Histocultures of Patient Head and Neck Tumors for Pharmacodynamics Studies

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This investigation was to establish a clinically relevant experimental model to evaluate the pharmacodynamics of drugs used for head and neck cancers. A total of 83 surgical samples of primary and lymph nodal metastatic tumors was obtained from 66 patients. Fragments of these tumors were cultured on a collagen gel matrix. The tumor cell labeling index (LI) was determined by [^{3}H]thymidine incorporation and autoradiography. Seventeen tumors (20%) were contaminated. About 80% of the remaining 65 tumors were successfully cultured for at least 2 weeks. The cultured tumor fragments retained the morphology and architecture of the freshly removed specimens; both tumor and stromal cells were present. The tumor cell LI after 2–3 weeks in culture, determined from the most proliferative area of the tissue, averaged 77 ± 12% for primary tumors and 78 ± 12% for nodal metastases. The activity of three clinically active agents, 5-fluorouracil (FU), cisplatin (DDP), and mitomycin C (MMC), was evaluated in 47 tumors. All three drugs inhibited the tumor LI. The concentrations needed to produce a 50% inhibition of the tumor LI (IC_{50}) were within the clinically achievable concentration range. The intertumor variation in the IC_{50} for FU (60-fold) was considerably greater than that for DDP and MMC (7- to 8-fold). The nodal metastatic tumors appeared to be less sensitive to FU than the primary tumors, while there were no apparent differences for DDP or MMC. Tumors from patients previously treated with chemotherapy and/or radiotherapy appeared less sensitive to FU and DDP than tumors from untreated patients, but the differences were not statistically significant. Interestingly, tumors from previously treated patients were significantly more sensitive to MMC than tumors from untreated patients. The maintenance of the morphology of the fresh tumor and the observed intertumor variability in IC_{50} values indicate the preservation of intra- and intertumor heterogeneity in the histocultures. In summary the viability and high success rate of growth of human head and neck tumors in organ-like culture and the chemosensitivity of the cultured tumors to clinically active agents at clinically achievable concentrations support the use of this experimental model for pharmacodynamic evaluation.

KEY WORDS: chemosensitivity; histoculture; head and neck tumors; pharmacodynamics.

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INTRODUCTION

The American Cancer Society estimates, for head and neck cancer, 73,100 new cases and 19,550 deaths in 1992. While a majority of the early disease is curable by surgery and radiation, over 75% of patients with advanced disease do not survive (1). In advanced disease, locoregional recurrences and distant metastases are common. Patients with relapse at the primary site, in regional nodes, or in distant sites have a poor prognosis, with a median survival time of about 6 to 10 months. Subclinical microscopic metastases can be found in up to 50% of cases during autopsy. Treatments for these patients include surgery and radiotherapy for locoregional control and systemic chemotherapy to eradicate tumors at distant sites outside the radiation field.

The status of chemotherapy has been reviewed by Wheeler (1). Several approaches have been used, including single agents, combinations of drugs, combinations of chemotherapy and radiotherapy, induction chemotherapy, regional therapy by intraarterial administration, and chemoprevention. The most commonly used agents include methotrexate, cisplatin (DDP),[7] bleomycin, 5-fluorouracil (FU), vinca alkaloids, cyclophosphamide, hydroxyurea, and doxorubicin. DDP is considered the most active single agent, and one of the most effective adjuvant and neoadjuvant protocols uses DDP plus FU, which gives a 4–10% complete response with increased survival. The low complete response rate indicates the need to improve treatment regimens further. Recent clinical trials for advanced stage, previously untreated, operable squamous cell carcinoma evaluated the role of a regimen using chemotherapy prior to or following surgery and postoperative radiation therapy and found that chemotherapy reduced the frequency of distant metastases but did not improve the survival rate (2). To increase the overall survival, effective control of both the primary and the metastatic tumors is necessary. Current and future national strategies in clinical head and neck cancer research involve evaluation of combinations of chemotherapy and radiation therapy including different drugs and different schedules. FU, DDP, and mitomycin C (MMC), because of their radiosensitizing effect, have been used in combination with radiation (3,4).

At present, the search for new drugs and effective combination chemotherapy with tolerable toxicity is primarily through Phase I and Phase II trials. This is a major task, is limited by available patients and financial resources, and does not readily assess the relative drug effectiveness against the primary versus the metastatic tumors. As an alternative, the histocultures of patient tumors can be used to evaluate drug activity. The histoculture method was first described by Hoffman and co-workers to culture human solid tumors (5–7). These investigators have conducted prospective clinical trials to evaluate the histoculture system as a predictive chemosensitivity assay. Our laboratory has used this system to evaluate the pharmacodynamics of MMC in human blad-

[7] *Abbreviations used:* FU, 5-fluorouracil; DDP, cisplatin; MMC, mitomycin C; LI, labeling index; IC_{50} and IC_{90}, drug concentrations needed to inhibit the tumor LI by 50 and 90%, respectively; AUC, cumulative product of concentration and exposure time.

