Prospective Correlative Chemosensitivity Testing in High-Dose Intraarterial Chemotherapy for Liver Metastases

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ABSTRACT

Clinical response of liver metastases treated by high-dose intraarterial chemotherapy (HDIAC) delivered via the hepatic artery was predicted by a modification of the human tumor colony-forming assay (HTCFA) originally described by Hamburger and Salmon [Science (Wash. DC), 197: 461-463, 1977]. In a first set of experiments, the immediate clinical response to HDIAC was determined in 12 patients with colorectal liver metastases. Biopsies were taken immediately before and after HDIAC, and cells were plated in the HTCFA. Three patients received intraoperative 4-epidoxorubicin and another 9 received mitomycin C by 15-min intraarterial infusions. Sensitivity in the HTCFA was defined as 50% inhibition of colony formation in tumors exposed to the chemotherapeutic agent, compared to the untreated controls. Clinical response was accurately predicted by the HTCFA in 11 of 12 cases.

Eight patients had a regression of disease following HDIAC treatment with mitomycin C, as evidenced by a reduction in serum carcinoembryonic antigen serum level (7 patients) or regression of tumor by computed tomography scan (1 patient). Three patients had no evidence of clinical response to epidoxorubicin, and their tumors were resistant to epidoxorubicin in the HTCFA. One tumor was sensitive to mitomycin C in the HTCFA, but serum carcinoembryonic antigen in the patient continued to increase following HDIAC. The HTCFA was also performed on untreated biopsies following incubation in vitro with the drug used for HDIAC. Results correlated with clinical response in all 12 cases. In a second set of experiments, the HTCFA was used to predict the long-term clinical response to HDIAC of 30 patients with liver metastases. One patient had breast cancer metastases, one patient had carcinoid liver metastases, 4 had liver metastases of malignant melanoma, and 24 patients had colorectal liver metastases. All 21 of the patients whose tumors were sensitive in vitro had clinical response, while 6 of 9 patients predicted by the HTCFA to be resistant had no clinical response. Our results demonstrate a high correlation between the HTCFA and clinical response.

INTRODUCTION

New forms of high-dose systemic (1) or regional (2) chemotherapy have been developed to overcome the resistance of solid tumors to conventional systemic chemotherapy. When high drug doses are applied regionally, certain tumors become more sensitive to anticancer drugs, as demonstrated with mitomycin C (3, 4). Since recent technical improvements allow repeated high-dose regional drug infusions at many different anatomic locations, new protocols in the high-dose range with cytotoxic agents or drug combinations are being developed and evaluated.

Most protocols involving systemic chemotherapy have been developed empirically. We decided to circumvent this time-consuming procedure by determining if in vitro chemosensitivity testing is an appropriate way to select the most effective drugs for regional chemotherapy of liver metastases. We investigated whether the HTCFA originally described by Hamburger and Salmon (5) can be used reliably to predict the clinical response to regional chemotherapy.

MATERIALS AND METHODS

Clinical Treatment and Evaluation. The clinical treatment with HDIAC was performed at the Justus-Liebig-Universität in Giessen, Federal Republic of Germany and procedures have been previously described (6). Treatment was started by operatively placing an Implantofix catheter (B. Braun, Melsungen, West Germany) with its tip into the hepatic artery via the gastroduodenal artery. After surgery, repeated HDIAC cycles (4-6) were performed with a combination of either mitomycin C (8 mg/m², day 1) and 5-fluorouracil (550 mg/m², days 2-6) or 4-epidoxorubicin (30 mg/m², day 1) and 5-fluorouracil (550 mg/m², days 2-6). The drugs were infused i.a. over a period of 1 h with an external Infusomate pump (B. Braun). The infusion tube was connected to a s.c. placed depot of the Implantofix catheter with a 23-gauge injection needle. I.a. drug levels in the hepatic artery ranged from 0.5-4 µg/ml for mitomycin C, 1-10 µg/ml for 4-epidoxorubicin, and 5-100 µg/ml for 5-fluorouracil. Response to treatment was determined either by reduction in serum CEA levels or by regression of tumor by computed tomography scan (7-10).

