Drug Sensitivity of Small Cell Gastrointestinal Carcinoma

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Summary. Small cell gastrointestinal carcinoma frequently denotes a poor prognosis. In this study, we carried out drug sensitivity tests in vitro, using four small cell gastrointestinal carcinoma cell lines and seven different anti-cancer agents, and the results were compared with those obtained in small cell lung carcinoma (SCLC) and common gastric carcinoma cell lines.

In addition to the SCLC lines, Lu-130 and Lu-139, the ECC18 cell line was sensitive to mitomycin C (MMC) and vincristine (VCR), while the other three small cell gastrointestinal carcinoma cell lines, ECC10, ECC12 and ECC4, were not sensitive to these agents. Only ECC4 cells were sensitive to 5-fluorouracil (5-FU). Our previous study showed that the ECC18 cell line had similarities to the biochemical variant of SCLC cells, and the three other small cell gastrointestinal carcinoma cell lines were similar to the morphological variant of SCLC cells. The present findings suggest that a regimen including MMC and VCR should be selected as the first choice for treatment of the biological variant of small cell gastrointestinal carcinoma, while most anti-cancer drugs in use at the present time are not effective in the treatment of the morphological variant of small cell gastrointestinal carcinoma. Thus, the development of new anti-cancer agents seems necessary for the morphological variant of small cell gastrointestinal carcinoma.

INTRODUCTION

Small cell gastrointestinal carcinoma is a rare tumor whose nature is not well known. It readily metastasizes to distant organs, frequently indicating a poor prognosis.¹-³ This tumor has been thought to be identical histologically and biologically to small cell lung carcinoma (SCLC).⁴ Chemotherapy has been shown to have a substantial impact on the management of patients with SCLC. A number of chemosensitivity tests for SCLC in vitro have contributed to the planning of clinical management.⁵⁻⁶ SCLCs have been studied in detail by using several different cultured cell lines. It is known that some cell lines derived from human colorectal carcinomas have endocrine properties.⁷,⁸ There is, however, no laboratory which possesses plural small cell gastrointestinal cell lines. Furthermore, there are only a few case reports describing clinical trials using chemotherapeutic agents in the treatment of small cell gastrointestinal carcinoma.¹²⁻¹⁴ The drug sensitivity of small cell gastrointestinal carcinoma remains poorly understood.

In this study, we examined the in vitro drug sensitivity of four small cell gastrointestinal carcinoma cell lines, comparing these with that of SCLC and common gastric carcinoma cell lines.

MATERIALS AND METHODS

Cell lines

Four cell lines derived from small cell gastrointestinal carcinoma, designated ECC18, ECC10, ECC12, and ECC4, were used for this study.¹⁰ For a comparative study, we used the human SCLC cell lines, Lu-130 (small cell carcinoma, classic type, lung) and Lu-139 (small cell carcinoma, classic type, lung),¹⁰ and common gastric adenocarcinoma cell lines, MKN28 (moderately differentiated tubular adenocarcinoma, stomach) and MKN45 (poorly differentiated adenocarcinoma, stomach).¹⁷ The Lu-130 and Lu-139 cell lines were obtained from the Riken Cell Bank (Tsukuba, Japan) (Table 1).

Drug sensitivity test

Weisenthal’s dye exclusion assay was used.¹⁸ This assay is closely correlated with MTT assay¹⁹⁻²¹ and easily performed without any expensive equipment.
Table 1. Cell lines used in this study.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Parent tumor</th>
<th>Type of cultured cella</th>
<th>Doubling time (h)</th>
<th>Previous chemotherapyc</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECC18</td>
<td>Esophagus</td>
<td>SC-BV</td>
<td>92</td>
<td>(-)</td>
</tr>
<tr>
<td>ECC10</td>
<td>Stomach</td>
<td>SC-MV</td>
<td>71</td>
<td>(-)</td>
</tr>
<tr>
<td>ECC12</td>
<td>Stomach</td>
<td>SC-MV</td>
<td>69</td>
<td>(-)</td>
</tr>
<tr>
<td>ECC4</td>
<td>Rectum</td>
<td>SC-MV</td>
<td>56</td>
<td>(-)</td>
</tr>
<tr>
<td>Lu-130</td>
<td>Lung</td>
<td>SC-C</td>
<td>78</td>
<td>N.D.</td>
</tr>
<tr>
<td>Lu-139</td>
<td>Stomach</td>
<td>SC-C</td>
<td>47</td>
<td>N.D.</td>
</tr>
<tr>
<td>MKN28</td>
<td>Stomach</td>
<td>Ad</td>
<td>28</td>
<td>(-)</td>
</tr>
<tr>
<td>MKN45</td>
<td>Stomach</td>
<td>Ad</td>
<td>30</td>
<td>(-)</td>
</tr>
</tbody>
</table>

a SC: Small cell carcinoma, Ad: Adenocarcinoma, Sq: Squamous cell carcinoma, well: well differentiated type, poor: poorly differentiated type.
b BV: Biochemical variant, MV: Morphological variant, C: Classic type, Ad: Adenocarcinoma, C N.D.: Not described.

Table 2. Reference concentrations of drugs used in vitro tests.

