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Originals

Selection of Postoperative Systemic Chemotherapy for Advanced Gastric Cancer Patients with Hepatic Metastasis Using the Histoculture Drug Response Assay

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SUMMARY

The usefulness of histoculture drug response assay (HDRA) before the start of antitumor chemotherapy was explored to predict the antitumor response of chemotherapy for progressive gastric cancer patients with hepatic metastasis. Cancer tissue was collected from 6 advanced gastric cancer patients with hepatic metastasis, to assess whether the tumor is sensitive to chemotherapeutic agents of 5-fluorouracil (5-FU), mitomycin C (MMC), docetaxel hydrate (TXT), and cisplatin (CDDP) by the HDRA method. The size of hepatic tumor was measured by CT scan 3 and 6 months after the start of the chemotherapy and examined the relationship between the selected chemotherapy and antitumor response. Of 6 patients investigated in the present study, 3 patients received monotherapy with TS-1®, which was found to be effective in 2 patients (66.7%). In the patient who received MMC monotherapy, the HDRA showed the cancer tissue to be less sensitive to any of antitumor agents tested, resultantly indicating no antitumor response. In the remaining 2 patients with combination therapy with TS-1® and MMC, the selected chemotherapeutic agents showed anti-tumor effect. The HDRA is useful to predict the antitumor response of postoperative adjunctive chemotherapy for advanced gastric cancer patients with hepatic metastasis, and plays an important role in selection of antitumor agents.

Key Words : histoculture drug response assay, chemotherapy, gastric cancer, metastasis

INTRODUCTION

While recent development of novel antitumor agents and administration methods has made great progress in antitumor chemotherapies, the therapeutic achievement still remains unsatisfactory for many types of solid cancers. In recent years, HDRA for tumor tissues isolated from individual patients has been attempted before starting an antitumor chemotherapy, to select proper effective antitumor agents for individual cancer patients.

In 1986, Hoffman et al. developed the HDRA method to assess the sensitivity of the concerned tumor to antitumor agents by three dimensional histoculture with ^{3}H-thymidine[1]. Thereafter, in 1992, Furukawa et al. modified the method of Hoffman et al. to establish a simplified HDRA method using 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl-2H-tetrazolium bromide assay

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Table 1 Clinicopathological features of patients with advanced gastric cancer.

Patient No.	Age	Sex	Stage	Histology	TNM classsification
1	72	M	IV	tub	T4N2M1
2	62	M	IV	tub	T3N3M1
3	83	M	IV	tub	T4N2M1
4	63	M	IV	por	T3N2M1
5	73	M	IV	por	T3N2M1
6	49	M	IV	pap	T2N3M1

Note. tub, tubular adenocarcinoma ; por, poorly differentiated adenocarcinoma ; pap, papillary adenocarcinoma.

(MTT) (HDRA with MTT : hereinafter comprehensively referred to as HDRA)[2]. In the present study, it was studied to clarify whether the HDRA method prior to antitumor chemotherapy is useful to predict the antitumor response of agents for gastric cancer.

METHODS

Patients

The present study was conducted in 6 advanced gastric cancer patients who underwent surgical extirpation of gastric cancer at First Department of Surgery in Dokkyo Medical University Hospital, during a period from 2000 to 2005. All the patients had hepatic metastasis of Stage IV in TNM Classification, and received the HDRA after the surgery. According to the HDRA results, the patients received monotherapy or combination therapy with TS-1®, MMC, CDDP and/or TXT. Clinical pathological characteristics in the patients are summarized in Table 1.

Judgment of antitumor response

Patients were subjected to CT scanning 3 and 6 months after the start of the chemotherapy. The antitumor response was judged as complete response (CR) for disappearance of tumor, partial response (PR) for shrinking the hepatic tumor by 30 % or more as compared to the baseline size before the start of the chemotherapy, and stable disease (SD) for no change in tumor diameter[3]. Growth of the tumor in size was judged as progressive disease (PD).

