Subrenal Capsule Assay Using Liver Cancer Specimens Obtained by Fine Needle Biopsy

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Summary: Twenty-seven liver cancer biopsies (20 hepatocellular carcinomas, 4 metastatic liver cancers and 3 cholangiocellular carcinomas) were obtained using a 21-gauge fine needle biopsy guided by ultrasonography. These cancers were subcutaneously transplanted to the subrenal capsular region of BDF1 mice premedicated with immunosuppressive agents to modulate the host immune reaction. The SRCA was based on the change in tumor size (ΔTS) and the tumor growth inhibition rate (TGIR). The transplantation rate of the 27 liver cancer specimens was 85% by ΔTS and 67% by TGIR. The efficacy rates of Adriamycin, cis-platinum, and mitomycin were respectively 71%, 58%, and 43% by ΔTS, and 73%, 56% and 50% by TGIR. Thus, liver cancer specimens obtained by fine needle biopsy and examined by SRCA had a fairly high transplantation rate, and this method can be useful for patients with inoperable liver cancer, as a general chemosensitivity test for anticancer drugs.

Key words: Liver cancer — Subrenal capsule assay (SRCA) — Chemosensitivity test — Fine needle biopsy — Hepatocellular carcinoma — Metastatic liver cancer — Cholangiocellular carcinoma

Introduction

A variety of treatments have been developed for inoperable liver cancer, including transcatheter arterial embolization (Yamada et al. 1983) and percutaneous ethanol injection (Fujimoto, 1988). Chemotherapy is, of course, another important non-surgical method of treatment.

In chemotherapy, the proper selection of anticancer drugs is essential to achieve effective treatment of the cancer without lowering the patient’s performance status, and many chemosensitivity tests have been devised for drug selection. The subrenal capsule assay (SRCA) reported by Bogden et al. (1978) is a useful in vivo test by which the evaluation of the effects of drugs is possible within a short period of time using only small specimens of cancer tissue.

Chemosensitivity testing of anticancer drugs for liver cancer by the SRCA has been reported only by Takahashi et al. (1988), who utilized human liver cancer cell lines and fresh hepatocellular carcinoma (HCC) tissues obtained from surgical specimens. We now report that specimens obtained from fine needle biopsies with ultrasonic guidance can be used in this assay. The results indicate that the application of this SRCA method is possible in patients with inoperable liver cancer.

Materials and methods

1. Experimental animals
BDF1 mice, aged 7-10 weeks, (Shizuoka Labo. Animal Center) were used as the experimental animals.

2. Tumor materials

The tumor materials included 20 HCCs, 4 metastatic liver cancers (2 colon cancers and 2 pancreas cancers), and 3 cholangiocellular carcinomas that were diagnosed histologically. Several tumor specimens (0.8 mm in diameter and 1-2 cm in length, Fig. 1) were obtained by 21-gauge fine needle aspiration biopsy with ultrasonic guidance (Majima et al. 1988).

3. SRCA techniques

The original method reported by Bogden et al. (1978) was employed. In brief, biopsy specimens were cut into small pieces of approximately 1 mm$^3$ and cultured in RPMI 1640 medium (Nissui, Tokyo, Japan) with 10% fetal bovine serum (GIBCO, Buffalo, NY) before implantation.

BDF1 mice were anesthetized with pentobarbital sodium (Abbot Laboratories, North Chicago, IL), and an incision was made through the skin and body wall. The left kidney was partially exteriorized, and a small slit was made in the renal capsule. The tumor fragment was loaded onto the tip of a 19-gauge venous canula, inserted through the slit, and deposited under the renal capsule. Immediately after implantation, the length (L) and width (W) of the implanted fragment were measured with a stereoscopic microscope. On the sixth day after implantation, each mouse was weighed and killed by cervical dislocation under anesthesia. The abdominal cavity was exposed, and the left kidney was removed and placed under a stereoscopic microscope for measurement of the final tumor size in units of 1 dmm (1 dmm=0.1 mm).

4. Administration of immunosuppressive agents

All experimental animals were given immunosuppressive agents. Cyclosporine A (CSA, 60 mg/kg) was administered subcutaneously daily for six days, starting from the first day after implantation. Cyclophosphamide (CPM, 150 mg/kg) was injected subcutaneously, 24 hrs. before tumor implantation, in 20 experiments and its activity was evaluated by the tumor response.