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der tumors. We reported that (a) the proliferative activity of bladder tumor histocultures correlated with the tumor aggressiveness in the hosts, (b) the antitumor effect of MMC in histocultures is achieved at clinically achievable drug concentrations, and (c) the response of the bladder tumor histocultures to MMC is in agreement with clinical experience, with a lower response in the more malignant tumors (9,10). These data in bladder tumors support the clinical relevance of the histoculture system for pharmacodynamic evaluation. Hoffman and co-workers evaluated the activity of FU and DDP against primary tumors from 10 head and neck cancer patients (8). Metastatic tumors were not evaluated. This previous study was to evaluate chemosensitivity, i.e., whether the tumor responded to the drugs, and was not designed to study the pharmacodynamics. The present study established and compared the growth in culture of the primary and nodal metastatic tumors from 66 head and neck cancer patients and evaluated the response of the cultured tumors to FU, DDP, and MMC.

MATERIALS AND METHODS

Chemicals and Supplies. Sterile pigskin collagen (Spongostan Standard) was purchased from Health Designs Industries (Rochester, NY), cefotaxime sodium from Hoechst-Roussel (Somerville, NJ), FU from Sigma Chemicals (St. Louis, MO), NTB-2 nuclear track emulsion from Eastman Kodak Co. (Rochester, NY), and [*methyl*-^{3}H]thymidine from ICN Biomedicals (Irvine, CA). DDP and MMC were gifts from Bristol-Myers Co. (Wallingford, CT). All other tissue culture medium and supplies were purchased from GIBCO Laboratories (Grand Island, NY). All chemicals and supplies were used as received.

Tumor Specimens. Human head and neck tumors were obtained through The Cooperative Human Tissue Network at The Ohio State University. Tumor stage was established preoperatively according to the American Joint Committee on Cancer (11). Tumor grade was determined by surgical pathologists. DNA contents were determined by flow cytometry as described previously (9).

Culture Conditions. Tumor specimens were prepared for culture within 2 to 4 hr postsurgery. The culture medium consisted of Eagle's minimal essential medium supplemented with 10% heat-activated fetal bovine serum, 0.1 m*M* nonessential amino acids, and the antibiotics, gentamicin (0.1 mg/mL) and cefotaxime sodium (95 μg/mL). The pH of the medium was 7.2. Prior to being placed into culture, the specimens were washed three times with complete medium. In the initial experiments, a high percentage of the cultures showed bacterial or yeast contamination. In later experiments, washes were done using a higher gentamicin concentration (1 mg/mL). This procedure significantly reduced the contamination. The tumor specimens were processed as described previously (9). In brief, the necrotic portions of the tumor were trimmed off and the nonnecrotic portions were cut into 1-mm^3 fragments. The fragments were mixed to ascertain randomization. Four to six tumor fragments were placed on a 1-cm^2 piece of collagen gel and cultured in six-well plates in a humidified atmosphere of 95% air, 5% CO_2 at 37°C. The labeling index (LI) of the tumors was determined by [^{3}H]thymidine labeling and autoradiography. Cultures were incubated with medium containing [^{3}H]thymidine with a specific activity of 60 Ci/mmol, at a concentration of 1 μCi/mL. After thymidine exposure, tissues were fixed, embedded in paraffin, and cut into 4-μm sections. The sections were placed on glass slides and processed for autoradiography. The tissue was scanned at low magnification (100×) using a Zeiss Axiovert 35 microscope (Carl Zeiss, Thornwood, NY) to find the most active area of incorporation and the LI of tumor cells in this area was determined by manual counting. LI was defined as the number of labeled tumor cell nuclei divided by the total number of tumor cell nuclei within a defined grid at 400× magnification. Because of the labor-intensive nature of this procedure, the LI was determined in one high-powered field per tissue fragment. On average, about 40 cells (range, 25 to 120 cells) were counted per fragment. Typically, 12 to 18 fragments were used for each treatment condition, and the data represented the average LI of all fragments (>300 cells counted). By choosing the most active area of cell growth to quantify tumor growth and drug effect, we standardized the selection of the areas for evaluation. By using 12–18 random samples per data point, the probability of obtaining a true representation of a heterogeneous tumor is greater than using a single sample. In drug-treated samples, the selection of the area with the highest proliferation would tend to favor the relatively drug-resistant areas. The drug effect is calculated from the inhibition of proliferation of the less chemosensitive cell populations and is more likely a conservative estimate rather than an overestimation.