HDRA

Surgically isolated gastric cancer tissue was kept in Hank's Balanced Salt Solution (HBSS) at 4℃. The tissue was washed with HBSS, chipped into small pieces weighing about 10 mg, weighed, and placed still on collagen gel of 24-well plate. The plate was cultured in 5 % $-CO_2$ incubator for 7 days. RPMI1640 containing 20 % fetal calf serum (FCS) and penicillin-streptomycin, amphotericin B (100 units/mL, 100 units/mL, and 0.25 μg/mL, respectively) was used as medium. Antitumor agents used were MMC, 5-FU, TXT, and CDDP. After incubating the plate, each well was added 100 μL of HBSS containing 0.06 % collagenase and 0.2 % MMT-phosphate buffered saline containing 50 mM sodium succinate respectively to react for 16 hours. After completion of the reaction, the reaction solution was discarded, and 0.5 mL of dimethylsulfoxide (DMSO) was added to each well to extract MTT-formazan from the tissue. One hundred μL of extract from each well was applied to a colorimeter for microplates to read absorbance at dominant wavelength 540 nm and control wavelength 630 nm. The measurement results were represented as Inhibition Index, I.I (%) of tumor growth inhibition rate, calculated by the following equation : I.I (%) = (1-T/C) × 100, where T was absorbance per gram of tumor tissue contacted with drug, and C was absorbance per gram of tumor tissue not contacted with drug. When I.I (%) was 50 % or higher, the HDRA was judged as positive.

RESULTS

The postoperative HDRA results are shown in Table 2. Of 6 patients investigated in the present study, 3 patients received monotherapy with TS-1®, which was found to be antitumor in 2 patients (66.7 %). In the patient who received MMC monotherapy, the HDRA showed the cancer tissue to be less sensitive to any of

Table 2 Evaluation of anti-cancer effect by HDRA

	HDRA (%)					Outcome (after chemotherapy)	
Patient No.	5-FU	MMC	CDDP	TXT	chemotherapy	3 months	6 months
1	68	66	45	55	TS-1	ND	PR
2	56	74	43		TS-1	ND	SD
3	61	65	37	63	TS-1	ND	PD
4	15	37	10	12	MMC	ND	PD
5	80	77	49	70	TS-1 + MMC	PR	ND
6	15	35	34	0	TS-1 + MMC	PD	PR

5-FU, 5-fluorouracil ; MMC, mytomycin C ; CDDP, cisplatin; TXT, docetaxel hydrate. PR, partial response ; SD, stable disease; PD, progressive disease ; ND, not determined.

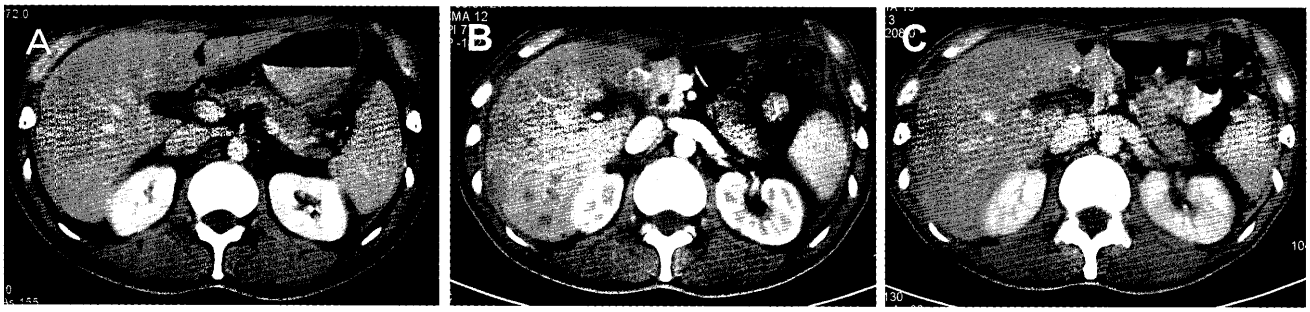

Figure 1 CT images of gastric cancer patient with liver metastasis. Data was from patient No.6. (A) Before surgical resection. (B) After surgical resection. Metastasized tumors are observed in the remnant liver. (C) After chemotherapy. Metastasized tumors were remarkably reduced in size.

antitumor agents tested, resultantly indicating no antitumor response. In the remaining 2 patients, combination therapy with TS-1® and MMC was found to be antitumor. A representative case (Patient No.6) is presented below.

Case presentation

A 49-year old male patient (No.6) was diagnosed to have anemia in health checkup, and referred to Dokkyo Medical University. The blood data read Hb 11.1 g/dL and tumor markers of CA19-9 1,250 U/mL and CEA 6.5 ng/mL. Gastroscopy revealed tumor accompanied by circular diffuse infiltration at the pyloric antrum. Biopsy diagnosed with moderately-differentiated adenocarcinoma. Abdominal CT scanning detected metastatic lesions in the hepatic left lobe and perigastric lymph node. According to the surgical findings, the diagnosis was LM (circ) type 3, cT3cN2cM1, cStage IV. Pyloric gastrectomy (D3) and outer extirpation of hepatic left lobe resulted in leaving tumor in the remaining liver, where the curability was C.