Chemosensitivity Determined with the HTCFA. The tumor material was transported to the tissue culture laboratory in Hanks' balanced salt solution immediately after excision. After removal of macroscopically nonmalignant and necrotic tissue, the tumor was minced into 1- to 2-mm pieces with scissors and digested enzymatically (11) overnight (10-12 h) at 4°C in a solution of Hanks' balanced salt solution with 0.3% DNase (Sigma Chemical Company, St. Louis, MO) and 0.14% collagenase (Sigma) with gentle stirring. We used 10-20 ml enzyme solution/g of tissue. A representative piece of the tumor biopsy was fixed in 5% buffered formaldehyde for histology. After enzymatic disaggregation, the cell suspension was filtered through 12 layers of sterile gauze. The enzyme solution was removed after centrifugation of the cell suspension at 200 xg for 10 min. Subsequently, the cell suspension was washed twice in McCoy's 5A medium with 10% FBS. The washed tumor cell suspension was adjusted to 3 x 10⁸ viable tumor cells/ml. We determined viability by trypan blue exclusion and cytological examination. Drug exposure in the HTCFA was performed with 1.5 x 10⁸ viable tumor cells in 1.5 ml of medium (McCoy's 5A, 10% FBS) over a period of 1 h at 37°C. The untreated control was processed in the same way, but without drug. Drug concentrations were 1 and 100 µg/ml for mitomycin C and 4-epidoxorubicin, and 100 and 1000 µg/ml for 5-fluorouracil. The lower test concentration was representative of clinically achievable serum concentrations (6). After the 1-h incubation at 37°C in vitro and the subsequent washing steps, the cells were seeded into 3 plastic Petri dishes in 0.3% agar over 0.5% agar feeder layer at a concentration of 5 x 10⁶ viable tumor cells/35-mm dish. In contrast to the method originally described by Hamburger and Salmon (5, 12), we did not use spleen-conditioned medium, mercaptoethanol, CaCl₂, or dextran. The serum source was FBS; the cultures were incubated at 37°C in a mixture of 5% CO₂ and air. The day after seeding, the culture dishes were evaluated using an inverted light microscope to identify cell aggregates as well as at weekly intervals to count colonies. A colony was defined as an outgrowth of 5000 cells.

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The abbreviations used are: HTCFA, human tumor colony-forming assay; HDIAC, high-dose intraarterial chemotherapy; FBS, fetal bovine serum; i.a., intraarterial(i.v.); CEA, carcinoembryonic antigen.
least 30 cells, and growth was sufficient for evaluation if at least 30 colonies had grown in the untreated control. The period up to the final evaluation lasted from 4–12 weeks. At the end of incubation, the inhibition of colony formation was determined by comparing the soft-agar growth of the drug-treated cultures with the untreated ones. In vitro sensitivity was defined as at least 50% inhibition of colony formation according to criteria described by Bertelsen et al. (13). A tumor was considered resistant to a drug if there was less than 50% inhibition of colony growth.

Following HDIAC, a second biopsy was obtained from twelve patients. Cell suspensions were prepared, and the cells were plated in the HTCFA. These cells presumably had been exposed in vivo to anticancer drugs during the course of HDIAC. The percentage of survival was calculated relative to the untreated (control) cells obtained from the first (pre-HDIAC) biopsy (Fig. 1).

RESULTS

In Vitro Response Rates. For colorectal liver metastases tested in the HTCFA, the in vitro sensitivity rate depended on the drug and its concentration. At a concentration of 100 μg/ml, 15 of 18 tumors were sensitive to epideroxicrubin and 15 of 17 to mitomycin C, where at 1 μg/ml, 5 of 15 tumors were sensitive to epideroxicrubin and 11 of 21 to mitomycin C. Since 5-fluorouracil can be given clinically at much higher doses than epideroxicrubin or mitomycin C, it was tested in vitro at 10-fold higher concentrations. A total of 12 of 18 tumors were sensitive to 5-fluorouracil at a concentration of 1000 μg/ml, and 5 of 15 were sensitive at 10 μg/ml.

Fig. 2 shows the mean percentage of colony survival versus drug concentration for nine colorectal tumors tested against mitomycin C. There is a clear dose response in these patients' tumors. Eight patients had a clinical response to mitomycin C, and one (patient 34) did not.

Comparison of in Vitro with in Vivo Drug Exposure. Tumors from 12 patients with colorectal metastases were biopsied prior to the initiation of HDIAC. Tumors were exposed in vitro to either mitomycin C or epideroxicrubin, and the percentage of colony survival was calculated relative to untreated (control) cells. Following HDIAC, a second biopsy was performed on each patient. Cells from these were plated in the HTCFA, and the percentage of survival was calculated relative to pre-HDIAC (control) cells. As shown in Table 1, 8 of 9 tumors exposed to mitomycin C in vitro were found to be sensitive to this drug, with less than 50% colony survival. Three tumors were resistant to epideroxicrubin, both in vitro and in vivo. Clinical responses were measured in all 12 patients. Seven patients treated with mitomycin C during HDIAC had decreases in CEA levels to less than one-half of the pretreatment value, and one patient negative for CEA had a response by computed tomography scan.

