In vivo chemotherapeutic profile of human gallbladder small cell carcinoma

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ABSTRACT

Small cell carcinoma of the gallbladder is very rare, but shows high malignant potential with frequent metastasis. Chemotherapeutic regimens for the treatment of gallbladder small cell carcinoma have not yet been established. In this study, we examined in vivo chemosensitivity tests for the GB-04-JCK human gallbladder small cell carcinoma, which were previously established as a serial-transplantable xenograft in nude mice. We used four anticancer drugs: docetaxel, irinotecan, nedaplatin and gemcitabine. Docetaxel maximally suppressed xenograft tumor growth in mice (P < 0.01), and showed complete tumor regression after chemotherapy day 35. Irinotecan and nedaplatin suppressed tumor growth without complete regression (P < 0.01). Gemcitabine did not affect tumor growth significantly. This in vivo experimental study proposed chemotherapeutic regimens for human gallbladder small cell carcinoma.

Gallbladder cancer is the fifth most common malignancy of the digestive system (12, 13, 18). Patients with gallbladder cancer are usually treated at an advanced stage, and the prognosis remains poor despite the development of modern diagnostic methods. Most of the gallbladder cancers are epithelial malignancies, i.e. carcinomas, and the majority of gallbladder carcinomas are differentiated adenocarcinomas (2). Small cell carcinoma of the gallbladder is rare, but should be separated from the ordinary gallbladder adenocarcinoma because of its characteristic morphological features and highly aggressive clinical behavior (17, 22). Small cell carcinoma frequently metastasizes in the early stage of disease, and surgery is occasionally abandoned at the first clinical examination in many patients (6).

Human tumor xenografts in immunodeficient mice are useful in vivo systems for studying the mechanisms of neoplastic proliferation and/or differentiation, as well as chemotherapeutic analyses (14). We have reported a number of xenografts of various human tumors in mice. Human tumor xenografts retained not only morphological characteristics, but also oncogenic alterations (15). We have also observed that in vivo chemosensitivity assays using human tumor xenografts are useful to establish the clinical chemotherapeutics of anticancer agents (11, 23, 25).

Chemotherapeutic regimens for the treatment of gallbladder small cell carcinoma have not yet been established although it clinically shows high aggressiveness with frequent metastasis (10). Therefore, clinicians are eager to develop a therapeutic strategy for gallbladder small cell carcinoma. Previously, we established the GB-04-JCK human gallbladder small cell carcinoma line as a serial-transplantable xenograft in nude mice (18). In this study, we clarify the in vivo chemotherapeutic profile of human gallbladder small cell carcinoma in order to propose clinical chemotherapeutic regimens.

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RESULTS

Tumor xenograft growth in mice

Tumor sizes and mouse body weights are shown in Figs. 1 and 2. The GB-04-JCK xenograft of human gallbladder small cell carcinoma grew well in the subcutaneous regions of the inoculated mice (Fig. 3). Doubling time of the xenograft was 8.83 days at the exponential phase of growth. During the observation periods, mice bearing the xenografts showed no apparent cachexic changes, and their body weight did not change significantly.

In vivo chemosensitivity of tumor xenograft

Results of the chemosensitivity tests of the GB-04-JCK gallbladder small cell carcinoma xenograft are shown in Table 1. The administration of 15 mg/kg of docetaxel showed the most effective therapeutic results, compared with other anticancer drugs. Relative tumor volume (RTV = TV28/TV0) was 0.2 ± 0.05 and the tumor regression rate (T/C% = 100 × treated tumor volume/control tumor volume) was 2.1% at day 28. Complete regression of the inoculated tumors was achieved with 15 mg/kg of docetaxel at day 35, resulting in 100% tumor-free period until day 65. Maximum body weight loss was 19.5% in the docetaxel treatment group.

Administration of 50 mg/kg of irinotecan suppressed tumor growth, but did not show complete tumor regression. T/C% of irinotecan was 34.1, 31.1 and 36.9% at days 14, 21 and 28, respectively. Maximum body weight loss was 0.4% in the irinotecan treatment group.

