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—Original Article—

EFFECTS OF ANTICANCER AGENTS OF THE INTESTINAL EPITHELIUM

A morphologic study

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Summary

In order to make clear the pathophysiology of digestive symptoms which are caused with anticancer agents, three anticancer agents, i.e., mitomycin C, 5-FU and cytosine arabinoside were administered pre-operatively, and the ultrastructure of the intestinal epithelium, especially that of absorptive cells, were studied on the specimens collected intra-operatively.

In view of the finding that severe degeneration occurred inside cytoplasms, chiefly their nuclei, it was surmised that the biosynthesis and supply of proteins such as digestive enzymes were not amply carried on; hence, the chemotherapy could give rise to severe digestion and absorption disorders. It appeared necessary to further study the dosage and dosage schedule of the anticancer agents, along with the necessity for taking ample care of patients presenting such disorders.

Key Words: *human jejunal epithelium, absorptive cell, anticancer agents, side effects, mitomycin C, 5-FU, cytosine arabinoside.*

Introduction

Today, importance is attached not only to surgical therapy but also to adjuvant chemotherapy as therapeutic means for patients with stomach cancer. In our department, too, intensive administration of anticancer agents has resulted in favorable long-term survival rates[1,2]. The occurrence of side effects, however, is the largest obstacle in the execution of cancer chemotherapy, and there are not a few cases where chemotherapy is withdrawn before an effective total dose has been administered[3,4]. Among other things, hematopoietic disorders and digestive disorders are side effects that most frequently occur, while there are few papers on basic studies of the etiology of digestive disorders from the chemotherapy. In order to make clear the pathophysiology of such digestive symptoms, morphologic observations were recently made of the jejunal epithelium on specimens from patients who had received preoperative adjuvant chemotherapy for stomach cancer, and the findings are presented hereunder.

Subjects and Methods

Among adjuvant chemotherapy regimens in employed in our department, MFC regimen whose side effects occurred relatively frequently was studied. The relations of the frequency of medication to the occurrence of side effects under this regimen are given in **Table 1**. Digestive symptoms were considerably frequently observed.

Table 1. Side effects of preoperative MFC treatment and their incidences. The figures in parentheses denote percentage (%)

Frequency of medication	Number of patients	Incidences of side effects	Anorexia	Lassitude	Nausea & vomiting	Diarrhea	Pyrexia	Abdominal pain	Leukopenia
10	3	3/3 (100)	1(33)	1(33)	3(100)	1(33)	0	1(33)	0
8	1	1/1 (100)	1(100)	1(100)	1(100)	0	0	1(100)	0
7	4	4/4 (100)	3 (75)	0	2 (50)	2(50)	0	1 (25)	2(50)
6	1	1/1 (100)	1(100)	0	1(100)	0	0	0	0
5	4	3/4 (75)	1 (25)	0	1 (25)	0	0	0	1(25)
3	40	20/40 (50)	7 (18)	4 (10)	10 (25)	5(13)	1(3)	5 (13)	0

In the present study, light-microscopic and electron-microscopic observations were made of the jejunal epithelium, especially the absorptive cells, on the specimens obtained one week after daily or dieb. alt. administration of MFC (4 mg of MMC, 250 mg of 5-FU and 40 mg of cytosine arabinoside) three to seven times in the pre-operative stage. From each of ten patients who had received anticancer agents pre-operatively for treatment of stomach cancer, consisting of eight patients who had received the administration of MFC three times, one who had received it five times, and another who had received it seven times, and also from each of ten patients with gastro-intestinal diseases who had not been medicated with anticancer agents pre-operatively, an epithelial specimen was collected of that portion of the jejunum which was about ten cm on the distal side of Treitz ligament during a gastroenterostomy by Billroth's II procedure, doubled fixed in 2% glutaraldehyde phosphate buffer (pH 7.4) and 1% osmic acid, dehydrated with alcohol, embedded with Epon resin, and then sliced into ultrathin sections with the Porter-Blum model MT2 microtome. The sections were then double stained with uranyl acetate and lead citrate, and observed with the Hitachi model HU-11Ds electron microscope. At the same time, some of the sections were stained with toluidine blue and also with hematoxylin and eosin for light-microscopic observation.