![Fig. 1. Schema of study design. A metastatic nodule in the liver was biopsied before initiation of HDIAC, and a second nodule was removed after completion of HDIAC. An aliquot of the first biopsy was plated directly in the HTCFA, and another aliquot was exposed to anticancer drug for 1 h in vitro before plating in the HTCFA. Cells from the second biopsy following 1 h HDIAC were plated directly in the HTCFA. Colony survival for cells exposed to drug, either in vitro or in vivo, was calculated as the percentage relative to untreated (control) cells. 37°, 37°C.](image)

![Fig. 2. Dose-response curves for mitomycin C. Cells from liver metastases of colorectal tumors were incubated in vitro for 1 h and plated in the HTCFA. Points, mean colony survival for nine tumors. Patient 34 had no clinical response (see Table 1). All eight others showed evidence of tumor regression.](image)

### Table 1 Correlation of HTCFA sensitivity in vitro and in vivo with clinical response following HDIAC

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Drug</th>
<th>Control</th>
<th>In vitro exposure</th>
<th>In vivo exposure</th>
<th>CEA post-HDIAC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>Mitomycin</td>
<td>122±3</td>
<td>30 (SF)</td>
<td>15 (S)</td>
<td>40 (S)</td>
</tr>
<tr>
<td>28</td>
<td>Mitomycin</td>
<td>1059±115</td>
<td>46 (S)</td>
<td>6 (S)</td>
<td>48 (S)</td>
</tr>
<tr>
<td>52</td>
<td>Mitomycin</td>
<td>301±42</td>
<td>35 (S)</td>
<td>7 (S)</td>
<td>20 (S)</td>
</tr>
<tr>
<td>56</td>
<td>Mitomycin</td>
<td>73±8</td>
<td>28 (S)</td>
<td>22 (S)</td>
<td>27 (S)</td>
</tr>
<tr>
<td>81</td>
<td>Mitomycin</td>
<td>68±3</td>
<td>13 (S)</td>
<td>0 (S)</td>
<td>10 (S)</td>
</tr>
<tr>
<td>83</td>
<td>Mitomycin/cis-</td>
<td>172±14</td>
<td>0/33 (S/S)</td>
<td>0 (S)</td>
<td>5 (S)</td>
</tr>
</tbody>
</table>

* CEA serum levels (percentage of pretreatment levels) after HDIAC.
* Mean ± SD of colony number of untreated control in soft agar.
* S, sensitive; R, resistant.
* CEA Negative, partial response by computed tomography scan.

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HTCFA following *in vitro* drug exposure. One tumor was sensitive to mitomycin C following *in vivo* treatment but there was no evidence of a clinical response in that patient.

High-Dose *in Vitro* Drug Exposure. Biopsies from 30 patients with liver metastases were obtained before HDIAC. Cells from these tumors were exposed *in vitro* to the same drugs subsequently used for HDIAC. For 23 patients with colorectal metastases, the percentage of colony survival measured in the HTCFA was correlated with serum CEA levels. There was a strong correlation between the percentage of colony survival and serum CEA levels following HDIAC (Fig. 3). The correlation coefficient was 0.80. Clinical correlation for all 30 patients is shown in Table 2. The HTCFA correctly predicted clinical outcome of HDIAC in 21 of 21 (100%) patients. Resistance was predicted accurately in 6 of 9 (67%) patients. The overall predictive accuracy for the assays was 27 of 30 (90%).

Fig. 4 demonstrates the potential value of the HTCFA as a predictor of response to HDIAC. Serum CEA levels were measured in a patient with colorectal cancer metastases before and after repeated courses of HDIAC. After a first course of treatment with epidoxorubicin plus 5-fluorouracil, CEA levels increased, indicating disease progression. After 1 month, the treatment regimen was changed to mitomycin C plus 5-fluorouracil. Four months following initial HDIAC treatment, the patient’s CEA level was only 44% of that of the pretreatment value, indicating a favorable response to mitomycin C plus 5-fluorouracil. Four months following initial HDIAC treatment, the patient’s CEA level was only 44% of that of the pretreatment value, indicating a favorable response to mitomycin C plus 5-fluorouracil. This patient’s tumor was correctly predicted by the HTCFA to be resistant to a combination of epidoxorubicin plus 5-fluorouracil, but sensitive to mitomycin C plus 5-fluorouracil.