Administration of 40 mg/kg of nedaplatin showed effective therapeutic results. T/C% of nedaplatin was 14.5% and 6.9% at days 14 and 21, respectively. At day 21, nedaplatin achieved complete regression in two of the five inoculated tumors. T/C% at day 28 was 9.3%, which was reflected by the regrowth of two tumors. Maximum body weight loss was 9.5% in the nedaplatin treatment group.

Gemcitabine did not affect tumor growth significantly as follows: T/C% at 120 mg/kg of gemcitabine administration was 97.3, 113.0 and 112.4% at days 14, 21 and 28, respectively. T/C% at 156 mg/kg of gemcitabine administration was 99.9, 98.2 and 115.1% at days 14, 21 and 28, respectively. T/C% at

MATERIALS AND METHODS

Xenograft line of human gallbladder small cell carcinoma in nude mice. Nude mice (female, 5 to 8 weeks of age, with a BALB/c background) purchased from CLEA Japan Inc. (Tokyo, Japan) were maintained under specific-pathogen-free conditions in accordance with the animal care guidelines of the Central Institute for Experimental Animals (14, 15).

The GB-04-JCK human gallbladder small cell carcinoma line, previously established as a serial-transplantable xenograft, was maintained through serial passages in vivo (for more than 20 years) as follows: Tumor specimens were obtained under sterile conditions from tumor-bearing nude mice, and after immersion in Dulbecco modified Eagle’s medium (DMEM), were cut into small fragments. The fragmented tumor tissues were subcutaneously inoculated into other mice using sterile injection needles (18).

Anticancer drugs. Four anticancer drugs were obtained as follows: docetaxel (TXT; Aventis Pharma, Tokyo, Japan), irinotecan (CPT-11; Yakult, Tokyo, Japan), nedaplatin (NDP; Shionogi, Osaka, Japan), and gemcitabine (GEM; Eli Lilly Japan, Kobe, Japan). All anticancer drugs were dissolved in saline and used for in vivo chemosensitivity tests.

In vivo chemosensitivity tests. GB-04-JCK was implanted subcutaneously as 2–3 mm tumor fragments in the right flank of nude mice. When the tumor reached 100 to 300 mm³ in volume, mice were divided randomly into test groups consisting of 5 mice per group (day 0). Administration doses of the anticancer drugs were defined based on the chemosensitivity test model using clinically equivalent doses (23, 25). Administration of the anticancer drugs (0.1 mL/10 g of mouse body weight) started at day 0. Docetaxel, irinotecan, nedaplatin and gemcitabine were separately given to mice bearing GB-04-JCK xenografts (treatment group). Saline was injected i.v. into the untreated control group. Tumor sizes and mouse body weights were measured twice a week until day 28. When the drugs affected tumor regression at day 28, we observed the tumor growth of the treatment group until day 60. Tumor volume (TV) was calculated according to the following formula: TV = length × width² × 1/2. Each tumor volume on day n was expressed as relative tumor volume (RTV) according to the following formula: RTV = TVn/TV0, where TVn is the tumor volume at day n, and TV(0) is the tumor volume at day 0. Tumor regression rate (T/C%) were evaluated by the following formula: T/C% = 100 × (mean RTV of treated group)/(mean RTV of control group). RTV was statistically evaluated using the Mann-Whitney U-test.
Chemotherapy for gallbladder small cell carcinoma

Most of the gallbladder tumors are epithelial malignancies, i.e. carcinomas, and the majority of gallbladder carcinomas are differentiated adenocarcinomas (2). Small cell carcinoma of the gallbladder is a relatively rare, but distinctive tumor, accounting for only a few percent of all primary gallbladder carcinomas. Albores-Saavedra et al. first described this disease in 1981 (4), and more than 50 cases have been reported (3, 5–7, 9). The histologic features of gallbladder small cell carcinoma are

Fig. 1  Tumor sizes of GB-04-JCK inoculated into nude mice. The GB-04-JCK xenograft of human gallbladder small cell carcinoma grew well in subcutaneous regions of inoculated mice (doubling time = 8.83 days).