Pharmacodynamic Studies. A common practice for evaluating drug activity *in vitro,* e.g., the human stem cell clonogenic assay, is to expose tumor cells to drug concentrations equivalent to 10, 100, and 1000% of the peak plasma concentrations for a selected time period. Drug sensitivity is concluded if the agent produces an arbitrarily defined effect (12). To compare the effect seen under the *in vitro* conditions with the clinical situations, the drug concentrations and exposure times used in the present study were selected based on the literature pharmacokinetic data and the administration rate of these agents. FU is often given by infusion over 5 days. The postinfusion drug exposure is relatively insignificant, due to the short half-life of <30 min (13). Hence a 5-day exposure time was used for FU. DDP and MMC are normally administered by intravenous bolus administration. The major half-life is 2–3 hr for DDP in ultrafiltered plasma (14,15) and 40–60 min for MMC (16). The exposure time for DDP (i.e., 10 hr) and MMC (3 hr) was approximately equal to four half-lives. However, initial studies showed that the 3-hr exposure to MMC produced minimal antitumor effect (see below). A longer exposure time of 24 hr was therefore also used.

Drug effect was quantitated as the inhibition of the LI. Cultures were maintained for a minimum of 4 days prior to drug exposure. Three to five drug concentrations were used per experiment depending on tissue availability. A total of 12–18 replicates was used for each drug concentration. These replicates, because of the mixing procedure, were taken from different portions of the tumor and were considered to be representative of the whole tumor. A pilot study determined that the collagen gel retained about 0.8 mL of fluid. In a total volume of 4 mL/well, this corresponded to a

20% dilution by residual medium in the gel. The drug solutions were prepared so that they corrected for this dilution effect and gave the desired concentration. Following exposure, the drug-containing medium was removed and the gel was rinsed three times with 4 mL of drug-free medium. Fresh medium containing [^{3}H]thymidine was added and the tumors were prepared for autoradiography, as described above.

Data Analysis. The concentration–effect relationship was analyzed by computer-fitting the experimental data to the modified E_{max} model [Eq. (1)], as described previously (10). E is the LI as percentage of control, C is the drug concentration, E_0 is the baseline effect in the absence of drug, K is the drug concentration at one-half E_0, and n is a curve shape parameter. IC_{50} and IC_{90} are the concentrations needed to produce 50 and 90% inhibition, respectively. The plot of LI, expressed as a percentage of control, versus the logarithm of drug concentration was analyzed using nonlinear least-squares regression (NONLIN, SAS, Cary, NC) and values for IC_{50} and IC_{90} were determined.

$$E = E_0 \cdot \left(1 - \frac{C^n}{K^n + C^n}\right) \quad (1)$$

Statistical evaluations were performed using paired or unpaired Student's t tests.

RESULTS

Patient and Tumor Characteristics. Surgical specimens of human head and neck tumors were obtained from the primary sites and the cervical lymph nodes. A total of 51 specimens of primary tumors and 32 specimens of nodal metastasis from 66 patients was studied (Table I). Among these, there were 17 pairs of primary and metastatic tumors. Some patients had prior drug treatment, but no chemotherapy was administered for at least 30 days before surgery.

Morphology of Histocultures. The LI as a function of thymidine exposure time was examined in seven tumors. The LI were 50 ± 22, 70 ± 17, and 73 ± 13% (mean ± SD) after 24, 48, and 96 hr of exposure to thymidine, indicating an increased LI with an increased exposure time. Because the concentration–effect relationship is established by the drug-induced inhibition of LI compared to the untreated control, a higher LI in the control gives a greater span of drug effect measurement. The 96-hr exposure time was chosen for subsequent studies, in part because it has been used in previous studies (5–10).

Figure 1 shows the micrographs of 14-day histocultures of a primary squamous cell carcinoma and a nodal metastatic tumor, from the same patient. Note the multicellular structure of the histocultures, the presence of lymphocytes in the nodal tumor, and the presence of stromal cells in both tumors. The black grains in the primary tumor cells were due to [^{3}H]thymidine labeling. Cell morphology prior to and after culture was compared. Most histocultures contained areas of viable and necrotic tumor tissue. Often viable and necrotic regions were dispersed in the same specimen. Areas of necrosis involved all cellular components and was interpreted to be due to tissue disruption during sample processing. Viable tumor cells were present on the surface and interior of the histocultures and were associated with viable fibroblasts and endothelial cells. In general, histology of viable regions of the histocultures was similar to that of the fresh uncultured tumor, i.e., no change in architecture, cell type, or

Table I. Tumor and Patient Characteristics[a]

	N
Grade[b]	
1	1
2	36
3	20
4	0
DNA content[c]	
Diploid	12
Aneuploid	16
Previous treatments[d]	
Radiation	16
Radiation plus chemotherapy	13
None or surgery only	35
Primary sites	
Larynx	13
Hypopharynx	15
Oropharynx	4
Floor of mouth	5
Tongue	18
Tonsil	4
Parotid gland	2
Retromolar/tongue/maxillary sinus	4
Esophagus	2
Neck biopsies[e]	2
Stage[b]	
1	6
2	7
3	13
4	20
Primary tumors	51
Nodal metastases	32
Recurrence[f]	
Yes	21
No	44
Age group	
25–29	1
30–39	2
40–49	5
50–59	22
60–69	26
70–79	10
Gender	
Female	23
Male	43

[a] A total of 83 tumors was obtained from 66 head and neck cancer patients. The tumors are categorized by their pathology and sites of the primary tumors. Patients are categorized by their age and gender. *N*, number.

