In vitro Chemosensitivity of Hepatocellular Carcinoma for Hepatic Arterial Infusion Chemotherapy Using the MTT Assay with the Combinations of Antitumor Drugs

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Summary: Despite the hepatic arterial infusion chemotherapy (HAI) has been advocated as an effective therapy for hepatocellular carcinoma (HCC) with multiple intrahepatic metastases, chemosensitivity of HCC for HAI with multidrug regimen has not been sufficiently investigated. The purpose of this study was to evaluate the in vitro chemosensitivity of HCC using 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay with the combinations of various antitumor drugs, and compared with the clinical results of HAI for patients with multiple intrahepatic recurrences of HCC. To evaluate the in vitro chemosensitivity of HCC to the combinations of antitumor drugs, 54 resected specimens of HCC were assayed using MTT assay with seven antitumor agents, 5-fluorouracil (5-FU), mitomycin C (MMC), adriamycin (ADM), etoposide (VP-16), cisplatin (CDDP), methotrexate (MTX) and CPT-11 (SN-38), exposed singly, or in combination. The results of in vitro assay were compared with the clinical results of 14 patients who underwent HAI for multiple recurrence of HCC, and also correlated with the experimental results of the combined use of CDDP and 5-FU in terms of pharmacokinetic reactions, i.e. synergism or antagonism. The MTT assay with the combinations of antitumor drugs represents an informative chemosensitivity test to HAI with multidrug regimen for recurrence of HCC.

Key words chemosensitivity test, tetrazolium salts, hepatic arterial infusion, hepatocellular carcinoma, pharmacokinetics

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common lethal malignancy in the world. While hepatic resection or liver transplantation only offers a chance of cure, resection was possible in less than a third of all patients with HCC, and the recurrence rate after surgery including both resection and liver transplantation is high in advanced cases [1-3]. Furthermore, 28.7 to 45.0% of the intrahepatic recurrence after...
resection manifested multiple nodules in the remnant liver, and sometimes with widespread distribution [2,4,5]. HCC with multiple widespread intrahepatic metastases is a devastating condition not amenable to various therapeutic modalities [2,6-8]. Although systemic chemotherapy has been of little value in contributing to survival benefit [9-11], hepatic arterial infusion chemotherapy (HAI) using an implantable reservoir has been advocated as an effective therapy to improve the prognosis of inoperable HCC [1,12-14]. Okuda et al. [14] reported that 71.0% overall response rate was achieved in patients with postoperative multiple recurrence of HCC using HAI with low dose administration of cisplatin and 5-fluorouracil (FP-HAI), however, only the patients who showed complete response could receive survival benefit, comparing with other patients whose responses were categorized in partial response, no change or progressive disease. Therefore, even though HAI may be an effective modality for postoperative multiple recurrence of HCC, the individual appropriate regimen of antitumor drugs and/or patient selection with the aim of the complete response would be necessary.

The rapid colorimetric assay described by Mosmann [15] for determining cell viability is measured by cell ability to convert soluble tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-Biphenyl-2H-tetrazolium bromide (MTT), to insoluble colored formazan. The MTT assay for chemosensitivity testing is a rapid and semi-automated quantitative assay, and has been used for screening the effects of antitumor agents on several cell lines [16]. Moreover, a better appreciation of the usefulness of the MTT assay as a predictive method for appropriate individual chemotherapy has been gained in treatments for leukemia [17], gastric cancer [18] or colorectal cancer [19].

However, with regard to the combination chemotherapy that is clinically used in common, there have been no simple and rapidly informative chemosensitivity tests so far and little has been known about chemosensitivity of HCC. The purpose of this study was to evaluate the in vitro chemosensitivity of HCC using MTT assay with the combinations of antitumor drugs.

MATERIALS AND METHODS

Cell lines
The well-characterized human hepatocellular carcinoma cell lines established and subcultured in the First Department of Pathology, Kurume University School of Medicine, Kurume, Japan, KYN-1 [20] and KYN-2 [21], were kindly provided.