<table>
<thead>
<tr>
<th>Anti-cancer agent (abbreviation)</th>
<th>Concentration (µg/ml)</th>
<th>Exposure time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etoposide (VP-16)</td>
<td>125</td>
<td>1</td>
</tr>
<tr>
<td>Cis-platin (CDDP)</td>
<td>1</td>
<td>Continuous</td>
</tr>
<tr>
<td>Adriamycin (ADM)</td>
<td>1.2</td>
<td>1</td>
</tr>
<tr>
<td>Mitomycin C (MMC)</td>
<td>0.5</td>
<td>Continuous</td>
</tr>
<tr>
<td>Methotrexate (MTX)</td>
<td>20</td>
<td>Continuous</td>
</tr>
<tr>
<td>5-Fluorouracil (5FU)</td>
<td>10</td>
<td>Continuous</td>
</tr>
<tr>
<td>Vincristine (VCR)</td>
<td>0.8</td>
<td>1</td>
</tr>
</tbody>
</table>

Tumor cells were exposed to seven different individual drugs known to be clinically effective against SCLCs or gastrointestinal tract carcinomas. These effectiveness were evaluated 4 days later. These drugs included Adriamycin (ADM), vincristine (VCR), methotrexate (MTX), cisplatin (CDDP), mitomycin C (MMC), 5-fluorouracil (5-FU), and etoposide (VP-16). The concentration and exposure time were determined in accordance with reports by Weisenthal et al.\textsuperscript{18} and Gazdar et al.\textsuperscript{7} (Table 2).

Each drug concentration was tested in triplicate or more, and in each experiment, at least 500 cells were counted. The cell survival rate was determined using the following formula:

\[
\frac{\text{No. of treated living tumor cells} + \text{No. of treated dead cells}}{\text{No. of untreated living tumor cells} + \text{No. of untreated dead cells}} \times 100(\%)
\]

In accordance with Weisenthal et al.\textsuperscript{18} the drug was considered to be effective against the cell line when the cell survival rate was less than 30% at the reference concentration. To confirm these results, a drug sensitivity test was performed at 10 and 1/10 times concentrations.

RESULTs

Small cell gastrointestinal carcinoma cell lines

The drug sensitivities of small cell gastrointestinal carcinoma are summarized in Table 3 and Fig. 1. The ECC18 cell line was sensitive to VCR and MMC, showing greater sensitivity when compared with other small cell gastrointestinal carcinoma cell lines in each concentration. The cell survival rates with VCR and MMC were 17.3% and 26.4%, respectively. However, ECC18 cells were resistant to the other five drugs. The ECC4 cell line was sensitive to 5-FU, and the cell survival rate was 18.9%. However, the ECC4
cell line was resistant to the other six drugs. The ECC10 cell line was resistant to all seven drugs. The ECC12 cell line had an intermediate degree of resistance to 5-FU (cell survival rate: 37.8%), but was resistant to the other six drugs.

**SCLC and common gastric cancer cell lines**

Drug sensitivities of SCLC and common gastric cancer are summarized in Table 4 and Fig. 1. Both the Lu-130 and Lu-139 cell lines were sensitive to VCR and MMC. Although the MKN28 cell line showed an intermediate degree of sensitivity to VP-16 (cell survival rate: 44.7%), it was resistant to the other five drugs. The MKN45 cell line showed an intermediate degree of resistance to MMC (cell survival rate: 33.0%) and VP-16 (cell survival rate: 40.5%), but was resistant to the other four drugs.

**DISCUSSION**

Patients with small cell gastrointestinal carcinoma frequently require chemotherapy. Despite this, we have little knowledge on the drug sensitivity of small cell gastrointestinal carcinoma. Jass et al.\(^4\) described small cell gastrointestinal carcinoma as being biologically identical to SCLC. However, only one small cell gastrointestinal carcinoma cell line, ECC18, showed similarities in drug sensitivity to the classic SCLC cell lines. The ECC18 cell line, as well as the Lu-130 and Lu-139 cell lines, was sensitive to MMC and VCR (Tables 3 and 4). Although it has been shown that MKN45 cells are more sensitive to MMC than MKN28 cells,\(^2\) the present study indicates that ECC18 cells are more sensitive to MMC than MKN45 cells. While MMC is usually used for patients with common gastric carcinoma, it has been seldom used for patients with small cell gastrointestinal carcinoma in Japan. There has been only one case report...
indicating that MMC is effective in patients with SCLC.23

Incidentally, there are a few reports indicating that a chemotherapeutic regimen including VCR is effective in cases of small cell gastrointestinal carcinoma.12,13 Our previous study showed that the ECC18 cell line corresponds to the biochemical variant of SCLC cells, while the ECC10, ECC12, and ECC4 cell lines belong to the morphological variant, according to the classification of SCLC.15,24 The biochemical variant, as well as the classic type, probably arises directly from true endocrine cells, while the morphological variant may occur via "neoplastic neometaplasia" from an adenocarcinoma cell to endocrine cells.19 Our findings indicate that a regimen including MMC and VCR should be selected for the treatment of small cell gastrointestinal carcinoma, which is a biochemical variant.

Although the ECC4 cell line showed considerable sensitivity to 5-FU, the drug was not effective against the ECC10 and ECC12 cell lines. Small cell gastrointestinal carcinoma cell lines of the morphological variant type were resistant to all drugs examined, except for 5-FU. These findings suggest that most drugs now in use are not effective for the treatment of the morphological variant of small cell gastrointestinal carcinoma. Thus, the development of new anti-cancer agents is necessary for effective treatment of the morphological variants of small cell gastrointestinal carcinoma.

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REFERENCES


