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A Trial in Estimating Chemosensitivity from Biopsy Specimen by Flow Cytometry

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To select the sensitive anticancer agents for the treatment of patients with carcinoma, chemosensitivity should be timely assessed from biopsy specimen for patients with gastric carcinoma.

Histogram of DNA analysis from biopsy specimens revealed the disappearance of G_2M accumulation in reflection of effects of anticancer drugs prior to the appearance of clinical signs.

In conclusion, DNA analysis from biopsy specimens is of great value in assessing the efficacy of anticancer drugs in the course of drug treatment and also in knowing the drug sensitivity to the tumor timely.

Introduction

Needless to say, it seems reasonable to consider that selection of anticancer drugs should be limited to a sensitive drug to malignant tumors.

There are many methods to assess the drug sensitivity in vivo and vitro study. However, at present, there is nothing of an ideal approach to determine the drug sensitivity precisely as well as to assess the efficacy of anticancer drugs in the course of drug treatment.

In this preliminary study, nuclear DNA determination from biopsy specimens was applied for assessment of biologic behavior in malignant tumors and the efficacy of anticancer drugs in comparison with DNA analysis between prior to or after administration of anticancer drugs.

On the other hand, long survivals of advanced cancer are only few in number in spite of the development of anticancer drugs. And also precise prediction of effects of potent anticancer drugs has to await development of the useful sensitivity test to anticancer drugs.

The purpose of this preliminary study is to certify the clinical application of cytometric DNA analysis for the assesment of the anticancer drug sensitivity from biopsied specimens.

Material and Methods

Endoscopic and roentgenologic examinations had been done before, during and after administration of anticancer drugs to assess the efficacy of anticancer drugs in combination with DNA analysis from biopsied specimens. The comparison of radiologic and endoscopic findings with the efficacy of anticancer drugs was made at each examination.

DNA analysis was conducted from biopsied speciments. Briefly, two or three biopsied specimens were made, adding 0.1% triton x-100 and micing with scissors, and were filtered through a 50mcm nylon mesh and stained with propidium Iodide (final concentration 50mcg/ml).

Cellular DNA content was measured by FACS scan IV.

Case report

Case 1: 62 years old, male

He complained of weightloss and dysphagia. He had a diagnosis of gastric cancer with Virchow' metastasis. Biopsy specimen of cervical nodes revealed poorly differentiated adenocarcinoma. ETP (THP-ADM 30mg/m^2 day 1, CDDP 40mg/m^2 day 2, 8, Etoposide 100mg/m^2 day 4, 5, 6) treatment was initiated at an interval of 4 weeks. Finally this treatment had been repeated twice. The values of CA19-9 (U/ml) and CEA (ng/ml) were gradually reduced, in particular, CA19-9 value fell to almost half of the pre-treatment values.

Roentgenologic finding was not improved as compared with those before and 1 month after anticancer drugs of ETP as shown in Fig. 1 (on the left). And also endoscopic finding failed to substantiate the efficacy of anticancer drugs (Fig. 1 on the right).

Meanwhile, a histogram of DNA content showed disappearance of G_2M accumulation as compared with that before administration of anticancer drugs as shown in Fig. 2. The effect of ETP treatment for poorly differentiated adenocarcinoma was represented as the disappearance of G_2M accumulation on histogram.

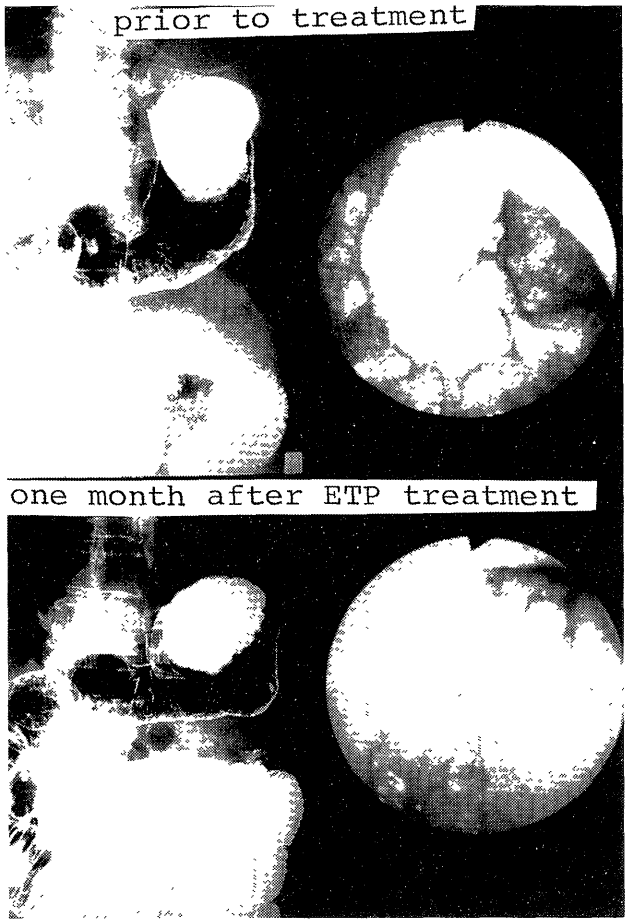

Fig. 1. Roentgenologic and endoscopic findings prior to and one month after ETP treatment