Patients
Between 1997 and 2002, a total of 54 patients with HCC, 12 females and 42 males, mean age 64.7 (range 22-82) years, who underwent surgical resection in the Department of Surgery, Kurume University Hospital, Fukuoka, were included in this study. None of the patients had received any previous treatments including transcatheter arterial embolization (TAE), transcatheter arterial chemoembolization (TACE) or other chemotherapeutic therapies. Informed consent was obtained from all patients preoperatively after they were given an explanation that a part of their resected specimens were to be submitted to chemosensitivity test described in this study. After patients were discharged from the hospital, follow-up examinations mainly consisted of tumor marker tests including serum alpha-fetoprotein and des-gamma-carboxy prothrombin, ultrasonography at least once every 1-3 months, computed tomography (CT) at least twice a year and, if necessary, hepatic angiography. Recurrence was defined clinically as new lesions with features of HCC by more than two imaging methods. The mean and median follow-up periods were 17.8 and 14.3 months, respectively (range 0.6-57.3 months). Data were obtained from the outpatient clinic or readmission records on recurrence, treatments for recurrent tumor and survival. The outcome was clear in all patients.

Antitumor drugs
The antitumor drugs tested were 5-FU, mitomycin C (MMC), adriamycin (ADM), etoposide (VP-16), CDDP, methotrexate (MTX) and CPT-11 (SN-38). 5-FU, MMC, ADM, VP-16, CDDP and MTX were commercially available. SN-38 (7-ethyl-10-hydroxycamptothecin), the active metabolite of CPT-11, was kindly provided by Yakult, Tokyo. All drugs were dissolved in saline, aliquotted to 10 X the final concentration and stored at −20°C. Drug solu-
Preparation and storage of 96-well microplates for the MTT assay with the combinations of antitumor drugs

Prior to assay, a 96-well microplates (NUNC, Roskilde) were prepared with each assigned well added by 15 μl of antitumor drug solution dissolved in saline singly, or in combinations of two or three drugs. Six antitumor drugs, i.e., CDDP, ADM, VP-16, 5-FU, MTX, MMC at final concentrations of 2.5, 0.6, 35.0, 60.0, 2.75, and 1.5 μg/ml, respectively, were used from 1997 until 2000. Final concentrations of antitumor drugs were determined according to the clinically achievable peak plasma concentration (PPC) reported by Scheithauer et al. [22]. From 2001, VP-16 was replaced by SN-38 and in vitro concentration of SN-38 was determined at a final dose of 30 ng/ml with reference to published peak plasma concentrations [23]. Each of control wells was added with the same volume of saline alone. The 96-well microplates prepared were stored at -20°C and used within 2 months from the first storage day. The microplates were allowed to warm to room temperature prior to assay.