Results

Light-microscopic findings of the small intestine: **Photo 1** shows a jejunal specimen from an untreated patient with anticancer agent. Finger-like elongated villi are regularly arranged in the annular folds of the mucous membrane, and there are many goblet cells among the absorptive epithelial cells with oval nuclei. On the specimen from a patient who received the administration of MFC three times every other day prior to the operation (**Photo 2**), the annular folds are flatter than those on the specimen from an unmedicated patient, and at the same time, the villi are arranged irregularly and sparse; furthermore, they have lost their heights markedly, some of them having been disrupted. Absorptive cells are obscurely demarkated from one another, and had pyknotic nuclei; cell bodies are basophilic, and heterochromic. Goblet cells are relatively decreased, too. These findings tend to be more striking in proportion to the increase in the dose of cancer chemotherapy.

Electron-microscopic findings of the small

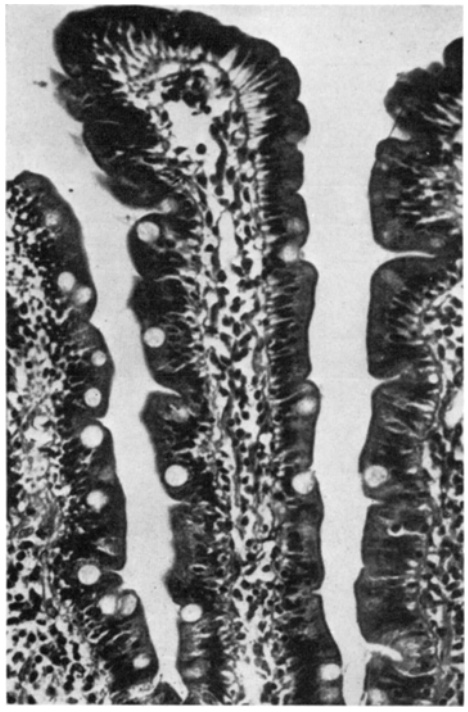

Photo 1. Villi of the jejunum on a specimen from a patient untreated with anticancer agents. The villi are elongated like fingers, and arranged regularly. (H & E stain, 250×)

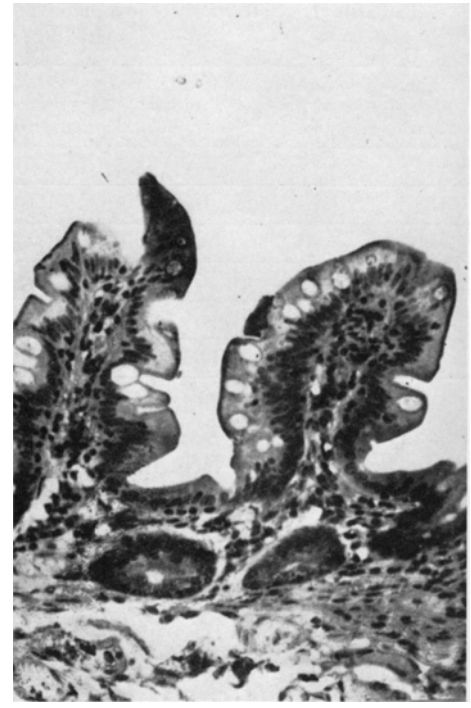

Photo 2. Villi of the jejunum on a specimen from a patient medicated with anticancer agents MFC three times. The villi are markedly flattened, are sparse, and are irregularly arranged. (H & E stain, 250×)

