Studies on Preoperative Cancer Chemotherapy

—Effects of Preoperative Intra-arterial Infusion of Methotrexate and Mitomycin C on Stomach Cancer—

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The latest results of the operative treatment for gastric cancer are fairly disappointing by comparison with those of mammary cancer and it is hardly probable that a further operative attempt to exterminate gastric cancer tissues might break by itself the deadlock. In order to break this deadlock, the steady progress has been made in regard to an early detection of gastric cancer and adjuvant cancer chemotherapy. The U. S. Veterans Administration Surgical Adjuvant Cancer Chemotherapy Study Group, under the chairmanship of Oscar Serlin, MD, reported that the use of ThioTEPA as an adjuvant to surgical removal of the stomach cancer increased significantly 2-year survival rates. On the other hand, Shapiro et al., and McDonald et al. have reported that antitumor drugs have a marked inhibitory effect on the growth of transplantable tumor cells injected into the blood stream of experimental animals. From the same point of view mentioned above, the preoperative cancer chemotherapy has been attempted in gastric cancer patients in our surgical department. It is the primary aim of the preoperative cancer chemotherapy to administer preoperatively antitumor drugs as much as possible without any complications in the postoperative course. There was no sign of tumor regression in all patients treated by the preoperative cancer chemotherapy because of a short interval between the chemotherapeutic treatment and surgery. However, even though the preoperative intra-arterial infusion could not demonstrate any tumor response, it might have a suppressive effect on metastasis and dissemination of cancer cells originated from surgery by inhibiting DNA biosynthesis of cancer cells.

Since thymidine is incorporated into DNA at the phase of DNA doubling prior to the next mitosis, tritiated thymidine (\( ^3 \text{H-thymidine} \)) provides a method for the detection of cells at this phase after exposure to the isotope. Consequently, \( ^3 \text{H-thymidine} \) is most adequate for studying the patterns of cellular proliferation of various tissues. Johnson et al., Lieb et al., and Wolberg et al. have reported differences in DNA synthetic activity by autoradiography of human tumors labeled in vitro with \( ^3 \text{H-thymidine} \). From these results, the use of \( ^3 \text{H-thymidine} \) is suitable for investigating the influence of preoperative cancer chemotherapy on gastric cancer cells.

The investigation described in this paper was undertaken to study the effect of the preoperative intra-arterial infusion with methotrexate and mitomycin C on the patterns of DNA biosynthesis in gastric cancer cells.

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MATERIALS and METHODS

Human subjects and doses of drugs: In the First Department of Surgery of Chiba University, Japan, 17 patients with resectable stomach cancer received the preoperative intra-arterial infusion therapy with methotrexate and mitomycin C. The doses of methotrexate and mitomycin C are shown in Table 2. The method of the preoperative intra-arterial infusion was described in the previous report. Since the intra-arterial infusion with mitomycin C used to cause severe abdominal pain, one ampoule of Buscopan compositum*, a spasmolytic and analgesic agent, was added to the infusion fluid containing mitomycin C. In addition, 80 to 100 mg of pyridoxal phosphate was administered intravenously for inactivating mitomycin C in the liver.

Controls not received the preoperative cancer chemotherapy consisted of 10 patients with resectable stomach cancer and 7 patients with gastroduodenal ulcer (Table 1).

Tissue preparation and incubation: Gastric cancer tissues, metastatic lymph nodes, and gastroduodenal epithelia were removed quickly from the resected specimens. Gastric cancer tissues and metastatic lymph nodes were deeply colored with Evans blue, with which the infusion area was visualized. Metastatic lymph nodes removed on insertion of polyethylene catheters were served as the control specimens not affected by the preoperative cancer chemotherapy (Table 1).

The removed tissues were placed immediately in Eagle's Minimum Essential Medium (Eagle's MEM) supplemented with 10% calf serum and were cut into thin slices, 1 mm in thickness, with 2 razor blades stood in double file. Pieces of cancerous tissues in the infusion group were fixed in 10% neutral formalin. Sections of these tissues were stained with hematoxylin and eosin, and compared with those of the same tumor that was incubated by the following method.

Within 30 minutes after excision, the specimens were incubated at 37°C either in physiological saline or in Eagle's MEM containing 1.0 μc/ml of 3H-thymidine (specific activity 5.0 C/mM) obtained from Radiochemical Centre, Amersham, England. Incubation in physiological saline was terminated after 1 hour and that in Eagle's MEM after 5 hours. Half of the specimens in the infusion group were incubated in Eagle's MEM. The other half in the infusion group and all specimens in the control group were incubated in physiological saline. A mixture of 95% oxygen and 5% carbon dioxide was bubbled through the incubation fluid to keep the specimens in motion and to provide oxygen.

Preparation of autoradiographs and counting: The specimens were fixed in 10% neutral formalin and embedded in paraffin. Sections 4 μ thick were cut perpendicularly to the surface. The slides were deparaaffinized and washed in running water for several hours in order to remove unbound ³H-thymidine. Under dark room conditions with controlled temperature and humidity, autoradiographs were made by the dipping method using Sakura NR-M2 emulsion. The slides were exposed for 2 weeks for grain counting and for 4 to 5 weeks for labeled cell counting. After each exposure at 4°C in a light-tight box containing desicator, the slides were developed and stained with hematoxylin and eosin.

The grain counts were made on 200 nuclei selected at random per specimen and the

*Buscopan compositum is manufactured by C.H. Boehringer Sohn, Ingelheim along the Rhein, Germany, and contains 20 mg of hyoscinc-N-butylbromide and 2.5g of sulpyrin.