[b] Ten tumors were not graded. The 21 recurrences and six other tumors were not staged. One patient had two tumors with different grades and stages.

[c] Ploidy data were not available on 38 tumors. Diploid tumors had a DNA content of 1, and aneuploid tumors had a DNA content higher or lower than 1.

[d] Treatment history was not available in two patients. Chemotherapy consisted of cisplatin, 5-fluorouracil, and/or alpha-interferon.

[e] The neck node biopsies were from unknown primary sites.

[f] Recurrence data were not available in one patient.

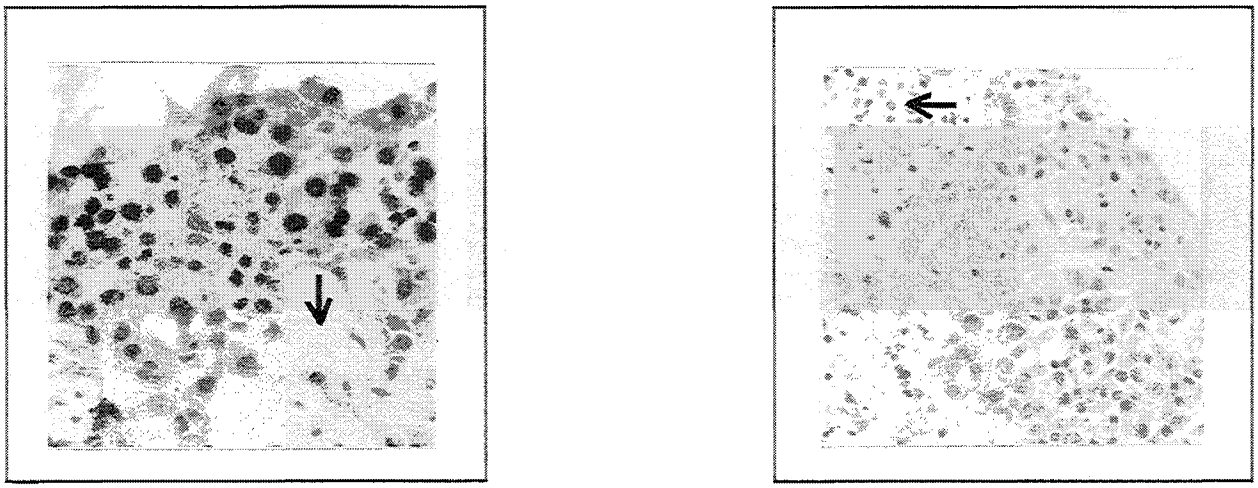

Fig. 1. Micrographs of 14-day histocultures of a primary squamous cell carcinoma (left; autoradiograph) and a nodal metastatic tumor (right; not an autoradiograph) from the same patient. The black grains on the nuclei in the primary tumors are due to the [^{3}H]thymidine labeling. Note the multicellular structure of the histocultures, the presence of stromal cells in both tumors, the presence of lymphocytes (smaller cells indicated by arrow) in the nodal tumor, and the necrotic region in the primary tumor (indicated by arrow). Hematoxylin and eosin counterstain.

degree of differentiation. Frequently the squamous carcinoma cells proliferated into the collagen supporting matrix (not shown). These cells had less organization and were more anaplastic than the cells in the tumor-associated connective tissue.

Culture Success Rate and Labeling Characteristics. Seventeen of the 83 tumor samples (20%) were contaminated and were excluded from the analysis. Washing of surgical specimens with additional gentamicin eliminated the contamination problem in the later samples. Of the remaining 66 samples, 14 did not show sufficient viable cells to be quantified or did not contain tumor cells. The remaining 52 samples (80% of 66) had >50% labeled nuclei. The mean LI was about 80% with a mean culture time of 12 days, which was the duration needed for drug evaluation studies. Some cultures were maintained for >6 months. While microscopic evaluation of the latter cultures showed maintenance of viable tumor cells, the fraction of viable and [^{3}H]thymidine-labeled cells in these cultures was significantly lower than in tumors cultured for less than 14 days.

