ANTITUMOR ACTIVITY OF QUINOCARMYCIN AGAINST CARCINOMA OF THE LUNG IN HUMAN TUMOR CLONOGENIC ASSAY

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Quinocarmycin monocitrate is a novel antitumor antibiotic isolated from Streptomyces melanovinaceus. We have utilized a human tumor clonogenic assay to test the antitumor activity of this drug against carcinoma of the lung and to compare its activity with those of mitomycin C or cisplatin, which are components of the clinically effective regimens in therapy for this disease. The overall in vitro response rate (defined as less than 50% survival of tumor colony forming units) for quinocarmycin at 0.1 and 1.0 μg/ml continuous exposure was 42% and 72%, respectively, which was superior to that of other drugs. Quinocarmycin and other antitumor drugs do not have identical spectra of antitumor activities in vitro, suggesting that this compound with good in vitro activity should be further developed for clinical trials.

Keywords — antitumor antibiotic; quinocarmycin; KW2152; human tumor clonogenic assay; lung carcinoma

INTRODUCTION

Quinocarmycin moncitrate (KW2152, NSC6014220D) is a new antitumor antibiotic isolated from Streptomyces melanovinaceus. It is a white crystalline powder; has a new chemical structure as shown in Fig. 1 and is soluble in water, ethanol and methanol. Its solution is rather stable, especially at pH 6.0.1

Quinocarmycin prolonged the life of leukemia P388 bearing mice using successive intraperitoneal administrations. Furthermore, it was found to possess antitumor activities against other experimental tumors, i.e., murine leukemia L1210, melanoma B-16, M-5076, sarcoma 180, Lewis lung carcinoma and human mammary carcinoma MX-1.2

The human tumor clonogenic assay (HTCA) has been used to predict clinical response to chemotherapeutic agents in individual patients3,4 and to test new antitumor drugs as an in vitro phase II study.5-8 In this study, quinocarmycin as several concentration was tested against carcinoma of the lung to determine whether the drug might have applicability against this tumor in clinical trials. In vitro activity of the compound was also compared with that of mitomycin C which is known to be an effective antitumor antibiotic in lung cancer therapy. Our data may prove useful for further experimental studies in relation to future phase I and II clinical trials of this compound.

MATERIALS AND METHODS

Tumor Specimens — After securing informed consents, solid tumor specimens were obtained from patients undergoing surgical procedures as part of their diagnostic evaluation or therapy. All patients had not received prior chemotherapy. Human pulmonary adenocarcinoma cell line PC-9 (kindly provided by Professor Y. Hayata, Tokyo Medical College) was also used in this study.

FIG. 1. Structure of Quinocarmycin

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Culture of Tumor Colony-Forming Cells

— The basic procedure of clonogenic assay system utilized in this study has been previously described. In brief, solid tumors were immediately minced finely with scissors in McCoy's 5A medium plus 10% heat-inactivated fetal bovine serum. The minced tumor was passed through a 120 mesh stainless steel screen to collect a single cell suspension. After two washes with McCoy's 5A medium, cells were suspended in 0.3% agar in enriched CMRL 1066 medium, supplemented with 15% horse serum.

Drug Sensitivity Study — Cells to be tested were mixed with various concentrations of quinocarmycin, which was generously supplied by Kyowa Hakko Co., Ltd. (Tokyo, Japan). One ml of this mixture was pipetted onto each of three 35-mm petri dishes containing 1 ml of 0.5% agar in enriched McCoy's 5A medium without conditioned medium. Cultures were incubated at 37 °C in a 7.5% CO₂ high-humidified atmosphere. Colonies were counted at day 9 using a computerized image analyzer for cell line cultures or at day 14 using an inverted phase microscope for primary tumor cultures.

RESULTS

Suspensions of tumor cells obtained from 41 patients with carcinoma of the lung were grown in the modified human tumor clonogenic assay. Of the 41 specimens, growth was sufficient (>30 colonies/plate) for assessing an antitumor activity of at least one concentration of the drug in 30 specimens, including 22 adenocarcinoma, 6 squamous cells, 1 large cell and 1 small cell. Results obtained in 30 lung carcinoma cells cultivated in the continuous presence of 0.1–10 μg/ml of the drug are shown in Table I. The effect of quinocarmycin was varied between

FIG. 2. Concentration-Dependent Growth Inhibition on Colony Formation of Carcinoma of the Lung by Quinocarmycin

The 5 × 10⁶ tumor cells from 24 individual patient specimens were cultured in HTCA in continuous presence of 0.1, 1 or 10 μg/ml of quinocarmycin. Each point represents the mean number of colonies surviving for three plates.

<table>
<thead>
<tr>
<th>Concentration of quinocarmycin (μg/ml)</th>
<th>Number of specimens tested</th>
<th>Number of responseᵃ⁾</th>
<th>Response ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>26</td>
<td>11</td>
<td>42</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>18</td>
<td>72</td>
</tr>
<tr>
<td>10</td>
<td>29</td>
<td>22</td>
<td>76</td>
</tr>
</tbody>
</table>

ᵃ⁾ In vitro response was defined as more than 50% decrease in colony number on drug-treated plates compared with control plates.
specimens and this variable effect was noted within tumors of the same histologic type and between different histologic tumor types. At 0.1 μg/ml in vitro responses, defined as less than 50% survival of tumor colony forming units (TCFUs), were noted against 9 cases of 19 adenocarcinoma and against 2/5 squamous cells, overall response ratio was 42%. A correlation between concentration and cytotoxicity was studied in specimens of which cell yield was sufficient for testing of multiple doses. The cytotoxicity of quinocarmycin was shown to be dependent on its concentration in 14 of 24 specimens as shown in Fig. 2. On the other hand, a subsequent plateau effect, wherein increased lethality with a higher concentration did not occur, was frequently observed in treatment with the drug, suggesting the presence of a subpopulation of cells resistant to the drug.

