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Sensitivity to Six Antitumor Drugs Differs Between Primary and Metastatic Liver Cancers

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Abstract—*Chemosensitivity to six different types of antitumor drugs was assayed using the succinate dehydrogenase inhibition (SDI) test, with regard to effects of 29 tissues from primary hepatocellular carcinomas (PHC) and 12 metastatic liver cancers. Succinate dehydrogenase activity in the PHC was significantly decreased by adriamycin (ADM), mitomycin C (MMC), aclacinomycin A (ACR) ($P < 0.01$), and 5-fluorouracil (5-FU), cisplatin (DDP) and carboquone (CQ) ($P < 0.05$), as compared to findings in tissues from the metastatic liver tumors. In PHC, chemosensitivity to antitumor drugs in the SDI test was positive in 58.6% of tissues exposed to ADM, 60.7% with MMC, 11.1% with 5-FU, 65.4% with DDP, 65.5% with ACR and 64.3% with CQ. On the contrary, the positive rates seen in metastatic liver tissues were 18.2% in DDP and 8.3% in CQ, and there was no positive chemosensitivity in tissues exposed to ADM, MMC, 5-FU and ACR. Therefore, PHC will show a better response than metastatic liver cancers to antitumor drugs.*

Our observations show that the selection of sensitive drugs is most important to improve the response rate and the survival time of patients. The SDI test proves useful for planning clinical management.

INTRODUCTION

THE PRESENT treatment of liver cancers in humans, including primary hepatocellular carcinoma (PHC) and metastases, is not very effective. When the tumors are localized and the hepatocellular reserve is adequate, surgical resection is a valid consideration [1]; however, not all patients benefit from resection. Systemic or intra-arterial chemotherapy for these liver cancers is disappointing, but a remarkable response can be obtained [2]. The efficacy of this chemotherapeutic treatment depends on multiple factors, such as the route of drug administration, the distribution of blood flow in the tumor and prolonged maintenance of effective concentration of drugs in the tumor tissue. The sensitivity of individual tumors to antitumor agents is also important. *In vitro* and *in vivo* trials to predict the chemosensitivity of individual tumors have been established [3, 4]. In the present study, we compared the chemosensitivity of PHC and metastatic liver cancer tissues to various antitumor agents, using the succinate dehydrogenase inhibition (SDI) test [5, 6], an approach based on the correlation between succinate dehydrogenase (SD) activity and cell viability. Tetrazolium salt was used as the hydrogen acceptor for the SD activity [7].

MATERIALS AND METHODS

PHC specimens from 29 Japanese patients (21 men and eight women), and metastatic liver cancer specimens from 12 patients (10 men and two women; primary sites were six colorectal carcinoma, three gastric carcinoma, two pancreatic carcinomas and one esophageal carcinoma) were obtained at surgery and immediately placed in McCoy's 5A solution.

The antitumor drugs tested were those in regular clinical use, adriamycin (ADM; 4 μg/ml), mitomycin C (MMC; 10 μg/ml), 5-fluorouracil (5-FU; 100 μg/ml), cisplatin (DDP; 20 μg/ml), aclacinomycin A (ACR; 4 μg/ml) and carboquone (CQ; 1 μg/ml), at ten times the peak plasma concentration [5]. These drugs were purchased from the following sources: ADM, MMC and 5-FU were from Kyowa Hakko Co., Ltd., Tokyo, DDP from Nihon Kayaku Co., Ltd., Tokyo, ACR from Sanraku-Ocean Co., Ltd., Tokyo and CQ from Sankyo Co., Ltd., Tokyo.

The SDI test was done as described [5, 6]. Briefly, the tumor tissues were cut with scissors and passed through a No. 32 stainless steel mesh into

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McCoy's 5A solution containing antibiotics, and then washed three times with this solution. The fragments were then suspended in minimal essential medium with L-glutamine (292 mg/ml), 10% fetal calf serum and antibiotics, plated in each of 35 mm plastic dishes (three dishes per test group and 4–6 in control), and then incubated at 37°C in a humidified 5% CO_2 atmosphere for 3 days. These fragments were then assayed for SD activity. 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT) was used as a hydrogen acceptor for SD activity [8]. The formazan formed from MTT was extracted with acetone containing 0.5% trichloroacetic acid and the absorbance of formazan was measured at 565 nm. The SD activity was presented as the optimal density (OD) per mg tissue protein. The tumor fragments with an OD of 0.5 on day 0 were plated in separate dishes and an OD over 0.1 in the control on day 3 was considered as the evaluable assay. The chemosensitivity was indicated by the percentage of SD activity of drug-treated cells compared to that of control cells, and was considered positive when the SD activity of the drug-treated cells decreased to below 50% of that in the control cells [5, 6].

