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Characterization of mitomycin C-induced gastrointestinal damage: changes in the gastric absorption of drugs in rats

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The effect of mitomycin C (MMC) pre-administration intravenously on the absorption of drugs from rat stomach has been examined by means of the in-situ loop technique. 48 h after the MMC-treatment, the absorption of salicylic acid, aspirin and sulphanilic acid was not influenced but that of sulphanilamide was significantly increased compared with the control. At 96 h, a differential effect of MMC on the absorption of each drug was seen: the absorption of weakly acidic drugs was significantly decreased while that of bases and strong sulphonic acid increased. The decreased absorption of salicylic acid and aspirin correlated with the reduced gastric mucosal blood flow. At 96 h there were severe haemorrhagic lesions in the gastric mucosae. The increase in absorption of poorly absorbed drugs could be ascribed to the increased permeability of the blood-gastric epithelium barrier as was evidenced by leakage of Evans Blue.

The gastrointestinal tract is a rapidly proliferating tissue that is severely damaged by antitumour drugs. The influence of such drugs on the intestinal absorption of other drugs and nutrients has been widely investigated, but less attention has been paid to their effects on the absorptive function of the stomach, where absorption of most acidic and weakly basic drugs administered orally takes place (Schanker et al 1957, 1958). Recently, Bright-Asare & Kauffman (1984) reported that gastric mucosal injury characterized by oedema, erythema, friability and ulceration of the mucosa, was produced in dogs being infused with 5-fluorouracil via the gastric artery. On the other hand, Nakamura et al (1983) found increased gastric mucosal permeability to phenolsulphonphthalein, a poorly absorbed compound, after indomethacin-induced mucosal damage in rats.

From these results, we postulated that gastric mucosal permeability would be changed by pretreatment with an antitumour drug, mitomycin C (MMC), because it causes diffuse ectasia, focal atypia, and distortion of glandular architecture in the mucosa of rat stomach (Philips et al 1960). We have therefore investigated the effect of treatment with MMC on the gastric absorption of several drugs in rats by means of the in-situ loop technique.

MATERIALS AND METHODS

Materials

Mitomycin C (MMC) was kindly supplied by Kyowa Hakko Kogyo Co., Tokyo, Japan. Salicylic acid, aspirin, sulphanilamide and sulphanilic acid were reagent grade commercial products. Evans Blue was purchased from Merck (GFR). All other chemicals were of finest grade available and were used without further purification.

Animals

Male Wistar rats, 180–220 g, were used. They had free access to water and diet for at least three days to acclimatize to laboratory conditions. Then they were weighed and given a bolus intravenous injection of MMC (3 mg kg^{-1}) in 0·9% NaCl (saline) into the left femoral vein under light ether anaesthesia. Control rats received a comparable volume of saline. Animals were maintained on 12 h light–dark cycles in cages with wide-mesh floors. All injections were given between 1200 and 1300h. Both groups were fasted for about 20 h before the experiments to remove gastric contents, but water was freely available.

Absorption experiments

Forty-eight or 96 h following the administration of MMC or saline, gastric absorption was examined by the in-situ loop method of Schanker et al (1957). Animals were anaesthetized with an intraperitoneal injection of sodium pentobarbitone, and the stomach cannulated to prepare an in-situ loop. 4 ml of drug solution dissolved in isotonic pH 1·1 buffer consisting of 0·1 M HCl and 0·05 M NaCl (Schanker et al 1957) was introduced into the gastric lumen. At the end of an absorption period (usually 1 h), the drug

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solution in the gastric lumen was collected as completely as possible, and the gastric lumen washed with the buffer. The amount of drug absorbed was calculated by the difference of the amount of drug in the initial and final mucosal solutions.

Measurement of gastric mucosal blood flow

Gastric mucosal blood flow was measured in animals anaesthetized with intraperitoneal sodium pentobarbitone (20 mg kg^{-1}) using the hydrogen gas clearance method (PHG 201, Unique Medical Co., Ltd, Tokyo, Japan) as described by Yamamoto et al (1984). The theoretical considerations are largely based on Kety's approach to blood-tissue exchange of inert gases (Kety & Schmidt 1945). Gastric blood flow was expressed as ml min^{-1} per 100 g tissue weight.

Leakage of Evans Blue

The leakage of Evans Blue from the blood circulation into the gastric lumen was studied in the control and MMC-pretreated rats. The operation was the same as the absorption studies. 4 ml of saline was introduced into the gastric lumen and 1% (w/v) Evans Blue in saline (0·5 ml/rat) was administered intravenously. After 1 h, the amount of the dye exsorbed into the gastric luminal solution was determined spectrophotometrically at 606 nm.