intestine: Absorptive cells of the jejunum on the specimen from a patient with a duodenal ulcer who was not pre-operatively treated with anticancer agents (**Photo 3**) are such that microvilli are arranged densely on the luminal side; cell nuclei are of slightly irregular oval shape, and located on the basal membrane side; the slightly dilated Golgi's apparatus are seen in the vicinity of the nuclei; there are many cell organoids such as mitochondria, free ribosomes, rough-surfaced and smooth-surfaced endoplasmic reticuli, and lysosomes are slightly increased; however, they are otherwise normal. Absorptive cells of the jejunum on the specimen from a patient who received the administration of MFC for treatment of stomach cancer three times every other day preceding operation show karyopyknosis, inhomogeneous aggregation of chromatin and marked atrophy of nucleoli (**Photo 4**). Mitochondria are markedly increased in number; lysosomes are also markedly increased; and there are mutiple vesicles (**Photo 5**). No abnormalities are observed in the other cell organoids in particular; microvilli are morphologically almost normal; the covering with polysaccharides is also normal. The opening of rough-surfaced endoplasmic reticulum is sporadically marked (**Photo 6**). The cells on the specimen from a patient who received the administration of MFC for seven consecutive days prior to operation are markedly degenearted as a whole, the nuclei being pyknotic and some having been lysed. The degeneration has not taken place uniformly in all cells arranged,

Photo 3. Absorptive cells of the jejunum on a specimen from a patient with duodenal ulcer who was not treated with anticancer agents. The cells are mostly normal in structure.

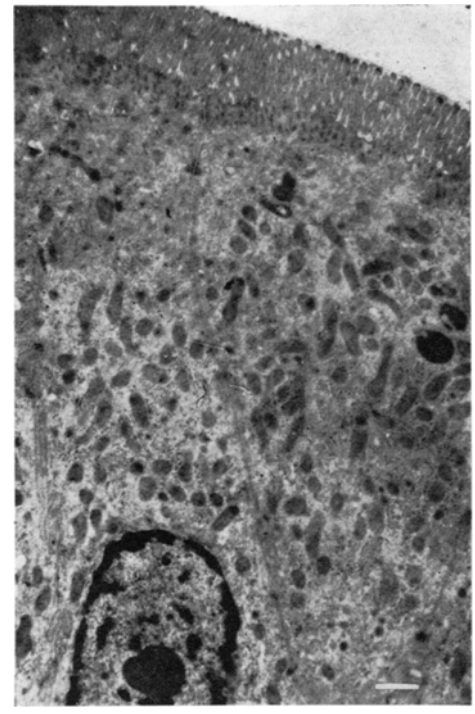

Photo 4. Absorptive cells of the jejunum on a specimen from a patient who received anticancer agents MFC three times every other day. Marked changes are seen in their nuclei.

however, and in some areas, markedly degenerated cells and apparently normal cells are arranged alternately (**Photo 7**). The electron density of the cell body is markedly increased in severely degenerated cells, and many white spot, vacuole-like matters which appear attributable to endoplasmic reticulum are seen (**Photo 8**). In some cells, the nuclear membrane is open; the location of crista is deviated; and mitochondria are dilated, being associated with their disarrangement. Lysosomes are also increased as compared with those on the specimen from a patient medicated three times (**Photo 9**). Microvilli in the degenerated cells morphologically are in no way different from those in normal cells.

Discussion

In recent years, Linß[5-7] made light-microscopic and electron-microscopic observations of the effects of actinomycin C on the small intestine in experiments on animals, but there are no papers on such studies of other anticancer agents. Actinomycin C is said to inhibit the biosynthesis of RNA and to cause servere digestive disorders[3]. He also observed the flattening of the annular folds and villi although his findings were not in entire agreement with our findings. Our electron-microscopic observations disclosed similar changes in cell organoids such as dilatation of mitochondria, opening of the rough-surfaced endoplasmic reticulum and increased lysosomes, but this anticancer agent appears to exert little effect on cell nucleus.