Table II summarizes the LI. There was no difference in the LI of primary and metastatic tumors. The LI were 76.7 ± 18.1% for stage 2 tumors (n = 5), 75.8 ± 14.4% for stage 3 tumors (n = 9), 75.4 ± 12.5% for stage 4 tumors (n = 15), 78.1 ± 11.2% for grade 2 tumors (n = 27), 74.4 ± 15.3% for grade 3 tumors (n = 12), 80.8 ± 11.9% for tumors with diploid DNA content (n = 11), 79.2 ± 10.3% for aneuploid tumors (n = 12), 78.6 ± 11.1% for tumors from patients previously treated with chemotherapy and/or radiotherapy (n = 20), 77.5 ± 13.9% for previously untreated tumors (n = 24), and 74.4 ± 11.3% for tumors from patients with uncertain treatment history (n = 6). The difference of the LI at 10 and 21 days was not significant. To avoid changes in tumor proliferation, all chemosensitivity experiments were completed within 14 days of culture.

Pharmacodynamic Evaluation. The pharmacodynamics of FU, DDP, and MMC were studied in a total of 47 primary and nodal metastatic tumors. Some of these specimens were of large enough size to evaluate two drugs as single agents. Five primary and four metastatic tumors showed a <30% inhibition by the drugs at the highest concentration used. Among these nine tumors, a pair of primary and metastatic tumors which did not respond to DDP responded to FU. The other 38 tumors responded to the tested drugs. The drug-induced inhibition of tumor cell proliferation was dependent on drug concentration.

Initial MMC pharmacodynamic studies used a 3-hr exposure time. Thirteen tumors (8 primary and 5 metastatic

Table II. Effect of Culture Length and LI[a]

	LI (%)								
	Overall[b]			10 days			21 days		
	N	Range	Mean ± SD	*N*	Range	Mean ± SD	*N*	Range	Mean ± SD
Primary	31	52–95	77 ± 12	20	52–95	76 ± 13	12	55–92	74 ± 13
Metastatic	21	52–99	78 ± 12	7	67–99	83 ± 10	5	53–95	73 ± 16
Total	52	52–99	78 ± 12	27	52–99	78 ± 13	17	53–95	75 ± 14

[a] Surgical tumor specimens from individual patients were cultured. The LI of primary and nodal metastatic tumors were determined. The LI of some tumors were determined several times, between day 7 and day 14 and on day 21.

[b] The LI after a culture period of 7 to 14 days.

tumors) were studied. Six tumors either were contaminated or did not contain sufficient labeled cells. Of the remaining seven tumors, only one showed a concentration-dependent response to MMC, with a maximum inhibition of 87% at a concentration of 3 μg/mL and a 50% inhibition at .42 μg/mL. The other six tumors gave a maximum response of 16–37% at the 3 μg/mL concentration. Later studies used a longer exposure time of 24 hr.

Figure 2 shows the representative concentration–effect relationships for FU, DDP, and MMC (24-hr exposure) in three different tumors. The curves were sigmoidal in shape and had a maximal effect approaching 100% inhibition. Table III summarizes the IC_{50} and IC_{90} values of the three drugs in 38 tumors with a total of 46 treatments. Sixteen tumors were used to evaluate simultaneously the pharmacodynamics of two drugs, i.e., FU and DDP in six primary and four metastatic tumors and FU and MMC in six primary tumors. The IC values of FU were lower than those of DDP in all 10 tumors, whereas the IC values of MMC were lower or about equal to those of FU in 5 of 6 tumors and higher in the remaining tumor. A comparison of the mean of IC_{50} values showed that on a μg/mL basis, MMC was more effective than FU while DDP was 5- to 20-fold less effective than FU and MMC. These pharmacodynamic data indicate that the head and neck tumors responded to all three drugs and that there was considerable intertumor variation in the chemosensitivity. Interestingly, the variation in IC_{50} was much higher for FU (60-fold) than for DDP and MMC (7- to 8-fold). Furthermore, when corrected for the total drug exposure, i.e., the product of concentration and exposure time ($C \times T$), the MMC effect was proportionally greater at longer exposure time, e.g., a $C \times T$ of 5.76 μg-hr/mL at a 24-hr exposure time produced a 50% inhibition, whereas a higher $C \times T$ of 9 μg-hr/mL at a 3-hr exposure time produced only a 30% inhibition.

Table III also compares the drug activity in tumors from previously untreated patients and patients previously treated with chemotherapy (FU, DDP, and/or α-interferon) and/or radiotherapy. The mean IC values for FU and DDP in the untreated tumors were lower than in treated tumors, but the differences were not statistically significant. Interestingly, the IC values of MMC were significantly lower in the previously treated tumors.

Comparison of Chemosensitivity of Primary and Nodal Metastatic Tumors. Histocultures of primary tumors and nodal metastases were compared for their sensitivity to FU, DDP, and MMC (Table IV). The intertumor variation for FU in both primary and nodal metastatic tumors was considerably higher than for DDP and MMC. Because of the large intertumor variation, the mean values for the primary and metastatic tumors were not significantly different. Four pairs of primary and nodal tumors from four patients were evaluated for FU activity. Three of four pairs showed a three- to five-fold higher IC_{50} for nodal tumors than primary tumors, while the remaining pair showed similar IC_{50}. While statistical analysis by Student's paired t test did not show a significant difference ($P = 0.10$), these data suggest a lower sensitivity of the metastatic tumors to FU.