Based on the HDRA results prior to antitumor chemotherapy (Table 2), combination therapy with MMC and TS-1® started. The dose of TS-1® was set to 100 mg/body/day (4-week treatment with 2-week drug withdrawal), and MMC 10 mg/body/day was systemically administered on day 1. Upon completion of 1st treatment course, tumor markers were remarkably increased (CA19-9 to 17,000 U/mL and CEA to 144 ng/mL), and CT scanning detected multiple metastatic tumors in the remaining liver (Figure 1). After second treatment course, dose reduction of MMC to 4 mg/body/day resulted in a decrease in tumor markers, lowering CA19-9 to 140 U/mL and CEA to 6.0 ng/mL at 6 months after starting of antitumor chemotherapy (Figure 2). CT scanning at that time point found the hepatic metastatic lesions to be significantly shrunk (Figure 1), where the antitumor response was judged as PR (shrinking rate : 75 %).

DISCUSSION

TS-1®, which is a combination drug containing di-

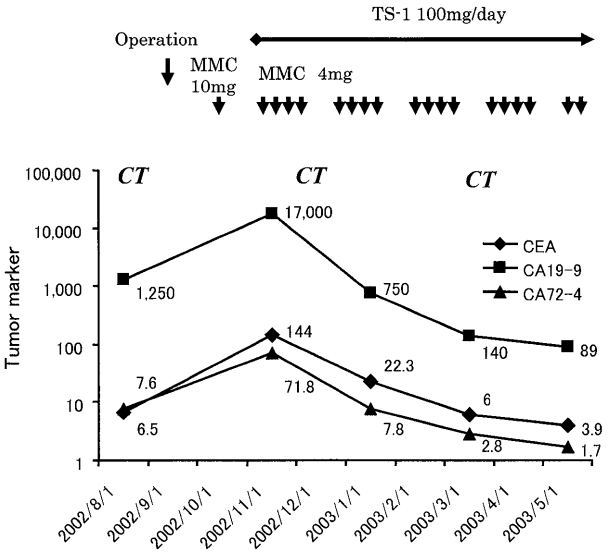

Figure 2 Representative clinical course of gastric cancer patient with liver metastasis. Data was from patient No.6.

hydropyrimidine dehydrogenase (DPD) inhibitor, is known to keep the blood concentration of 5-FU high[4], and monotherapy with TS-1® has been reported even to show high response rate (46.5%) against gastric cancer[5,6]. The safety and antitumor response of combination therapy with TS-1® and other antitumor agents are not known, yet, although the antitumor response rate of combination therapy with TS-1® and CDDP has been reported to be as high as 74% in Phase I/II study[7], and Phase III study is ongoing at present[8]. On the other hand, there are no therapeutic methods established for multiple hepatic metastasis of gastric cancer, and therefore, various treatment methods such as hepatic intra-arterial chemotherapy and microwave coagulation therapy are being tried in addition to systemic chemotherapy. The antitumor response of monotherapy with TS-1® in the case of hepatic metastasis has been reported to be 35.1%[5], and in fact in the present study, the monotherapy was found to be antitumor in 2 of 3 patients. On the other hand, the following cases have been recently reported : combination therapy with TS-1® and CDDP was highly antitumor against hepatic metastasis[9~11] and combination therapy with TS-1® and TXL increased the antitumor response, suggesting that combination therapy using antitumor agents with different action mechanism increases the antitumor response. However, the protocol of combination chemotherapy for gastric cancer still remains to be established. Regarding the anti-tumor effect, TS-1® is supported to be a key drug for the chemotherapy for gastric cancer. Therefore, in the present study we applied TS-1® alone or a combination of TS-1® and another drug according to the data from HDRA although we failed to apply TS-1® in case 4 because the patient's condition rapidly got worse. As a result, uncommon combination therapy with TS-1® and MMC showed anti-tumor effect in the two patients with such therapy. Thus, the HDRA is likely to be a useful method to seek for new combination therapy with TS-1®.

The HDRA of excised gastric cancer tissues is being tried in this institution to select postoperative adjunctive chemotherapy. In the present preliminary study, antitumor agents expected to be hopeful in the HDRA were found to be antitumor in 4 of 6 patients. Thus, correct selection of antitumor agents which are expected to be effective is quite important for improvement in the therapeutic antitumor response. In reverse, selection of proper antitumor agents by the HDRA is useful even to avoid continuous treatment with non-promising agents. In the case presented above, it was considered that the treatment should be discontinued for decreased WBC count at completion of 1st course. However, the treatment was continued according to the HDRA results, with the dose of MMC being reduced, and consequently, this combination therapy was found to be antitumor. As suggested above in the present study, the HDRA method is likely to play a great role in selection of postoperative adjunctive chemotherapy.

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