In association with the disclosure that 5-FU, when used in combination with mitomycin C

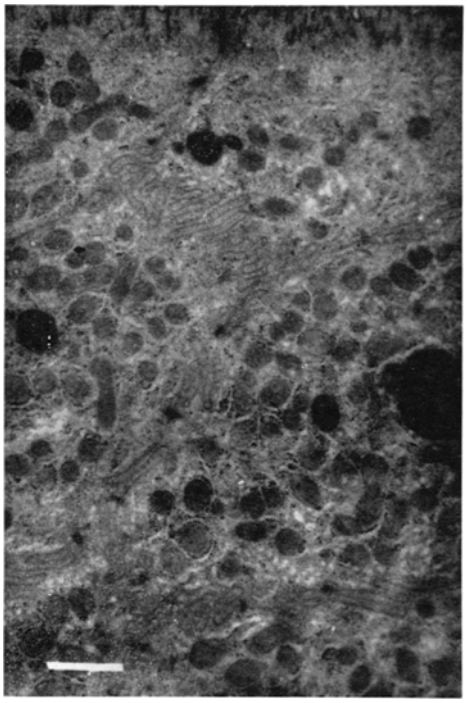

Photo 5. Absorptive cells from the jejunum on a specimen from a patient who received anticancer agents MFC every other day three times. Mitochondria and lysosomes are increased.

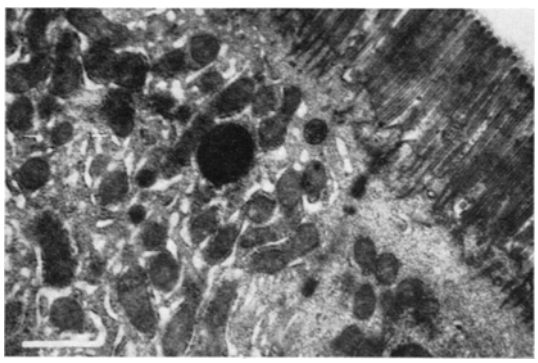

Photo 6. Absorptive cells from the jejunum on a specimen from a patient who received anticancer agents MFC every other day three times. Some cells have the open rough-surfaced endoplasmic reticulum.

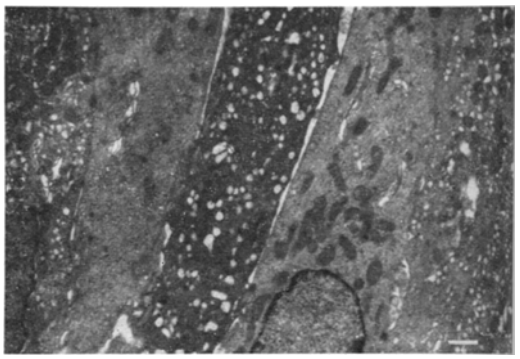

Photo 7. Absorptive cells of the jejunum on a specimen from a patient who was medicated with anticancer agents MFC every day seven times. Apparently normal cells and degenerated cells are alternately arranged.

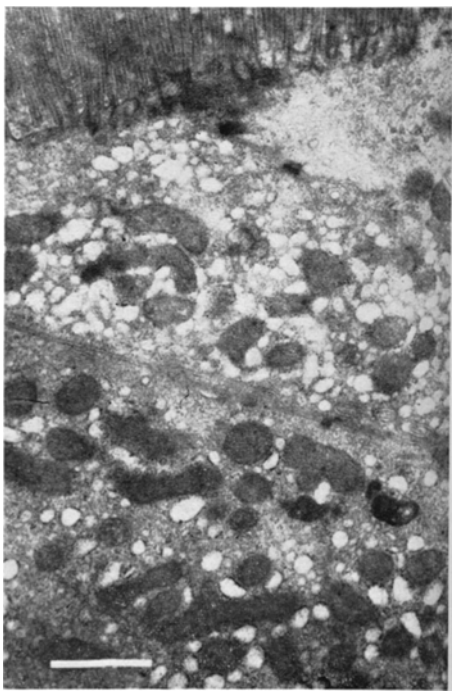

Photo 8. Absorptive cells of the jejunum on a specimen from a patient who was treated with anticancer agents MFC every day seven times. The opening of rough-surface endoplasmic reticulum is striking.