Direct action of MMC on the gastric mucosal permeability

To investigate the direct action of MMC on gastric mucosa, the gastric lumen was pretreated for 1 h with 4 ml of 1 mM MMC dissolved in pH 8·3 isotonic bicarbonate buffer, which was used for protecting MMC against gastric acidity (Wakaki et al 1958). After thorough wash-out of MMC, the absorption experiment was performed as above.

Analytical method

Salicylic aid was extracted with chloroform after adding concentrated HCl, transferred to the aqueous phase (0·1 M NaOH) and determined spectrophotometrically at 295 nm. No salicylic acid was detected in the mucosal solution by HPLC in preliminary experiments. Aspirin was determined after being hydrolysed to salicylic acid by boiling the acidic solution for 30 min. Sulphanilamide and sulphanilic acid were diazotized and coupled with 2-diethyl-aminoethyl-1-naphthylamine. The coloured derivatives were extracted with isoamylalcohol by the addition of sodium chloride and the organic phase was determined spectrophotometrically at 555 and 560 nm.

Statistical analysis

The results were analysed statistically with the Student's *t*-test. $P < 0.05$ was considered significant.

RESULTS

Macroscopical observations revealed few outward signs of disturbance by 48 h after the administration of MMC. Diarrhoea was rarely present. But 96 h after the dosing, animals were lethargic and severe watery diarrhoea was often present. On laporotomy, gross distention of stomachs and intestines was found both being filled with evil-smelling liquid. Some stomachs were removed and opened along the greater curvature after fixation of the muscular outer surface by 1% (v/v) formaldehyde solution (Brodie & Hanson 1960). The gastric mucosa 48 h after the MMC had no pronounced modifications except that the corpus seemed to be somewhat red. However, at 96 h gross haemorrhagic lesions in the corpus and antrum could often be seen. No gross lesion developed in the forestomach.

Table 1 summarizes the effect of MMC-pretreatment on the absorption by the stomach of the organic acids and bases examined. In the control animals, weak acids such as salicylic acid and aspirin, were well absorbed from the buffer solution. On the other hand, sulphanilic acid, a strong sulphonic acid, and sulphanilamide, a base, were poorly absorbed from this strongly acidic solution. At 48 h after dosing with MMC, the absorption of each drug was uninfluenced, except that of sulphanilamide was significantly increased compared with the control. At 96 h there was a differential effect of MMC on the absorption of each drug. The absorption of salicylic

Table 1. Effect of mitomycin C pretreatment on the gastric absorption of drugs.

	% Absorbed in 1 h†		
Drugs	Controls	MMC 48 h‡	MMC 96 h‡
Salicylic acid§	47·0 ± 2·2 (6)	45·6 ± 1·3 (5)	40·7 ± 1·2 (7)*
Aspirin	46·9 ± 2·2 (5)	50·4 ± 1·1 (6)	30·3 ± 1·7 (5)**
Sulphanilamide	1·9 ± 0·5 (5)	6·2 ± 0·6 (10)**	7·3 ± 0·7 (8)**
Sulphanilic acid	5·3 ± 0·7 (6)	6·6 ± 1·2 (6)	10·6 ± 1·8 (6)*

† Absorption of each drug was estimated by means of in-situ loop technique (pH 1·1).

‡ Absorption experiments were performed on rats 48 or 96 h after a bolus intravenous injection of mitomycin C (MMC) (3 mg kg^{-1}).

§ In the case of salicylic acid, each value represents the absorption percentage in 30 min. Results were expressed as the mean ± s.e. with the number of experiments in parentheses. The data were analysed by means of Student's *t*-test. Statistical significance: $^{*}P < 0.02$; $^{**}P < 0.001$.

acid and aspirin was significantly decreased while that of sulphanilamide and sulphanilic acid increased. The wet weight of stomachs was not significantly changed, i.e. that of control and MMC-treated rats was 0·973 ± 0·019 (control; mean ± s.e., n = 41), 0·931 ± 0·025 (MMC 48 h; n = 30) and 0·850 ± 0·024 g (MMC 96 h; n = 24), respectively. In separate experiments, the buffer solution (pH 1·1) containing no drug was introduced into the gastric lumen and the pH of the luminal solution measured after 1 h. The mean pH of the gastric luminal solution was virtually identical in the three groups (pH in control and MMC-treated rats, 1·22 ± 0·02 (control; mean ± s.e.), 1·27 ± 0·03 (MMC 48 h) and 1·39 ± 0·05 (MMC 96 h), respectively; n = 4 per group). Consequently, the changes in gastric absorption of the drugs were not due to the change in gastric pH.

To ascertain that the changes described were derived from pretreatment with MMC, the relation of suppression of aspirin absorption to dose of MMC was characterized in animals that had received various doses of MMC 96 h before experiments.