MTT assay

Chemosensitivity of fresh surgical specimens and cell lines were evaluated in vitro, using the modified MTT-assay [15,24,25]. The tumor specimens were obtained by hepatectomy and stored in Dulbecco’s Modified Eagle Medium (DMEM) (Gibco BRL Life Technologies, Rockville, MD) supplemented with 10% fetal bovine serum (FBS) (Gibco BRL Life Technologies), 100 μl penicillin (MEIJI SEIKA, Tokyo) and 100 μg/ml Streptomycin (MEIJI SEIKA, Tokyo). Tumor specimens were immediately transferred from the operating room to our laboratory, weighed, scissor-minced, washed with medium and pretreated enzymatically for 30 min at 37°C using 0.5 mg/ml pronase (Kaken Pharmaceutical, Tokyo), 0.2 mg/ml collagenase type I (Sigma, St. Louis,) and 0.2 mg/ml DNase (Sigma). Then filtered through a 70 μm nylon mesh (FALCON 2350, Becton Dickinson, NJ) centrifuged at 1000 rpm, 5 min. The tumor cell pellet was resuspended in a serum-free DMEM and viable tumor cell count was determined by trypan blue dye exclusion method. The solution was diluted to 1-5×10⁶ cells/ml, then plated in 96-well microplates with volumes of 135 μl in the wells containing one drug, 120 μl in the wells containing two drugs and 105 μl in the wells containing three drugs. The control wells were filled with cells and saline added up to the same volume as antitumor drug(s) and the wells containing saline alone were prepared to measure the background optical absorbance. Backgrounds, controls and wells for antitumor drug(s) were set up in quadruplicate. Following the cell incubation for 72 hrs at 37°C in a humidified atmosphere of 95% air and 5% CO₂, 15 μl of 0.5% MTT (Sigma) solution, dissolved in PBS and filtered through a 0.20 μm filter (MILLEX-GV, Millipore, MA), were added to each well except background wells, and cells were reincubated for a further 4 hrs at 37°C. Microplates were then centrifuged at 1000 rpm for 5 min, and the supernatant in each well was aspirated carefully, so as not to disturb the MTT-formazan crystal. 150 μl of dimethyl sulfoxide (DMSO) (Wako, Tokyo) was added to each well to solubilize the formazan product, and the absorbance at 540 nm of the solution in each well was determined using a multiwell scanning spectrophotometer (IMMUNOMIN (NJ-2300), Nalge Nunc International, NY). The mean absorbance was calculated from quadruplicate absorbance. The inhibition rate (IR) was calculated using the following formula.

IR (%)=[1–(T−B)/(C−B)]×100

where T, C and B represent the mean absorbance of the drug-treated, control and background wells, respectively.

Mean absorbance of control tumor minus blank (C − B)<0.1 was defined as inadequate for assessment because of an insufficient amount of viable cells as a control. When the inhibition rate was greater than 50%, the tumor cells were defined as sensitive to the drug(s) tested. The efficacy rate was calculated as follows:

Efficacy rate=no. of drugs evaluated as effective for one drug or drugs combined/no. of drugs assayed for one drug or drugs combined

As for the KYN-1 and KYN-2, these two cell lines were cultured and maintained in each flask with DMEM supplemented with 10% FBS at 37°C in a 5% CO₂ incubator. When the growth of cells reached the exponential phase, culture flasks were rinsed with PBS, treated using 0.25% trypsin-EDTA for 5 min at 37°C, then the cells were resuspended in DMEM with 10% FBS. Suspended cells were washed with DMEM with 10% FBS twice by centrifugation at 1000 rpm for 5 min. The number of viable tumor cells was counted by trypan blue dye exclusion.
method, then the solution was diluted to a concentration of $5 \times 10^5$ cells/ml. Following steps and calculation of the IR for KYN-1 and KYN-2 were the same as for the resected specimen.

**HAI and assessment of tumoral response**

Of 54 patients whose primary tumor was resected and examined by the MTT assay, 14 patients who manifested intrahepatic recurrence after surgery were treated by HAI. For HAI, a 4 or 5 French heparin-coated catheter was introduced into the proper or common hepatic artery using the Seldinger technique. The gastroduodenal artery and the right gastric artery were occluded by steel coils to prevent gastroduodenal injury due to antitumor agents. After the catheter was connected to the injection port, the device was implanted in a subcutaneous pocket in the right lower quadrant. Chemotherapy regimen was practically determined by individual treating doctors without reference to the results of the chemosensitivity assay.

The therapeutic response was assessed by enhanced computed tomography, ultrasonography and digital scanning angiography 4 weeks and 3 months after treatment. Maximum response within 3 months was rated according to WHO [26] as ‘complete response’, ‘partial response’, ‘no change’ or ‘progressive disease’. complete response and partial response were defined as ‘effective’, while no change and progressive disease were defined as ‘ineffective’, then the results of HAI and sensitivities determined by the MTT assay were compared and statistically examined using the chi squared test. A P value of $< 0.05$ was considered statistically significant.