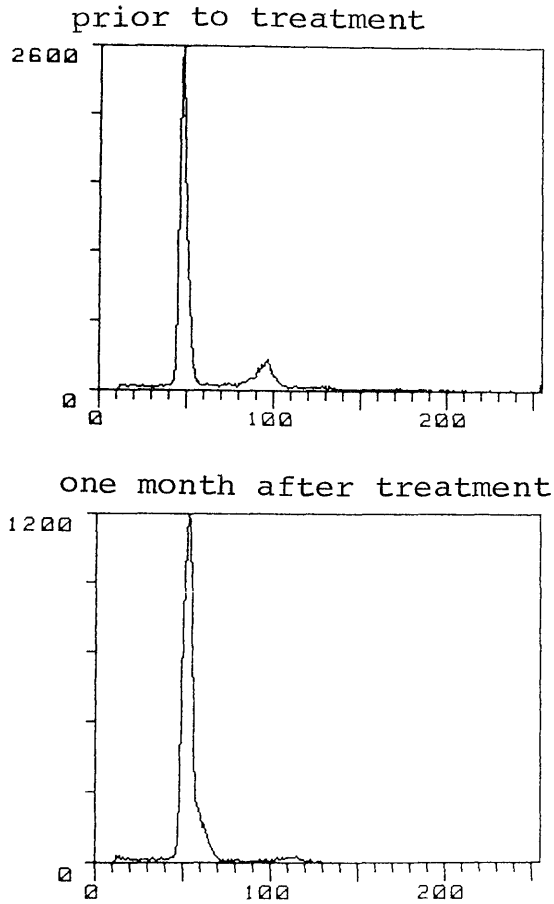

Fig. 2. Histogram one month after ETP treatment revealed disappearance of G_2M accumulation as compared with that before ETP treatment (upper)

Case 2: 63 years old, male

He complained of anemia and dysphagia. He had carcinoma of the stomach (scirrhus type) from the cardia to the antrum with ascitic fluid as shown in Fig. 3. Histology revealed poorly differentiated adenocarcinoma.

At laparotomy, it was defined that carcinoma of the stomach involved directly the pancreas, the duodenum and the transverse colon with diffuse peritoneal dissemination (P_3). Therefore, gastrectomy was abandoned. Continuous hyperthermic peritoneal perfusion therapy was performed for 50 minutes with a 42 ℃ saline of 3000ml to 4000ml, which contained CDDP (300mg) and etoposide (300mg).

After the 2nd month of the treatment, roentgenologic and endoscopic findings were not changed as shown in Fig. 3, and thereafter these were no altered 3 and 4 months after treatment as shown in Fig. 4. DNA histograms from biopsy specimens of gastric lesion were shown in Fig. 5. When compared with that prior to the treatment, accumulation of G_2M was already reduced on day 3 after the treatment in spite of a presence of the G_2M phase under magnification. Thereafter, DNA histogram showed the appearance of G_2M accumulation 3 months later, although it failed to be found

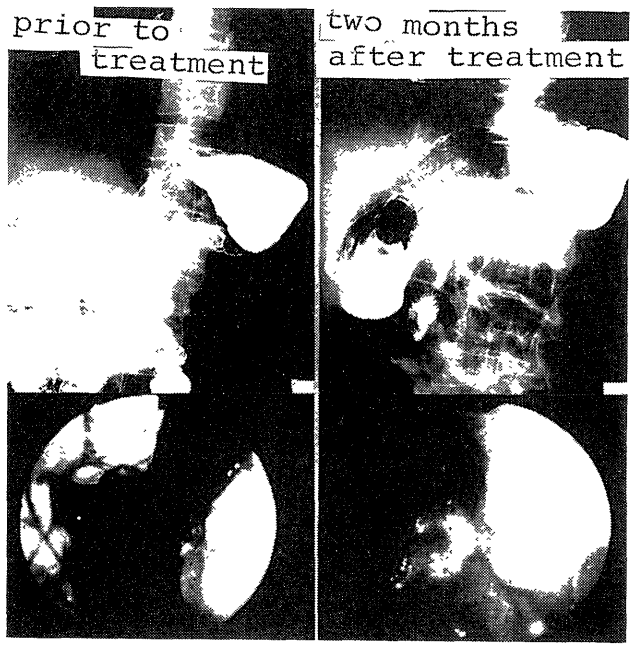

Fig. 3. Roentgenologic and endoscopic findings prior to and after continuous hyperthermic peritoneal perfusion (CHPP)

until 2 months after the treatment as shown in Fig. 5.