Inspecting individual specimens of the 21 mitomycin C-resistant tumors, 8 (38%) were sensitive to quinocarmycin and of the 19 cisplatin-resistant tumors, 9 (47%) were sensitive to quinocarmycin (Table II).

In order to learn characteristics such as time- and concentration-dependency of the antitumor activity of quinocarmycin, PC-9 cells were exposed to this drug at various concentrations for 1 or 24 h. Figure 3 depicts the dose response curve of quinocarmycin in HTCA. Quinocarmycin showed a 50% inhibition concentration (IC₅₀) of TCFUs at 0.74 and 81 μg/ml after brief exposures of 24 and 1 h, respectively. A time-schedule dependency index (TDI) was calculated by dividing the IC₅₀ with 1-h exposure by that with 24-h exposure. The TDI value of quinocarmycin was 109 which indicates a high time-schedule dependency of the inhibiting activity of quinocarmycin in HTCA system. This result coincided to that in an animal experiment where quinocarmycin was effective schedule-dependently against P388 leukemia by daily successive administration.¹³

DISCUSSION

The antitumor activity of quinocarmycin has been reported in various transplanted murine tumors. In this study the overall response ratio of quinocarmycin against freshly isolated lung cancer cells at 0.1 μg/ml with continuous exposure was 42%, which is a high level of in vitro activity compared with other drugs screened previously in the cloning system.¹⁰ Furthermore, the effective growth inhibition of quinocarmycin in several specimens resistant to other antitumor drugs indicates the possibility of non-cross resistance.

These data may have to be interpreted more carefully. First, in this study for new drug screening purposes, less than 50% survival of TCFUs

<p>| Table II. Frequency of Response to Quinocarmycin in Tumor Specimens Resistant to Standard Drugs |
|-------------------------------------|---------------------------------|---------------------------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Resistant drug</th>
<th>Number of specimens tested</th>
<th>Number of response quinocarmycin</th>
<th>Response ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitomycin C</td>
<td>21</td>
<td>8</td>
<td>38</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>19</td>
<td>9</td>
<td>47</td>
</tr>
</tbody>
</table>

a) Resistance criteria was defined as failure to reduce colony formation to 50% of control by continuous exposure of drug at 0.1 μg/ml.  
b) Definition for response was as detailed in Table I.
was a criterion for in vitro drug sensitivity in order to make the system more sensitive. This criterion may overpredict a drug efficacy in the HTCA system as compared with a more stringent definition of less than 30% survival, which was utilized for predicting patients who would respond to a particular conventional chemotherapeutic drug.31 However, a recent clinical study analyzing cytotoxicities of anticancer drugs retrospectively with the cloning system revealed that a decrease of more than 50% in TCFUs correlated with the response in patients with carcinoma of the lung.11 Second, to assess the therapeutic potential of new anticancer agent, it is essential to choose appropriate concentrations of the drug for in vitro testing. Preclinical toxicity studies with quinocarmycin have been carried out in experimental animals. The LD₅₀ for mice after a single dose intravenous injection of quinocarmycin was 16.6 mg/kg.29 Single or 5 successive administrations with LD₃₀ or 1/3 × LD₁₀ respectively, did not show significant depression of white blood cells and platelets.30 In an ongoing pharmacokinetic study in humans, the maximal tolerated dose has not yet been reached. An optimal dose in the phase II study was proposed to be 2 mg/m² each day for 5 d. The peak plasma concentration of quinocarmycin averaged 135 ng/ml, when 2 mg/m² was given to patients (M. Morimoto, personal communication). In former studies with the human tumor clonogenic assay system, the use of about one-tenth of the peak plasma concentration in patients has been shown to be an appropriate dose for predicting clinical response.12 The concentration, even the lowest, of quinocarmycin used in this study is in the neighborhood of the peak plasma concentration. However, when the cytotoxicity was compared with that of other drugs at their peak plasma concentrations, quinocarmycin (0.1 µg/ml; 42%) was slightly inferior to mitomycin C (1 µg/ml; 67%) but identical to cisplatin (1 µg/ml; 42%). Third, this study using PC-9 cells disclosed that quinocarmycin belongs to a time-dependent drug group. The antitumor activities of such drugs were inclined to be overestimated in the assay system with long drug exposure time, as previously shown by Matsushima et al.13 Therefore, further in vitro studies should include low concentrations and short drug exposure experiments, including cytotoxic studies in humans.

The mode by which quinocarmycin acts as an anticancer drug has been investigated recently.14 The drug caused significant inhibition of ribonucleic acid (RNA) synthesis after a short time exposure. In P388 leukemia cells exposed for 1-h to the drug, the 50% inhibitory concentration for RNA synthesis was 10⁻⁷ M, 30-fold less than that for deoxyribonucleic acid (DNA) synthesis. Such information and our results taken together suggest that quinocarmycin is an active antitumor antibiotic with potential utility for treatment of carcinoma of the lung.

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REFERENCES