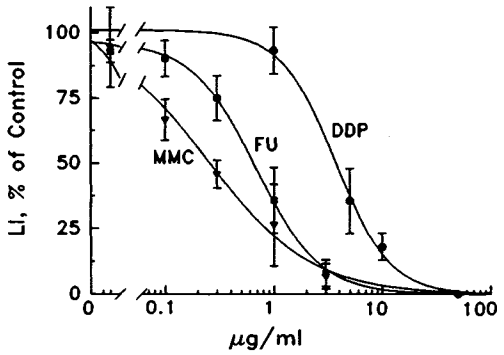

Fig. 2. Representative concentration–effect curves of FU (120-hr exposure; squares), DDP (10-hr exposure; circles), and MMC (24-hr exposure; triangles) in three head and neck tumors. Symbols represent mean ± SD of 12–18 replicates for each experiment. The lines are the computer-fitted lines using Eq. (1).

DISCUSSION

When comparing the IC values of MMC in human bladder tumor histocultures with the literature values determined with monolayer and spheroid cultures of established human bladder tumor cell lines, the drug activity in tumor histocultures was found to be 10 to 5300-fold lower than that in monolayers and 7- to 20-fold lower than that in spheroids (10). The large difference in chemosensitivity between the multicellular tumor histocultures and the monolayers may be due to different drug penetration and the intratumor heterogeneity in the human tumors, and it highlights the need of using a clinically relevant model for drug evaluation.

The advantages of the histoculture system have been discussed elsewhere (5–10). These include the use of patient materials and the maintenance of intra- and intertumor heterogeneities. As discussed previously, the histocultures consist of a mixture of oxygenated subpopulations in the periphery and hypoxic subpopulations in the center, similar to that seen in human solid tumors. Because the response to radiation depends on the oxygenation status (17), the histoculture system represents a good experimental model to evaluate radiotherapy, which is a major treatment modality for head and neck cancer.

Results of the present study demonstrate a high success rate of establishing histocultures of head and neck tumors (80% of uncontaminated samples). DNA synthesis was maintained in the histocultures. The LI of viable neoplastic cells in primary and nodal metastatic tumors was similar at about 80%. There were no correlations between the thymidine LI and the tumor pathology. It is conceivable that the 96-hr exposure time used in the thymidine labeling may be relatively long compared to the cell cycle time, resulting in an accumulative LI rather than an instantaneous LI that may be more accurate in representing the proliferative activity. Other proliferation indices, such as the proliferating cell nuclear antigen, are being evaluated in our laboratory.

The three drugs which have demonstrated clinical activity against head and neck cancer, i.e., FU, DDP, and MMC, inhibited the LI of the histocultures. The IC values showed considerable intertumor variation, with the greatest variation for FU. The cause of these variations was not apparent. Data in Table III suggest a trend of higher IC values,

Table III. Pharmacodynamics of Drug-Induced Inhibition of Tumor Cell Proliferation[a]

	FU (μg/mL)		DDP (μg/mL)		MMC (μg/mL)	
	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
Untreated						
Mean ± SD	0.47 ± 0.39	2.96 ± 4.02	2.71 ± 0.71	7.56 ± 1.88	0.29 ± 0.12	3.13 ± 1.50
Range	0.05–1.39	0.06–1.31	1.58–3.45	5.10–9.93	0.16–0.48	0.53–5.35
N	10	10	5	5	7	7
Treated						
Mean ± SD	0.77 ± 0.84	5.13 ± 7.22	4.30 ± 2.75	17.3 ± 19.7	0.15 ± 0.11	0.40 ± 0.17
Range	0.10–3.14	0.16–26.1	1.01–8.36	2.31–55.9	0.07–0.37	0.12–0.59
N	13	13	6	6	6	6
P	NS	NS	NS	NS	0.05	0.002
Overall[b]						
Mean ± SD	0.75 ± 0.82	4.23 ± 5.83	3.49 ± 2.08	12.4 ± 14.3	0.24 ± 0.13	1.83 ± 1.71
Range	0.05–3.14	0.06–26.1	1.01–8.36	2.31–55.9	0.07–0.48	0.12–5.35
N	25	25	12	12	14	14

[a] Surgical specimens were obtained from previously untreated patients or patients treated with radiotherapy and/or chemotherapy (FU, DDP, and/or interferon). The histocultures were exposed to FU, DDP, and MMC for 120, 10, and 24 hr, respectively. The concentration–effect curves were computer-fitted to Eq. (1) to obtain IC for 50 and 90% inhibition of tumor LI.