In our laboratory, the sensitivity of the SDI test was compared to that of *in vivo* systems such as the subrenal capsule assay [6] and ATP assay [9]. A close relationship was apparent.

Statistical analysis of difference in SD activity between primary and metastatic liver cancers was determined by Student's *t*-test. A *P* value of less than 0.05 was considered significant.

RESULTS

SD activity in the 29 PHC and 12 metastatic liver cancer tissues, and exposed to six antitumor drugs is shown in Fig. 1. The mean percentages of SD activity in PHC and metastatic tumor tissues were 50.8 ± 22.6%, 72.8 ± 15.6% for ADM ($P < 0.01$), 45.6 ± 23.2%, 74.6 ± 14.0% for MMC ($P < 0.01$), 69.0 ± 15.4%, 80.6 ± 15.0% for 5-FU ($P < 0.05$), 42.3 ± 21.5%, 55.7 ± 8.8% for DDP ($P < 0.05$), 43.3 ± 23.1%, 68.2 ± 11.2% for ACR ($P < 0.01$) and 44.7 ± 21.9%, 63.0 ± 14.3% for CQ ($P < 0.05$), respectively. PHC had a significantly lower SD activity than secondary liver cancer, to all the six anticancer drugs.

In PHC, the SD activity of the drug-treated cells decreased to below 50% of that of control cells in 17 of 29 patients (58.6%) in the case of ADM, 17 of 28 (60.7%) in MMC, three of 27 (11.1%) in the 5-FU, 17 of 26 (65.4%) in DDP, 19 of 29 (65.5%) in ACR and 18 of 28 (64.3%) in CQ. PHC in tissues from four patients was sensitive to all the six drugs tested, yet sensitivity to any drug was nil in PHC from eight patients. The remaining 17 were sensitive

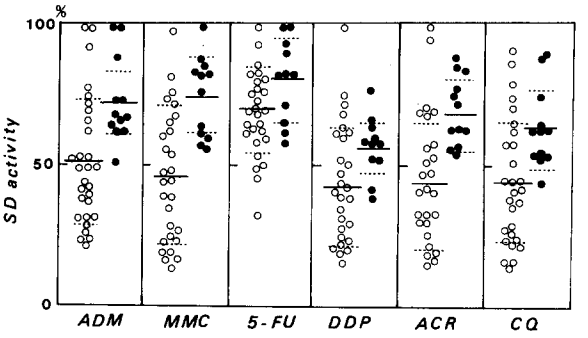

Fig. 1. SD activity in PHC and metastatic liver cancer tissues exposed to antitumor drugs. The chemosensitivity is indicated by the percentage of SD activity of drug-treated cells compared to that of control cells. Solid and dotted bars represents the mean ± S.D. There was a significant difference in ADM, MMC and ACR ($P < 0.01$), *and 5-FU, DDP and CQ* ($P < 0.05$) *between PHC and metastases. PHC: primary hepatocellular carcinoma* (○), *metastases* (●); *SD; succinate dehydrogenase, ADM; adriamycin, MMC; mitomycin C, 5-FU; 5-fluorouracil, DDP; cisplatin, ACR; aclacinomycin A, CQ; carboquone.*

to only some of the drugs. In terms of metastatic liver carcinoma, none of the tissues from 12 tumors had a sensitivity to ADM, MMC, 5-FU and ACR. Only two (18.2%) and one (8.3%) specimen of the 12 tumors were sensitive to DDP and CQ, respectively.

DISCUSSION

In patients with PHC, the median survival time is 22 months (range 9–93 months), and in those with metastases, this time is 18 months (range 4–113) [10]. Systemic chemotherapy for liver cancers has met with little success. In a prospective randomized clinical trial [11], no response was seen in any of 48 patients with PHC who were given 5-FU systemically. Johnson *et al.* [12] found that intravenous administration of ADM induced a remission in 32% of 44 patients with PHC. Okuda *et al.* [13] reported that the median survival of 169 untreated patients with PHC was only 1.6 months from the time of the diagnosis. Systemic or intra-arterial administration of MMC significantly prolonged survival in patients with advanced PHC. The recent trend in chemotherapy has been to give an antitumor drug via the hepatic artery so that a higher level of the agent will reach tumor cells. When 5-FU was prescribed intra-arterially, the response rate was 73% in patients with metastasized liver cancers [14]. Despite this relatively high response rate, there is little evidence to suggest that intrahepatic chemotherapy actually prolongs the survival [15].