**Quantitative analysis of combined effect of CDDP and 5-FU for KYN-1 and KYN-2 in vitro**

Combined effects of CDDP and 5-FU for KYN-1 and KYN-2 were assayed according to the median effect principle described in detail by Chou and Talaly [27]. The median effect equation states that:

$$f_J = \frac{D}{D_m}^m (1)$$

where $D$ is the dose, $f_a$ and $f_u$ are the fractions of the system affected and unaffected, respectively by the dose $D$, $D_m$ is the dose required to produce the median effect (analogous to IC$_{50}$), and $m$ is a Hill-type coefficient signifying the sigmoidicity of the dose-effect curve. Since by definition, $f_a + f_u = 1$, the median effect equation above can be linearized by taking the logarithms of both sides, i.e.

$$\log[(f_a^{-1} - 1)^{-1}] = m \log(D) - m \log(D_m)$$

Inhibition rates of KYN-1 and KYN-2 were determined by the MTT assay in the presence of doubling dilutions of CDDP (0.31-10.0 $\mu$g/ml) and/or 5-FU (7.5-240.0 $\mu$g/ml), ranging from 0.125 to 4.0 $\times$ PPC, with a constant molar ratio mixture of CDDP and 5-FU (1:1). Each drug (s) was set up in quadruplicate. Results were entered into a Statview J-4.5 spreadsheet on a Macintosh computer, and the data were analyzed with this software. Analysis of the results by the plot of the fractional inhibitions ($f_i$), the
median effect plot, gave the following parameters: for CDDP, 5-FU and the combination of CDDP and 5-FU, m, Dm and the correlation coefficient (r). The procedure for determining synergism, summation and antagonism at any effect level (i.e., for any fa value), involves the following steps: 1) for a given degree of effect (i.e., a given fa value representing x per cent affected), calculate the corresponding doses [i.e., (D),, (D),2 and (D),,2] by using the alternative form of equation (1), D,=Dm [(f,/(1-f,))]m, 2) calculate the combination index (CI) by using following equation:

\[ CI=(D)\cdot/(D)\cdot1+(D)\cdot2/(D)\cdot1(D)\cdot2 \]

where (D)\cdot, and (D)\cdot2 are from step 2), and (D)\cdot1,2 [also from step 2)] can be dissected into (D)\cdot1 and (D)\cdot2 by their known ratio, (P/Q). Thus, (D)\cdot,=(D)\cdot1,2×P/(P+Q) and (D)\cdot2=(D)\cdot1,2×Q/(P+Q). In this case, P=Q=1. CI values that are smaller than, equal to, or greater than 1, represent synergism, summation and antagonism, respectively.

**RESULTS**

**Chemosensitivity of specimens obtained from surgery**

Forty of 54 specimens (74.1%) were successfully assayed and analyzed in this study. The reason for all the 14 unsuccessful assays was an insufficient amount of viable cells in control. Each efficacy rate of the seven antitumor drugs using the MTT assay was shown in Fig. 1. The effective drug combinations were listed in Table 1.

Analysis of correlation between the clinical results of HAI for intrahepatic recurrence and the chemo sensitivity determined by the MTT assay for the primary HCC.