**DISCUSSION**

Because colorectal carcinomas are individually heterogeneous in their sensitivity to anticancer drugs, a predictive test system that will allow the individual tumor drug sensitivity to be determined before the start of therapy is necessary. The HTCFA is widely applied in predictive drug testing for systemic chemotherapy. Overall, clinical sensitivity has been correctly predicted in 50–80% of the cases and resistance in more than 90% (13–21). Up to now, the HTCFA results have been obtained in systemic chemotherapy from testing of many tumor types. While validity of the HTCFA initially seemed to be promising, it has been difficult to assess the absolute benefit to the individual (22–24).

In colorectal carcinoma, one of the most common human tumors, clinical correlations were assessed by Bertelsen et al. (13). In 39 cases, 38% were sensitive and 62% resistant to 5-fluorouracil *in vitro*. In 80% of the tumors tested, clinical response could be predicted correctly. Predictive accuracy for sensitivity was 60% and for resistance 92%; however, Agrez and Lieber (25) reported far lower *in vitro* sensitivity rates of 6–20% for colorectal cancer.

Several factors might contribute to the limited validity of the HTCFA in predicting response to systemic chemotherapy. One critical question is whether the tumor biopsy is representative for the target to be treated. It is known that biopsies from different metastatic sites in the total body system may react differently to chemotherapy (26, 27). Tumor heterogeneity could present practical limitations to the use of HTCFA in predicting response to HDIAC. In the series of twelve paired biopsies (taken before and after HDIAC) reported in this study, several histopathological variables were measured. Paired tumors were identical as to histological grading, degree of vital and necrotic tissue in the specimen, and viability of the cells used for the HTCFA.

A second question is whether the pharmacokinetics of the HTCFA is representative of the *in vivo* situation. We believe our system is a considerable improvement over attempts to predict clinical responses to systemic chemotherapy. Drug concentration and exposure time at the tumor site were well defined. Liver metastases are mainly supplied by the arterial blood system, and during HDIAC anticancer drugs are delivered under predetermined and consistent conditions. When the HTCFA is used to predict systemic chemotherapy, the *in vitro* and *in vivo* pharmacological parameters often vary greatly (13, 22–24).

**Table 2.** *In vitro/in vivo* correlations between HTCFA and HDIAC

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Resistance</th>
<th>Sensitivity</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>21</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Resistance</td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

* Prediction accuracy for sensitivity = 21 of 21 (100%).
* Prediction accuracy for resistance = 6 of 9 (67%).
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17, 22–24) causing a discrepancy in the in vitro and in vivo results.

A third problem to consider is the rate of false-positive and false-negative predictions for any given assay. The HTCA has been found to predict resistance to systemic chemotherapy very accurately (92–99%). Its prediction of sensitivity is not as good (50–70%). This relatively high (30–50%) rate of false-positive predictions has been attributed to technical problems with the HTCA. The false-positive rates may be due in part to a failure to achieve maximal systemic levels of anticancer drugs at the tumor site. This may result from several factors, including local restrictions in blood flow to the tumor and the manifestation of toxic side effects, which may lead the oncologist to lower the amount of anticancer drug given (28). Drewinko et al. (29) pointed out that colorectal carcinomas resistant to standard levels of chemotherapeutic agents may respond when higher concentrations of drug are administered; thus, tumors that are sensitive in the HTCA but resistant to the same drugs given systemically may respond to higher drug doses in vivo. In our test system, the in vitro and in vivo pharmacological parameters were similar with regard to drug concentrations and exposure times. Drug concentrations tested in the HTCA were in the range of achievable i.a. levels and thus were considerably higher than those previously recommended for use in the HTCA (17, 24). The 1-h drug exposure time in vitro also corresponded to the 1-h infusion time during HDIAC. A duplication in vitro of the pharmacokinetics during HDIAC was probably responsible for the high predictive accuracy of our system.

Additional evidence of the validity of our system for predicting response to HDIAC is the fact that the sensitivity of liver metastases to chemotherapeutic agents was independent of whether these cells were exposed to the drug in vitro or in vivo. Possinger (30) made similar observations. In his system, tritiated thymidine and tritiated uridine uptake were used as in vitro and in vivo response markers in systemic chemotherapy of malignant effusions. The inhibition of DNA and RNA precursor uptake after drug exposure during systemic chemotherapy was found to correlate well with clinical response; however, only resistance could be accurately predicted by drug incubation in vitro.

Our results indicate that patients with liver metastases may benefit from HDIAC if active drugs are selected with HTCA. So far we have tested only three drugs, mitomycin C, epidermorubicin, and 5-fluorouracil. By expanding the test drug panel, it is possible that the sensitivity rates in the HTCA and clinical responses following HDIAC could be increased. We are developing a protocol to determine whether the HTCA can be used to help select second-line drugs for epidermorubicin for those patients who have failed standard first-line chemotherapy.

REFERENCES

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