The present study showed that the PHC was significantly more sensitive to six antitumor drugs than were tissues from a metastasized tumor. More than half of the PHC were sensitive to the drugs tested, except for 5-FU. On the contrary, there was little chemosensitivity to any drug in metastatic

tissues. Thus, PHC will probably show a better response to antitumor drugs. The SDI test clinical correlation was noted in some clinical cases [16]. To improve the response rate and the survival time, a sensitive drug has to be selected. When the clinical state does not respond to any drug, other modes of therapy such as chemotherapy combined with transcatheter arterial embolization [17] have to be considered. The SDI test used in the present study is a simple, inexpensive and rapid technique for screening antitumor drugs, and hence proves useful for planning clinical management.

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REFERENCES

1. Nordlinger B, Parc R, Delva E, Quilichini MA, Hannoun L, Huguet C. Hepatic resection for colorectal liver metastases. Influence on survival of preoperative factors and surgery for recurrences in 80 patients. *Ann Surg* 1987, **205**, 256–263.
2. Okuda K, Ohnishi K, Nomura F. Hepatic tumors. In: Arias IM, Frenkel M, Wilson JHP, eds. *The Liver Annual*. Elsevier, Amsterdam, 1983, 336–364.
3. Griffin TW, Bogden AE, Reich SD, Antonelli D. Initial clinical trial of the subrenal capsule assay as a predictor of tumor response to chemotherapy. *Cancer* 1983, **52**, 2185–2192.
4. Weisenthal LM, Lippman ME. Clonogenic and non-clonogenic *in vitro* chemosensitive assays. *Cancer Treat Rep* 1985, **69**, 615–632.
5. Maehara Y, Anai H, Kusumoto H, Sugimachi K. Poorly differentiated human gastric carcinoma is more sensitive to antitumor drugs than is well differentiated carcinoma. *Eur J Surg Oncol* 1987, **213**, 203–206.
6. Anai H, Maehara Y, Kusumoto H, Sugimachi K. Comparison between succinate dehydrogenese inhibition test and subrenal capsule assay for chemosensitivity testing. *Oncology* 1987, **44**, 115–117.
7. Dallne G. Viability and tetrazolium salt in ascites tumours. *Nature* 1960, **186**, 176.
8. Mosmann T. Rapid colorimetric assay for growth and survival application to proliferation and cytotoxic assays. *J Immunol Methods* 1983, **65**, 55–63.
9. Anai H, Maehara Y, Kusumoto T, Sugimachi K. Comparison of the chemosensitivity of human neoplastic tissues between succinate dehydrogenase inhibition test and ATP assay. *Clin Chim Acta* 1987, **166**, 107–109.
10. Danopoulos ED, Danopoulou IE. Eleven years experience of oral urea treatment in liver malignancies. *Clin Oncol* 1981, **7**, 281–289.
11. Falkson G, Moertel CG, Lavin P. Pretorius FJ, Carbone PP. Chemotherapy studies in primary liver cancer. A prospective randomized clinical trial. *Cancer* 1978, **42**, 2149–2156.
12. Johnson PJ, Williams R, Thomas H, Sherlock S, Murray-Lion IM. Induction of remission in hepatocellular carcinoma with doxorubicin. *Lancet* 1978, 1006–1009.
13. Okuda K, Obata H, Nakajima Y, Ohtsuki T, Okazaki N, Ohnishi K. Prognosis of primary hepatocellular carcinomas. *Hepatology* 1984, **4**, 3S–6S.
14. Watkins E, Khazei AM, Nahra KS, Surgical basis for arterial infusion chemotherapy of disseminated carcinoma of the liver. *Surg Gynecol Obstet* 1970, **130**, 581–603.
15. Oberfield RA, McCaffrey JA, Polio J, Clouse ME, Hamilton T. Prediction of response of tumor and host to cancer chemotherapy. *Cancer* 1979, **44**, 414–423.
16. Kondo T. Prediction of response of tumor and host to cancer chemotherapy. *Natl Cancer Inst Monogr* 1971, **34**, 251–256.
17. Sakurai M, Okamura J, Kuroda C. Transcatheter chemo-embolization effective for treating hepatocellular carcinoma. A histopathologic study. *Cancer* 1984, **54**, 387–392.