Characteristics of 14 patients who had undergone resection of primary HCC followed by the MTT

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**TABLE 1. Effective drug combinations of two and three drugs for resected HCC specimens**

<table>
<thead>
<tr>
<th>Drugs combined</th>
<th>Efficacy rates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDDP + ADM</td>
<td>48.7</td>
</tr>
<tr>
<td>CDDP + MMC</td>
<td>43.6</td>
</tr>
<tr>
<td>ADM + MMC</td>
<td>39.5</td>
</tr>
<tr>
<td>VP-16 + ADM</td>
<td>34.6</td>
</tr>
<tr>
<td>CDDP + VP-16</td>
<td>32.1</td>
</tr>
<tr>
<td>CDDP + 5-FU</td>
<td>28.2</td>
</tr>
<tr>
<td>CDDP + SN-38</td>
<td>27.3</td>
</tr>
<tr>
<td>CPT-11 + MMC</td>
<td>27.3</td>
</tr>
<tr>
<td>MMC + 5-FU</td>
<td>21.6</td>
</tr>
<tr>
<td>ADM + 5-FU</td>
<td>21.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drugs combined</th>
<th>Efficacy rates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDDP + SN-38 + MMC</td>
<td>63.6</td>
</tr>
<tr>
<td>CDDP + ADM + VP-16</td>
<td>57.7</td>
</tr>
<tr>
<td>ADM + MMC + VP-16</td>
<td>57.7</td>
</tr>
<tr>
<td>CDDP + 5-FU + MMC</td>
<td>56.8</td>
</tr>
<tr>
<td>SN-38 + MMC + MTX</td>
<td>54.5</td>
</tr>
<tr>
<td>CDDP + ADM + MMC</td>
<td>54.1</td>
</tr>
<tr>
<td>CDDP + ADM + MTX</td>
<td>54.1</td>
</tr>
<tr>
<td>CDDP + MMC + MTX</td>
<td>54.1</td>
</tr>
<tr>
<td>CDDP + 5-FU + VP-16</td>
<td>46.2</td>
</tr>
<tr>
<td>ADM + 5-FU C + VP-16</td>
<td>46.2</td>
</tr>
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</table>
TABLE 2. Correlation of results between HAI for multiple intrahepatic recurrence and MTT assay for primary HCC

<table>
<thead>
<tr>
<th>Age/Sex</th>
<th>Intervals between the surgery and liver metastasis (mo.)</th>
<th>HAI Regimen</th>
<th>Outcomes of HAI</th>
<th>Results of MTT assay</th>
<th>Correlation between MTT assay and HAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>77/M</td>
<td>21.0</td>
<td>CDDP + 5FU</td>
<td>CR</td>
<td>effective</td>
<td>TP</td>
</tr>
<tr>
<td>47/M</td>
<td>3.6</td>
<td>CDDP + 5FU</td>
<td>PR</td>
<td>effective</td>
<td>TP</td>
</tr>
<tr>
<td>66/M</td>
<td>7.0</td>
<td>CDDP + 5FU</td>
<td>CR</td>
<td>effective</td>
<td>TP</td>
</tr>
<tr>
<td>62/M</td>
<td>1.0</td>
<td>CDDP + 5FU</td>
<td>PR</td>
<td>effective</td>
<td>TP</td>
</tr>
<tr>
<td>59/M</td>
<td>1.7</td>
<td>CDDP + 5FU</td>
<td>NC</td>
<td>ineffective</td>
<td>TN</td>
</tr>
<tr>
<td>59/F</td>
<td>21.3</td>
<td>ADM + MMC</td>
<td>CR</td>
<td>ineffective</td>
<td>FN</td>
</tr>
<tr>
<td>65/F</td>
<td>1.4</td>
<td>CDDP + 5FU</td>
<td>NC</td>
<td>ineffective</td>
<td>TN</td>
</tr>
<tr>
<td>71/M</td>
<td>10.6</td>
<td>CDDP + 5FU</td>
<td>NC</td>
<td>ineffective</td>
<td>TN</td>
</tr>
<tr>
<td>69/F</td>
<td>6.5</td>
<td>CDDP + 5FU</td>
<td>PR</td>
<td>effective</td>
<td>TP</td>
</tr>
<tr>
<td>64/M</td>
<td>3.0</td>
<td>CDDP + 5FU</td>
<td>NC</td>
<td>ineffective</td>
<td>TN</td>
</tr>
<tr>
<td>70/M</td>
<td>1.0</td>
<td>CDDP + 5FU</td>
<td>PR</td>
<td>ineffective</td>
<td>FN</td>
</tr>
<tr>
<td>55/M</td>
<td>3.3</td>
<td>CDDP + 5FU</td>
<td>NC</td>
<td>ineffective</td>
<td>TN</td>
</tr>
<tr>
<td>56/M</td>
<td>6.3</td>
<td>CDDP + 5FU</td>
<td>CR</td>
<td>Effective</td>
<td>TP</td>
</tr>
<tr>
<td>60/F</td>
<td>31.2</td>
<td>ADM + MMC</td>
<td>PR</td>
<td>Effective</td>
<td>TP</td>
</tr>
</tbody>
</table>