Fig. 1 shows that the magnitude of the decrease in aspirin absorption was dependent on the dose of MMC. However in the rats treated with 0·3 mg kg^{-1} of MMC, there was no significant decrease in aspirin absorption.

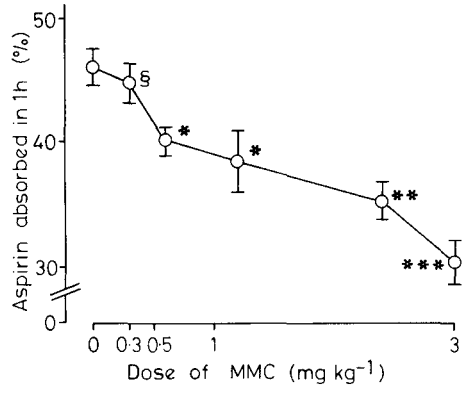

FIG. 1. Dose-dependent decrease in the aspirin absorption from the rat stomach. Its absorption from the in-situ gastric loop was determined at 96 h after an intravenous administration of 0·3, 0·6, 1·2, 2·4 and 3 mg kg^{-1} mitomycin C. Symbols represent the mean ± s.e. of at least five experiments. Statistical significance: (§) not significant; (*) $P < 0·05$; (**) $P < 0·01$; and (***) $P < 0·001$.

To examine the mechanism by which the gastric absorption of salicylic acid and aspirin was decreased by pretreatment with MMC, the mucosal blood flow was measured. As shown in Fig. 2, the flow was

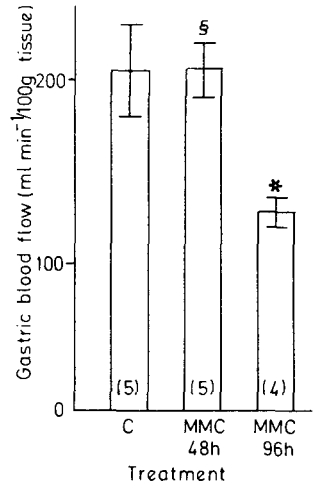

FIG. 2. Effect of mitomycin C pretreatment on the gastric mucosal blood flow in rats. Animals were given mitomycin C (3 mg kg^{-1}) intravenously and their gastric mucosal blood flow was measured 48 or 96 h after by means of the hydrogen gas clearance method. Results were expressed as the mean ± s.e. (ml min^{-1}/100 g tissue). Numbers in the columns represent the number of experiments. Statistical significance: (§) not significant; and (*) $P < 0·05$.

almost unchanged 48 h after the dosing, but it was significantly reduced at 96 h; the mean values at this time were about 62% of those from control rats. The time-courses of changes in the gastric mucosal blood flow and in the absorption of salicylic acid (and aspirin) followed the same pattern.

The effect of MMC on the leakage of Evans Blue from the blood capillaries into the gastric lumen was studied to examine the changes in the permeability of the blood-gastric epithelium barrier (Fig. 3). At 48 h after MMC injection, the amount of Evans Blue leaked had already increased significantly. A 2-fold increase in the amount of dye excreted into the gastric lumen compared with the controls was observed in rats 96 h after MMC.

To investigate whether MMC directly interacted with the gastric mucosal membrane and thereby altered its permeability characteristics, the mucosa of the control was pretreated with MMC solution (1 mM) for 1 h and then absorption experiments were performed as usual; MMC had no significant effect on the absorption of these two drugs (Fig. 4).

DISCUSSION

It is well known that most antitumour drugs induce undesirable gastrointestinal side-effects including anorexia, weight loss and diarrhoea. An early study of MMC toxicity showed that in-vivo administration

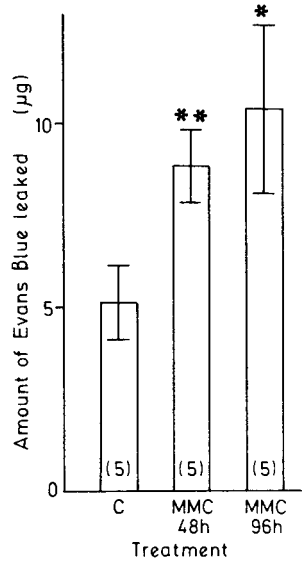

FIG. 3. Leakage of Evans Blue into gastric lumen of control and mitomycin C-treated rats. Animals were administered with mitomycin C (3 mg kg^{-1}) intravenously. 48 or 96 h after dosing, the stomach was cannulated to prepare an in-situ loop and 4 ml of 0·9% NaCl (saline) was introduced into the gastric lumen. Evans Blue in saline (1% (w/v)) was administered intravenously (0·5 ml/rat). After 1 h, the dye that had leaked into the gastric lumen was determined spectrophotometrically. Results are expressed as the mean ± s.e. Numbers in the columns represent the number of experiments. Statistical significance: (*) $P < 0·05$; and (**) $P < 0·02$.