[b] Includes tumors from patients with uncertain treatment history (two for FU, one for DDP, and one for MMC).

i.e., a lower sensitivity, in tumors from previously treated patients to FU and DDP, compared to the untreated patients. This is in agreement with the general belief that recurrent tumors are more chemoresistant. Interestingly, the previously treated tumors were significantly more sensitive than the untreated tumors to MMC. This suggests MMC as a treatment choice for recurrent tumors. Further studies on a larger sample size are needed to confirm these data.

The present study showed that the histoculture system could be used to compare the chemosensitivity of primary and metastatic tumors. The data suggest that there was no difference in the response of the primary and metastatic tumors to DDP and MMC, but there was a trend of a lower chemosensitivity of the metastatic tumor to FU. Ensley and co-workers evaluated the patient response in relation to the DNA content in tumors and found that in patients who received chemotherapy as initial therapy, complete response was obtained in 80% with aneuploid primary tumors and 2% with diploid primary tumor (18,19). They also reported a significant increase in the frequency of DNA diploid tumors in the nodal metastases, as compared to the primary tumors (20). It was proposed that the poorer prognosis and poorer response of nodal metastases may be due in part to the lower sensitivity of diploid tumors to chemotherapy (20). The relationship of DNA ploidy and drug sensitivity was not established in the present study, due in part to the relatively small number of tumors studied. Further studies are needed to compare the chemosensitivity of primary and metastatic tumors and to identify agents and approaches to treat effectively the metastatic tumors as well as the primary tumors.

The two important parameters for pharmacodynamic evaluation are drug concentration and exposure time. Compared to the peak or steady-state plasma concentrations after intravenous bolus injection or continuous infusion of the clinically used doses for FU (steady-state concentration of 0.5 μg/mL), DDP (peak concentration of 3 μg/mL), and MMC (peak concentration of 0.5 μg/mL) (12–15), the IC_{50} of these drugs found in the present study are within the clinically achievable concentration range. The exposure times were selected based on the clinical pharmacokinetic data and the administration rate of these agents. Whether the exposure time is critical to the drug effect depends on many fac-

Table IV. Comparison of Chemosensitivity of Primary and Metastatic Tumors[a]

	IC_{50} (μg/mL)		
	FU	DDP	MMC
Primary tumors			
Mean ± SD	0.68 ± 0.74 (*N* = 18)	3.77 ± 2.42 (*N* = 7)	0.25 ± 0.13 (*N* = 9)
Median	0.47	3.05	0.26
Range	0.05–3.08	1.58–8.36	0.07–0.41
Nodal tumors			
Mean ± SD	0.96 ± 1.04 (*N* = 7)	3.12 ± 1.68 (*N* = 5)	0.21 ± 0.16 (*N* = 5)
Median	0.53	2.91	0.16
Range	0.12–3.14	1.01–5.64	0.10–0.48

[a] Head and neck tumor histocultures were exposed to FU, DDP, and MMC for 120, 10, 24 hr, respectively. The concentration–effect curves were computer-fitted to Eq. (1) to obtain the IC_{50}.

tors, including the cytotoxic mechanism, tumor sensitivity to drug, drug accumulation in tissue, and rate of drug penetration and activation. We have shown that an increase in exposure time decreases the IC of MMC in human bladder tumors (10). The present study showed that for MMC in head and neck cancer, the exposure time was a critical factor. A 3-hr exposure produced only minimal activity, while a 24-hr exposure produced a disproportionally greater activity. Further studies are needed to evaluate the relative importance of concentration and exposure time. These pharmacodynamic data are important to identify the most effective regimen. For example, demonstration of a critical role of a long exposure time on the activity of MMC or other agents in head and neck cancer will support the use of a continuous infusion over a bolus injection.

In summary, data from the present study indicate that the head and neck tumor histocultures responded to the clinically active agents at clinically achievable concentration and validate the histoculture system for drug activity evaluation. It is noted that the predictive value of an *in vitro* chemosensitivity assay is validated only by prospective clinical trials. Important aspects of treatment that can be addressed by the histoculture system include (a) evaluation of treatments against primary and metastatic tumors, (b) determination of radiation dose and drug concentration and exposure time necessary to produce a desired effect, and (c) evaluation of combinations for additive or synergistic effects.