5. Evaluation of the transplanted tumor specimens and drug activities

Both the change of tumor size ($\Delta$TS) assay developed by Griffin et al. (1983) and the tumor growth inhibition rate (TGIR) assay developed by Battle Columbus Laboratories (Ovejera et al. 1978) were employed in these experiments.

According to the former method, the $\Delta$TS was calculated as follows: $\Delta$TS=$(L+W)/2$; $\Delta$TS=TS$_6$-TS$_0$, where TS on day 0 was TS$_0$ and TS on day 6 was TS$_6$. Evaluation of $\Delta$TS in the control group was possible if it was above -0.5 dmm. A $\Delta$TS in the animals given anticancer drugs was an effective response if it was
below -1.0 dmm. According to the latter method, the tumor volume (TV) was calculated as follows: $TV = (L \times W^2) / 2$, this relationship on day 0 was defined as $C_0$ in the control group and $T_0$ in the group receiving anticancer drugs.

The TV on day 6 was defined as $C_6$ and $T_6$. The growth rate ($C_6/C_0$) was evaluated when it was above the value of 1, and TGIR was calculated as follows: $TGIR = (1 - (T_6/T_0 + C_6/C_0)) \times 100\%$. Effective growth inhibition was considered to be occurring, when the TGIR was greater than 50%.

6. Administration of anticancer drugs

Cis-platinum (CDDP), Adriamycin (ADM), and Mitomycin (MMC) were given by single intravenous injections on day 1. The drug doses was as follows: CDDP, 10 mg/kg; ADM, 10 mg/kg; and MMC, 8 mg/kg. These were the maximum tolerated doses for BDF1 mice to isolate truly negative drugs. Anticancer drugs were tested on 16 hepatocellular carcinomas, 2 metastatic liver cancers from the colon, and 2 cholangiocellular carcinomas.

Results

1. Evaluation of the transplanted tumor specimens

The evaluable rate of CSA-treated tumors was 86% (6/7) in the $\Delta$TS assay and 57% (4/7) in the TGIR assay. The mean $\Delta$TS value was 0.57 (-0.75 to 2.1) dmm, and the mean tumor growth rate was 1.59 (0.88 to 3.29). The evaluable rate of 20 tumors treated with CPM was 85% (17/20) with the $\Delta$TS assay, and 70% (14/20) with the TGIR assay. The mean $\Delta$TS value was 0.98 (2.4 to 5.3) dmm, and the mean tumor growth rate was 1.55 (0.45 to 5.1). No significant difference was found between the CSA and CPM groups (Fig. 2).

2. Activity of the anticancer drugs (Table 1)

Applying the $\Delta$TS method, the drug activity of ADM was 71% (10/14), of CDDP was 58% (7/12), and of MMC was 43% (3/7). Using the TGIR method, the activities were ADM, 73% (8/11); CDDP, 56% (5/9); and MMC, 50% (3/6).

![Tumor growth rate](image)

**TABLE 1**

<table>
<thead>
<tr>
<th>Anticancer drug</th>
<th>$\Delta$TS</th>
<th>TGIR</th>
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<tbody>
<tr>
<td>ADM</td>
<td>10/14 (71%)</td>
<td>8/11 (73%)</td>
</tr>
<tr>
<td>CDDP</td>
<td>7/12 (58%)</td>
<td>5/9 (56%)</td>
</tr>
<tr>
<td>MMC</td>
<td>3/7 (43%)</td>
<td>3/6 (50%)</td>
</tr>
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ADM: adriamycin; CDDP: cis-platinum; MMC: mitomycin.
3. Changes in body weight

The changes in body weight of the mice between the first and sixth days were measured. The mean decrease in body weight was 2% in the control mice, and no group of drug-treated mice was found to have a body weight loss of more than 20%. The mean values for ADM, CDDP, and MMC were, respectively, -5%, -14%, and -11%.

4. Histological examination

The histology of the control group is shown in Fig. 3 on the sixth day after transplantation of cancer specimens to the subrenal capsular region in BDF1 mice. Fig. 3 shows a cholangiocellular carcinoma (a) after CSA and an HCC (b) after CPM. Cellular infiltration into the interstitial spaces was minimized by the administration of immunosuppressive agents, and the histology of the implanted cancer in the subrenal capsular zone was similar to that of the original tissue.