Fig. 2  Body weights of GB-04-JCK-inoculated nude mice. The body weights of xenograft-inoculated mice did not change significantly.

Fig. 3  Histologic features of GB-04-JCK xenograft in nude mice. The carcinoma cells grow in a solid-sheet pattern, and have hyperchromatic nuclei, finely dispersed chromatin and inconspicuous nucleoli (Hematoxylin & eosin staining).

203 mg/kg of gemcitabine was 96.8, 84.7 and 92.1% at days 14, 21 and 28, respectively.

DISCUSSION

A standard chemotherapeutic regimen for gallbladder small cell carcinoma has not yet been established. In this study, we examined in vivo chemosensitivity tests of GB-04-JCK human gallbladder small cell carcinoma, previously established as a serial-transplantable xenograft in nude mice (18). Docetaxel maximally suppressed GB-04-JCK xenograft tumor growth in mice, and showed complete tumor regression. This is the first in vivo experimental study proposing chemotherapeutic regimens of gallbladder small cell carcinoma.
who experienced complete remission over a period of six months (7). Moskal et al. reviewed five patients treated with combined surgery and chemotherapy in the literature, and proposed chemotherapy using 5-fluorouracil, leucovorin, streptozotocin, doxorubicin, cisplatin and etoposide for the treatment of gallbladder small cell carcinoma (17). Pavithran et al. reported that a patient with this tumor showed partial remission with 5-fluorouracil and cisplatin treatment (21); however, the tumor recurred three months later and showed no apparent chemotherapeutic response.

Human tumor xenografts in immunodeficient mice are useful because they provide sufficient amounts of tumor tissue for analysis of the in vivo biological characteristics of human neoplasms (1, 8, 20, 24). Human tumor xenografts generally retain their original characteristics including morphological phenotypes and biological features, as well as their

<table>
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<tr>
<th>Group</th>
<th>dose (mg/kg)</th>
<th>Schedule</th>
<th>day</th>
<th>RV (Mean±SD)</th>
<th>T/C (%)</th>
<th>Maximum BW loss (%)</th>
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<td>Dosetaxel (TXT)</td>
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<td>Iriontecan (CPT-11)</td>
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<td>1.0 ± 0.17</td>
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<td>5.0 ± 0.53</td>
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<td>7.8 ± 1.96</td>
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<td>Gemcitabine (GEM)</td>
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<td>203</td>
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a) Administered i.v. injection
b) Relative tumor volume
c) Treated/control
d) % of initial body weight
e) Significantly reduced tumor growth by comparing control group (P < 0.01, one-side)
*ND, No data. The mice of the control group were sacrificed at day 28 and no further data was not obtained.
oncogenic alterations (14, 15). Previously, we have reported that in vivo chemosensitivity assays using human tumor xenografts are useful to establish clinical chemotherapeutics of anticancer agents (11, 23, 25). The GB-04-JCK xenograft analyzed in this study retains the characteristics of the original human tumor very well, and is thought to be a good system for studying the chemotherapeutics of anticancer agents against human gallbladder small cell carcinoma. In these experiments, the tumor xenograft showed maximum sensitivity to docetaxel, and was relatively sensitive to irinotecan and nedaplatin.

Docetaxel, irinotecan, and nedaplatin are usually used for the treatment of pulmonary small cell carcinoma. On the other hand, the treatment of extra-pulmonary small cell carcinoma, including gallbladder small cell carcinoma, has not yet established although it shows highly aggressive clinical behavior. Based on the present study, these anticancer drugs are thought to become candidates of chemotherapeutic regiments for the treatment of extra-pulmonary small cell carcinoma, especially gallbladder small cell carcinoma.

REFERENCES