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REFERENCES

1. R. H. Wheeler. Chemotherapy of patients with recurrent head and neck cancer. *In* C. Jacobs (ed.); Carcinoma of the Head and Neck: Evaluation and Management, Kluwer, Boston, 1990, pp. 171–193.
2. A. A. Forastiere, B. Metch, D. E. Schuller, J. F. Ensley, L. F. Hutchins, P. Triozzi, J. A. Kish, S. McClure, E. VonFeldt, S. K. Williamson, and D. D. Von Hoff. Randomized comparison of cisplatin plus fluorouracil and carboplatin plus fluorouracil versus methotrexate in advanced squamous cell carcinoma of the head and neck: A Southwest Oncology Group study. *J. Clin. Oncol.* 10:1245–1251 (1992).
3. M. Rotman and H. Aziz. Concomitant continuous infusion chemotherapy and radiation. *Cancer* 65:823–835 (1990).
4. J. B. Weissberg, H. S. Yung, R. J. Papac, C. Sasaki, D. B. Fischer, R. Lawrence, S. Rockwell, A. C. Sartorelli, and J. J. Fischer. Randomized clinical trial of mitomycin C as an adjunct to radiotherapy in head and neck cancer. *Int. J. Radiat. Oncol. Biol. Phys.* 17:3–9 (1989).
5. A. E. Freeman and R. M. Hoffman. *In vivo*-like growth of human tumors *in vitro*. *Proc. Natl. Acad. Sci. USA* 83:2694–2698 (1986).
6. R. A. Vesico, C. H. Redfern, T. J. Nelson, S. Ugoretz, P. H. Stern, and R. M. Hoffman. *In vivo*-like drug responses of human tumors growing in three-dimensional gel-supported primary culture. *Proc. Natl. Acad. Sci. USA* 84:5029–5033 (1987).
7. R. M. Hoffman, K. M. Connors, A. Z. Meerson-Monosov, H. Herrara, and J. H. Price. A general native state method for determination of proliferation capacity of human normal and tumor tissues in vitro. *Proc. Natl. Acad. Sci.* 86:2013–2017 (1989).
8. K. T. Robbins, N. M. Varki, A. M. Storniolo, H. Hoffman, and R. M. Hoffman. Drug response of head and neck tumors in native-state histoculture. *Arch. Otolaryngol. Head Neck Surg.* 117:83–86 (1991).
9. T. D. Schmittgen, J. L.-S. Au, M. G. Wientjes, R. A. Badalament, and J. R. Drago. Cultured human bladder tumors for pharmacodynamic studies. *J. Urol.* 145:203–207 (1991).
10. T. S. Schmittgen, M. G. Wientjes, R. A. Badalament, and J. L.-S. Au. Pharmacodynamics of mitomycin C in cultured human bladder tumors. *Cancer Res* 51:3849–3856 (1991).
11. O. H. Beahrs, D. E. Henson, R. V. P. Hutter, and M. H. Myers (eds.). *American Joint Committee on Cancer Manual for Staging of Cancer,* 3rd ed. J.B. Lippincott, Philadelphia, 1988, pp. 27–62.
12. D. S. Alberts and H.-S. G. Chen. Tabular summary of pharmacokinetics parameters relevant to in vitro drug assay. *In Cloning of Human Tumor Cells;* Alan R. Liss, New York, 1980, pp. 351–359.
13. J. L. Grem. Fluorinated pyrimidines. In B. A. Chabner and J. M. Collins (eds.), *Cancer Chemotherapy: Principles and Practice,* J.B. Lippincott, Philadelphia, 1990, pp. 130–224.
14. L. A. Zwelling and J. W. Kohn. Platinum complexes. In B. Chabner (ed.), *Pharmacologic Principles of Cancer Treatment,* W.B. Saunders, Philadelphia, 1982, pp. 309–339.
15. R. Goel, P. A. Andrews, C. E. Pfelfle, I. S. Abramson, S. Kirmani, and S. B. Howell. Comparison of the pharmacokinetics of ultrafilterable cisplatin species detectable by derivatization with diethyldithiocarbamate of atomic absorption spectroscopy. *Eur. J. Cancer* 26:21–27 (1990).
16. J. Verweij, J. D. den Hartigh, and H. M. Pinedo. Antitumor antibiotics. In B. A. Chabner and J. M. Collins (eds.), *Cancer Chemotherapy: Principles and Practice,* J.B. Lippincott, Philadelphia, 1990, pp. 382–396.
17. C. N. Coleman, D. J. Glover, and A. T. Turrisi. Radiation and chemotherapy sensitizers and protectors. In B. Chabner and J. M. Collins (eds.), *Cancer Chemotherapy: Principles and Practice,* J.B. Lippincott, Philadelphia, 1990, pp. 424–448.
18. J. Ensley, Z. Maciorowski, M. Hassan, H. Pietraszkiewiez, L. Heilbrun, J. A. Kish, E. Tapazoglou, J. R. Jacobs, and M. Al-Sarraf. *Cytometry* 10:334–338 (1989).
19. J. Ensley, Z. Maciorowski, H. Pietraszkiewicz, *et al.* Prognostic impact of cellular DNA parameters in neoadjuvant and adjuvant trials of patients with head and neck cancer. In S. Salmon (ed.), *Adjuvant Therapy of Cancer,* Grune & Stratton, New York, 1990, pp. 101–108.
20. D. L. Burgio, J. R. Jacobs, Z. Maciorowski, M. D. M. Alonso, H. Pietraszkiewicz, and J. F. Ensley. DNA ploidy of primary and metastatic squamous cell head and neck cancers. *Arch. Otolaryngol. Head Neck Surg.* 118:185–187 (1992).