Histocultures of Patient Head and Neck Tumors for Pharmacodynamics Studies

Jessie L.-S. Au,1,2,5,6 M. Guillaume Wientjes,1,2,5 Thomas J. Rosol,3 Antoinette Koolemans-Beynen,1,5 Eric A. Goebel,4 and David E. Schuller4,5

Received February 12, 1993; accepted April 5, 1993

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 [3H]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 (IC50) were within the clinically achievable concentration range. The intertumor variation in the IC50 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 IC50 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.

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; IC50 and IC90, drug concentrations needed to inhibit the tumor LI by 50 and 90%, respectively; AUC, cumulative product of concentration and exposure time.
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-3H]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 post surgery. The culture medium consisted of Eagle’s minimal essential medium supplemented with 10% heat-activated fetal bovine serum, 0.1 mM 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-mm³ fragments. The fragments were mixed to ascertain randomization. Four to six tumor fragments were placed on a 1-cm² piece of collagen gel and cultured in six-well plates in a humidified atmosphere of 95% air, 5% CO₂ at 37°C. The labeling index (LI) of the tumors was determined by [³H]thymidine labeling and autoradiography. Cultures were incubated with medium containing [³H]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 Axiowert 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