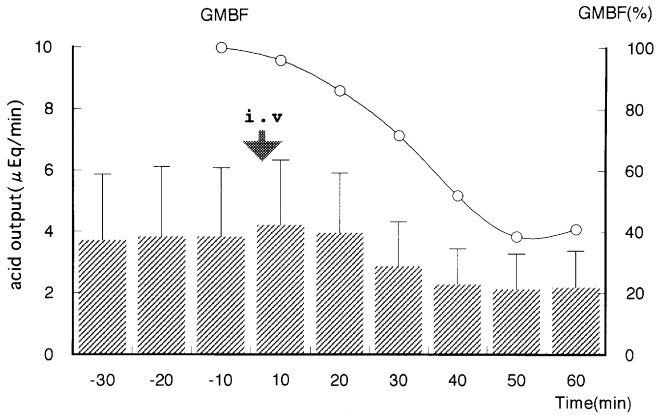

Fig. 3 Gastric acid secretion during administration of 200 mg/kg 5-FU

Effect of 5-FU on acid secretion

When 200 mg/kg 5-FU was administered intravenously to pylorus-ligated rats, the gastric pH tended to increase and acid secretion tended to decrease (Fig. 3). The effect on acid secretion was not significant compared with the basal acid secretion. The changes in gastric acid secretion paralleled the changes in GMBF.

Gross morphology

In rats receiving high-dose 5-FU, the gastric mucosa was characterized by edema and diffuse reddening. Rats receiving pirenzepine had essentially normal mucosa. None of the stomachs showed signs of acute or chronic ulceration or bleeding.

Histology

With the exeption of superficial hyperemia, no mucosal damage or inflammation was noted. No changes were found in liver or heart muscle preparations.

Discussion

Blood flow is important in maintaining the condition of the gastric mucosa. To our knowledge, there are no previous investigations on the change in GMBF with intravenous anticancer drugs. Anesthetized animals were used in this study to avoid stress reactions and effects on the chemoreceptor trigger zone. Even though 200 mg/kg 5-FU is a high dose, one-third of the ED_{50} with intravenous administration, respiration and heart rates were stable during the experiment.

We found that 5-FU had a direct toxic effect on the gastric mucosa. Edema and reddening of the gastric mucosa were observed. No significant change was seen with a low dose of 5-FU, but GMBF significantly decreased with a high dose of 5-FU in a dose-dependent manner.

The selective antagonist of the muscarinic acetylcholine receptor, pirenzepine, was given at the same time as high-dose 5-FU to investigate its protective action against mucosal toxicity. The dose of pirenzepine used, (1 mg/kg) had previously been shown to increase GMBF and to inhibit gastric secretion in rats [6]. The increase in GMBF induced by pirenzepine is mediated in rats by an endothelium-derived relaxing factor (EDRF) [5]. Nitric acid as an EDRF has been identified [7]. Kitagawa et al. have reported that the continuous direct arterial infusion of collagenase from the splenic artery to the stomach causes a reduction in endothelial cells in the submucosal vasculature. The endothelial cells play an important role in the regulation of the gastric mucosal circulation [4]. We suggest that 5-FU may damage the vascular endothelial cells and inhibit the secretion of EDRF.

There are a limited number of studies on acid secretion relative to chemotherapy. Bright-Assare and Kauffman have reported that infusion of 5-FU into the left gastric artery has no effect on histamine-stimulated gastric acid secretion in dogs [1]. However, Fabrin et al. have reported a decline in the gastric potential difference in pigs and a tendency for gastric pH to increase after combination chemotherapy, including fluorouracil, cyclophosphamide, and epirubicin [2]. In the present study, intravenous administration of high-dose 5-FU produced a tendency for gastric pH to increase and acid secretion to decrease, but the latter was not significant compared with basal acid secretion. The changes in gastric acid secretion paralleled the changes in GMBF. We could not determine whether the decreasing acid secretion was secondary to the reduction in GMBF. In another study, acid secretion after cisplatin (2 mg/kg) administration significantly decrease ($P < 0.05$) in rats (data not shown). Anticancer drugs may have an inhibiting effect on acid secretion.

Although it is generally thought that negative effects on cell turnover can cause gastric mucosal injury, such injury would not be expected within 1 h following drug infusion, the observation period in our study. The pathogenesis of gastric mucosal injury remains obscure, but we speculate that GMBF reduction is responsible. If a decrease in GMBF is prolonged for several hours, ulceration and bleeding may occur.

In summary, a decrease in GMBF may be a factor in the gastric mucosal injury induced by chemotherapy. Concomitant administration of pirenzepine may be a useful prophylaxis for chemotherapy induced gastric mucosal injury.

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