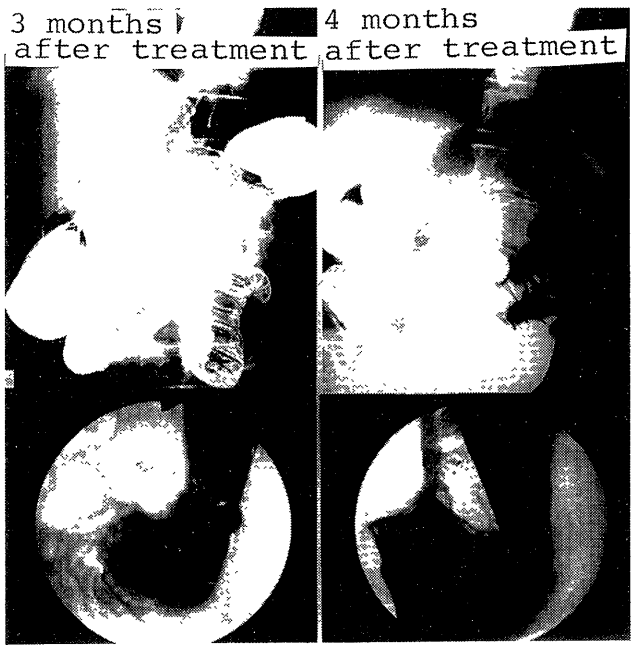

Fig. 4. Roentgenologic and endoscopic findings 3 and 4 months after CHPP. During these periods, no significant improvement was indicated

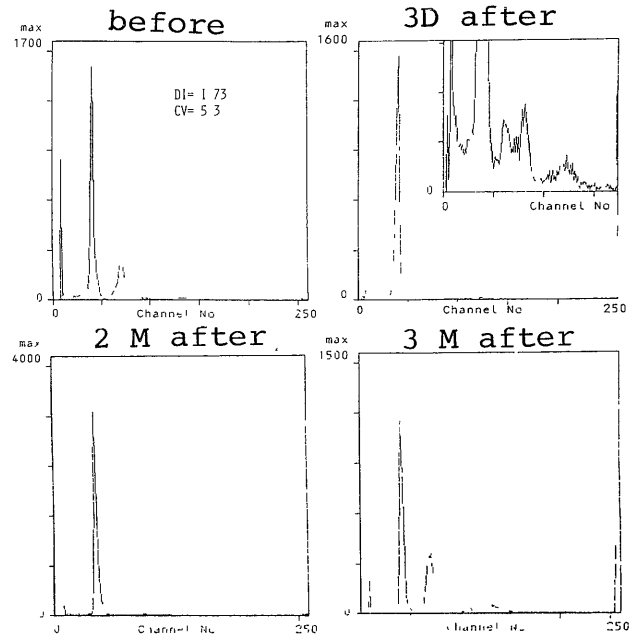

Fig. 5. Histogram revealed disappearance of G_2M accumulation on day 3 after CHPP in spite of the remaining G_2M accumulation under a magnification. At two months after CHPP, a G_2M accumulation was no longer seen on histogram but at three months after CHPP a phase of G_2M accumulation was appeared again.

Discussion

It is considered that the effect of anticancer drug should be predicted in case of the maximum uptake into the tumorous tissues. However, there have been few reports as to whether or not the drug concentration directly provides the influence of anticancer effects.[1]

Recently, flow cytometric DNA analysis was widely used for assessment of nuclear DNA content in a short time. The efficacy of drugs used should be determined by the following factors and methods such as the drug concentration of tissues,[2] the degree of cellular degeneration,[3] inhibition rate of TS activity,[4] changes in the cell cycle by flow cytometry[5] and chemosensitivity test by SDI[6] and SRC.[7]

The advantages toward DNA analysis by flow cytometry are that 1) changes in the same sites are able to be serially observed, 2) changes in DNA synthesis are able to certify even though histologic alteration failed to be elucidated, 3) small sample size is sufficient to evaluate changes in DNA synthesis.

The cellular DNA content reflects indirectly the number of chromosoms, and the DNA content in malignant tumors increases remarkably. It is caused by amplification of oncogens, increasing number of chromosoms, multipolar mitosis, polymorphism of oncogenic structure in cell growth and shift of the cell line.

Biopsy specimens are able to obtain timely in the course of the treatment of anticancer drugs. DNA histogram from biopsy specimens showed the disapperance of G_2M accumulation in reflection of inhibition of DNA synthesis by anticancer agents.[8,9] It is suggested that drug sensitivity to the tumor be first indicated on changes in the peak of the G_2M phase by anticancer agents of which the effects influence a pattern of G_2M accumulation.

Needless to say, the more the effects of anticancer drugs are enhanced, the more DNA synthesis should be inhibited. There are reports concerning no differences in the drug sensitivity between diploidy and aneuploidy patterns. On the other hand, it has been documented that aneuploid tumors are more sensitive to anticancer drugs in renal cell carcinomas or ovary carcinomas.

In fact, it is accepted that radiation therapy is effective for aneuploid tumors. In this series, the drug sensitivity has been shown as the result of the disappearance of G_2M accumulation on histogram. It implies that anticancer drugs sensitive to the tumors effectively act on aneuploid cells of malignant tumors. As a result, DNA ploidy patterns vary from aneuploidy to diploidy pattern because of the remaining diploidy cell group.

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