CR: complete response; PR: partial response; NC: no change; PD: progressive disease; FN: false negative, i.e., the outcome of HAI was effective, though the result of MTT was 'ineffective'; TP: true positive, i.e., the outcome of HAI was effective and the result of MTT was 'effective'; TN: true negative, i.e., the outcome of HAI was ineffective and the result of MTT was 'ineffective'.

**Fig. 2.** Effects of CDDP and 5-FU administered to mice bearing KYN-1 and KYN-2 xenografts.

a) Growth curves of KYN-1 not treated (closed circle) (n=7) or treated (open circle) (n=7) with CDDP and 5-FU. Significant difference was observed in TGR between the control and treated group (*p=0.0101).

b) Growth curves of KYN-2 not treated (closed circle) (n=8) or treated (open circle) (n=8) with CDDP and 5-FU. Statistical difference was not observed between the control and treated group. Each point shows the mean and standard deviation.

assay for resected specimen, and subsequently developed multiple intrahepatic recurrences underwent HAI were summarized in Table 2.

**Combination effect of CDDP and 5-FU in vivo**

Chemosensitivity of KYN-1 and KYN-2 to CDDP and 5-FU in combination was confirmed by the MTT assay prior to in vivo assay: KYN-1 was evaluated as sensitive, however, KYN-2 was evaluated as resistant to CDDP and 5-FU in combination (data not shown). The effects of CDDP and 5-FU against the xenografts of KYN-1 and KYN-2 are illustrated in Fig. 2.
EVALUATION OF COMBINED-DRUG MTT FOR HEPATOCELLULAR CARCINOMA

Fig. 3. Drug combination index (CI) with respect to fraction affected (fa) for the inhibitory effect of a combination of CDDP and 5-FU in (a) KYN-1, (b) KYN-2 cell line.

Median effect plots of CDDP and 5-FU separately or in combination showed a good linearity for both KYN-1 and KYN-2 (data not shown). As to KYN-1, the CI shows a marked synergism, CI<1, between CDDP and 5-FU at all values of fractional inhibition. Conversely, antagonism, CI>1, was observed in KYN-2 between the same two drugs at all values of fractional inhibition.

Quantitative analysis of combined effect of CDDP and 5-FU for KYN-1 and KYN-2 in vitro

Computer-generated graphical presentations of the combination indexes of CDDP and 5-FU for KYN-1 and KYN-2 are given in Fig. 3.

DISCUSSION

One of the reasons why chemosensitivity testing has not been incorporated into standard oncologic therapy has been the lack of a good drug sensitivity assay for combination chemotherapy that has been commonly used in the clinical setting. A number of studies concerning the combined effect, i.e., synergism, summation or antagonism, of several antitumor drugs on fresh human tumor specimens or established cell lines have been reported and it has been confirmed that there exists individual diversity not only in the chemosensitivity but even in the combined effect of various antitumor drugs [17,28-30]. However, a simple method suitable for screening individual combined effects has not yet been developed. Morabito et al. [17] asserted that the MTT assay is suitable for evaluating the in vitro chemosensitivity of chronic lymphocytic leukemia (CLL) B-cells to 'multidrug regimen compounds'. Thus, we made a hypothesis that MTT assay with antitumor drugs combined may reflect the combined effect of the solid malignant tumor and this study was undertaken. Preliminary results of our study, however, showed a high compatibility of 85.7% in predicting accuracy, i.e., coincidence of the results of MTT assay and the clinical effects of corresponding HAI chemotherapy.