in the treatment of experimental animal tumors, exerted a synergistic life-prolonging effect[8], two agent combination therapy with 5-FU and mitomycin C or MFC therapy with cytosine arabinoside in addition to the said two agents has often been employed for the treatment of adenocarcinoma, and in our department, too, favorable results have been

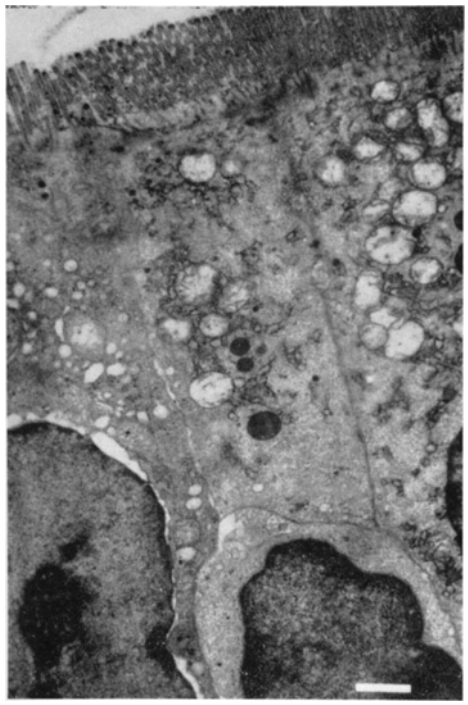

Photo 9. Absorptive cells of the jejunum on a specimen from a patient who received anticancer agents MFC every day seven times. Cells with open nuclear membrane and swelled mitochondria are noted.

attained with the therapy. Mitomycin C biochemically is a DNA synthesis inhibitor, while it is said that the mode of its action is to act on cancer cells in the inactive stage to degenerate and rupture them. It is commonly said that the agent experts a marked inhibitory effect on the hematopoietic organs but causes less digestive disturbances. It is also said that 5-FU competes with uracil to selectively enter the DNA synthesizing pathway of tumor where it inhibits the methylation of deoxyuridylic acid to thymidylic acid: it thus exerts an anticancer effect as a DNA synthesis inhibitor, and this agent causes severe digestive disturbances. Further, cytosine arabinoside, being a kind of pyrimidine nucleoside, is a metabolism antagonist which inhibits the DNA synthesizing process, and also exerts a potent inhibitory effect on the marrow[3].

It appeared that because, as described in the foregoing, each component agent of MFC cancer chemotherapy has a DNA synthesis inhibitory action, the therapy brought about a severe degeneration inside cancer cells, chiefly around their nuclei. The increased frequency of administration of the anticancer agents was associated with the severest changes in the cell nuclei, some nuclei having been lysed; secondary degeneration of cell organoids occurred inside cell bodies, and lysosomes were markedly increased in the supranuclear part of the cell body, leading to a surmise that the high-molecular substances were being actively uptaken and the degenerated substances in the cell were being actively disposed of.

In view of these findings, it could be considered that the biosynthesis and supply of proteins such as digestive enzymes were not sufficiently carried on, and severe digestion and absorption disorders could result. The doses of the anticancer agents were mostly paralleled with the degrees of damages to cells; however, because there were patients who showed severe damages to cells in spite of less frequent medication, it appeared that there would be some individual variations in the damage to cells. Severe damages to cells were observed in patients presenting severe diarrhea, irrespective of the size of dose administered, and it will be necessary to take enough care of such patients. It also appeared necessary to further study the selection, dosage and dosage schedule of anticancer agents to be used.

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