Discussion

Bogden et al. (1978) first described the SRCA method. The tumor was implanted into the subrenal capsular space of nude mice and the effect of anticancer drugs was evaluated after a period of 11 days. In 1979, they also reported that the use of normal mice made it possible to evaluate drug effects in six days (Bogden et al. 1979). Since that time, many studies have been performed, and it appears that the chemosensitivity tests might predict clinical results (Bogden et al. 1984). The main advantages of the SRCA are the possibility of rapid evaluation and earlier clinical investigation, using only a small amount of cancer tissue.

Resected cancer specimens have usually been used for the SRCA in clinical cases to monitor the efficacy of postoperative chemotherapy. There are, however, many inoperable liver cancers, frequently accompanied by liver cirrhosis.

Chemosensitivity testing of anticancer drugs was explored using biopsy specimens of liver cancer and the SRCA. The present method warrants consideration as a test that can be performed before chemotherapy, because the SRCA with biopsy material poses a minimal burden on patients with inoperable liver cancer.
The evaluable tumor growth rate in the present study was 83% by the ΔTS method and 67% by the TGIR method, these were similar to the rates in other studies using surgical specimens. Takahashi et al. (1988), however, reported that the evaluable rate of surgical specimens with HCC was only 50% by the TGIR method. This rate was a little lower than that for other types of cancer, because it was difficult to obtain good specimens after a variety of preoperative treatments were performed. The present study yielded results characteristic of cancer specimens without previous treatment. This method can obtain good specimens without ischemia.

A technical advantage in the present study is that it was easy to obtain and dissect specimens. It is extremely difficult to cut resected specimens into 1 mm³ sections, although several methods have been reported (Sakai et al. 1987). In this study, as shown in Fig. 1, the specimens obtained by 21-gauge fine needle aspiration biopsy were 0.8 mm in diameter and 1-2 cm in length. By cutting the specimens into a set length, 1 mm³ tissue sections were easily obtained.

The administration of immunosuppressive agents is essential to increase the evaluable tumor rate by suppressing the host response, as clarified by histological studies (Edelstein et al. 1983). The immunosuppressive agents that are usually administered include CSA, CPM and breclinine, and Inoue et al. (1987) reported no difference in the effects of the three agents on tumor growth until the ninth day after administration. Terashima et al. (1988) also reported that the administration of CPM produced a similar histology to the administration of CSA within the first six days after implantation. Terashima et al. (1988) also reported that the administration of CPM produced a similar histology to the administration of CSA within the first six days after implantation. There are several potential problems with the SRCA method. A decrease in the body weight of the mice may occur due to toxicity of the immunosuppressive agents or a deterioration in the overall condition of the animals. Overestimation of the effects of anticancer drugs in combination with immunosuppressive agents may occur, and anticancer drugs may have immunosuppressive effects themselves, e.g., CSA may increase the antitumor effect of ADM (Dzieka et al. 1986). The death of a number of the implanted mice due to the administration of the anticancer drugs can also be a problem. In the present study, a dose of 150 mg/kg CPM was administered 24 hours before implantation, and a reasonable rate of suppression of host reactions until the sixth day after implantation was recognized in the 20 mice when a chemosensitivity examination was performed. As shown in Fig. 3, the cellular infiltration into the interstitial space was controlled, and the decrease in the body weight of the mice was found to be less than 20%.

The ΔTS and TGIR methods were used as criteria for evaluation of the test results. Other techniques include the method of Sakai et al. (1987) in which thickness and volume were indicators, and a method using histologic criteria. In the SRCA method, Terashima et al. (1987) reported that the effectiveness of anticancer drugs tended to be overestimated by the TGIR method in rapidly growing tumors and by the ΔTS method in slowly growing tumors. In the present study, the specimens were slightly smaller than 1 mm³, and the TGIR method seemed more appropriate than the ΔTS method for the evaluation. As shown in Table 1, however, no significant differences of the drug activities between the two methods were observed.

Only three drugs were employed in the present study. The variety of anticancer drugs frequently used in chemotherapy for liver cancer is not large, however, and those which are often administered (especially by intraarterial or intravenous
therapy) are ADM, MMC, and CDDP.

Chemosensitivity testing for inoperable liver cancer patients using ultrasonic biopsy and the SRCA method was effective. Further studies are needed for the full application of the SRCA method to liver cancer patients.

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References