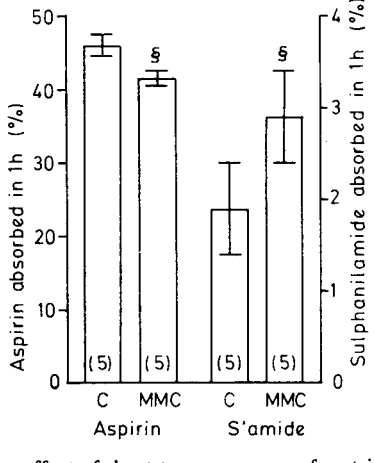

FIG. 4. The effect of short-term exposure of gastric mucosa to mitomycin C on the absorption of aspirin and sulphanilamide. The stomach of rats not having mitomycin C was filled with 1 mM mitomycin C (dissolved in pH 8·3 isotonic bicarbonate buffer). After 1 h, the stomach was thoroughly washed with HCl–NaCl buffer (pH 1·1) and then the absorption experiment was performed by means of the in-situ loop technique (pH 1·1). Results are expressed as the mean ± s.e. Numbers in the columns represent the number of experiments. Statistical significance: (§) not significant.

of the drug caused lesions in the epithelium of the intestine and stomach in rats (Philips et al 1960). Komuro et al (1975) suggested that MMC reduced the absorptive surface area of small intestine, which caused malabsorption of a wide range of nutrients and drugs. However, there have been few papers about the effects of MMC on gastric absorption. We have presented herein basic information that should aid the clarification of antitumour drug-induced disorders of the gastric absorptive function.

As shown in Table 1, the absorption of drugs from the stomach was significantly changed within 96 h of dosing with MMC. That is, the absorption of the well-absorbed drugs salicylic acid and aspirin decreased, while that of poorly absorbed sulphanilamide and sulphanilic acid increased. The absorption of aspirin was decreased by MMC preadministration dose-dependently (Fig. 1), suggesting that the changes in absorption were derived from gastric damage induced by MMC.

To clarify the inhibitory effect of MMC on salicylic acid and aspirin absorption, its effect on gastric mucosal blood flow was investigated by means of the hydrogen gas clearance method (Fig. 2). Since both the absorption and blood flow change followed the same time-course pattern, it was indicated that the transport of these non-dissociated lipid-soluble molecules was restricted, at least in part, by the reduced blood flow. The findings of Yamamoto et al (1984) that the decreased absorption of salicylic acid from the rat stomach during systemic anaphylaxis was induced by reduction of the blood flow, lends further support to the present conclusion. The leakage of Evans Blue from the blood circulation into the gastric lumen was measured in control and MMC-pretreated rats as an index of the blood-gastric epithelium permeability (Fig. 3). A significant increase in the leak of dye was observed in the MMC-pretreated rats, indicating that the blood-gastric epithelium barrier had become more permeable by 48 h after administration of MMC. Nakamura et al (1983) reported that gastric absorption of a poorly absorbed drug, phenolsulphonphthalein, was much increased in rats with gastric mucosal damage induced by oral indomethacin. So it may be reasonable to consider that sulphanilamide and sulphanilic acid, even in their ionic forms, could pass through gastric mucosal membrane more easily at this stage.

Recently, Pinkerton et al (1985) suggested that methotrexate had a topical effect on small intestinal transport independent of its effect on crypt cell kinetics. MMC is suggested to be active in dividing

cells and behave as an alkylating agent to inhibit DNA synthesis (Lown 1979), moreover, RNA synthesis is also affected by it at high concentrations.

To examine the direct effect of MMC on gastric mucosa, the tissue was exposed to 1 mM MMC for 1h. The lack of the effect on the absorption of aspirin and sulphanilamide suggested that there was no direct action. However, changes in the mucosal permeability and the blood flow played a role in the absorption processes at 48 and 96 h after administration of MMC. Therefore, it is considered that other changes might occur in the intraluminal or membrane factors that control the absorption of drugs. Changes in systemic physiological conditions might also contribute to the absorptive function of the stomach.

Nothing definite is known about the mechanisms by which antitumour drugs cause mucosal damage and also reduce blood flow although they have been known to induce gastrointestinal lesions and ulcerations in clinical situations. Cell renewal studies indicated that the stomach turns over its epithelial cells at a rate similar to the intestine, the proliferative cell cycle for gastric mucous epithelial cells being 2 to 3 days, and mucosal replacement 4 to 6 days (Lipkin 1981). In rodent and man, parietal and zymogen cells renew more slowly than mucous epithelial cells. Taking such a high proliferative rate of cells and observed lack of direct action of MMC into consideration, the toxic effects induced by MMC in the gastric mucosa would occur as a result of mitotic arrest in the dividing cells.

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