Morabito et al. [17] also reported that a synergistic effect was observed in vitro when CLL B-cells obtained from patients were simultaneously exposed to those antitumor drugs that showed ineffectiveness in single use, such as vincristine, prednisone or epirubicin. Our study indicated that the greater the number of drugs combined, the higher each efficacy rates of drugs increased, as is the advantageous principle of the combination chemotherapy in clinical use. In addition, 45.1% of the drugs in single use evaluated 'ineffective' were converted to those 'effective' when used in combination with another drug. Therefore, it is suggested that the combined use of 'ineffective' drugs does not necessarily mean the 'ineffective' drug combination and the chemosensitivity tests so far might neglect the efficacy of combined use of those drugs evaluated 'ineffective'.

There have been many controversies concerning the differences in chemosensitivity between primary tumors and their metastases [31-34]. Kurihara et al. [34] reported that a chemosensitivity assay of primary gastric cancer was useful for evaluating the effectiveness of antitumor agents for arterial infusion or systemic chemotherapy against liver metastasis. Kusuzaki et al. [32] also reported that regardless of whether the pulmonary metastatic tumors of osteosarcoma were synchronous or metachronous, they showed the same change in their DNA ploidy pattern and chemosensitivity as the primary tumor. In
our study, multiple intrahepatic recurrences of 14 patients who underwent HAI occurred within three years after surgery and those recurrence patterns were considered to be metastatic tumors rather than multicentric occurrence on the basis of several diagnostic images [5,35], and the outcomes of HAI were significantly correlated with the results of the MTT assay for primary tumor. Therefore, it is possible that intrahepatic metastases may bear the inherent nature of chemosensitivity of the primary tumor unless any chemotherapy was conducted during the period between surgical resection and subsequent intrahepatic metastases.

One of the pharmacokinetic advantages of HAI is the locoregional delivery with a high concentration of antitumor drugs. Cancer cell in monolayer chemosensitivity assay under PPC drug concentration setting are exposed to a high concentration of antitumor drugs. Therefore, it is possible that intrahepatic metastases may bear the inherent nature of chemosensitivity of the primary tumor unless any chemotherapy was conducted during the period between surgical resection and subsequent intrahepatic metastases.

One of the pharmacokinetic advantages of HAI is the locoregional delivery with a high concentration of antitumor drugs. Cancer cell in monolayer chemosensitivity assay under PPC drug concentration setting are exposed to a high concentration of antitumor drugs, therefore, this assay may be similar to the internal environment of the liver receiving the administration of HAI. Link et al. [36] reported that the human tumor colony-forming assay indicated high predictive accuracy, 86%, for the effectiveness of HAI against various liver tumors, and also described that the high drug concentration chosen in their study could be achieved in the clinical HAI. The drug concentration in this study and the results obtained were similar to those of Link et al. [36].

In this study, the combinations of drugs tested were determined in an arbitrary fashion. Even though the majority of drug combinations were not associated with those in common clinical use and a practical regimen based on the results of MTT remains unclear, a brand-new modulation of conventional drugs could be created from the results of MTT.

In conclusion, the preliminary results of this study suggest that the MTT assay in PPC setting may become a simple and rapidly informative chemosensitivity test for HAI against multiple intrahepatic recurrences of HCC, providing individualized chemosensitivity assessments for HAI with combination chemotherapy regimens. However, a prospective study is needed to clarify the efficacy of our assay for HAI against multiple intrahepatic recurrence of HCC.

REFERENCES


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