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Synergistic interaction between cisplatin and tamoxifen delays the emergence of cisplatin resistance in head and neck cancer cell lines

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Abstract The interaction between cisplatin (cDDP) and tamoxifen (TAM) was evaluated in the human head and neck squamous-carcinoma cell lines UM-SCC-10B and UM-SCC-5. Synergy between cDDP and TAM was demonstrated in the UM-SCC-10B cell line. Concordant with the synergistic effect between cDDP and TAM, the rate of development of resistance to cDDP was delayed when selections were performed in the presence of TAM. However, in the UM-SCC-5 cell line, TAM was neither synergistic nor did it delay the development of cDDP resistance. The difference with respect to the synergistic interaction of cDDP with TAM and the effect on the development of cDDP resistance in the UM-SCC-10B and UM-SCC-5 cell lines was not related to any significant difference in the accumulation of the cDDP analog [3H]-*cis*-dichloro(ethylenediamine)platinum(II) (DEP), drug sensitivity [concentrations inhibiting colony formation by 50% (IC_{50} values) were 6.5 and 7.2 μ*M* for cDDP and 3.5 and 3.2 μ*M* for TAM, respectively], the number of estrogen and progesterone receptors (negative in both cell lines), the number of antiestrogen binding sites (404 ± 85 and 353 ± 24 fmol/mg protein, respectively), or the affinity of TAM for these binding sites (1.7 and 1.5 n*M*, respectively). Importantly, however, we demonstrated that TAM can delay the emergence of resistance to cDDP in head and neck carcinomas and that this effect is linked to the nature of the interaction between cDDP and TAM.

Key words Cisplatin · Tamoxifen · Synergism
Antiestrogen binding sites · Head and neck cancer

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Introduction

There has been considerable interest in understanding the nature of the interaction of antiestrogens and cisplatin (cDDP). Recent clinical studies have indicated that the addition of tamoxifen (TAM) or toremifene to cDDP-containing regimens can increase clinical response rates [29–31], and in vitro studies have indicated a synergistic interaction between the cytotoxic effects of TAM and cDDP [31, 34]. TAM is a nonsteroidal antiestrogen that has been successfully used to treat breast cancer. Most of the work concerning its mechanism of action supports the view that TAM produces its biological effects by competitively inhibiting the binding of estradiol to intracellular estrogen receptors (ER) [10, 13, 25, 41]. However, there is a growing body of laboratory evidence demonstrating that TAM can affect non-ER-mediated events as well [11, 41, 47, 48] and that these events are involved in the antitumor activity of TAM. Indeed, some clinical trials have reported responses in ER-negative patients [5, 38], raising the possibility that non-ER-related antitumor mechanisms might be therapeutically important.

Recent studies have identified a class of antiestrogen binding sites (AEBS) that may mediate and/or modulate the actions of antiestrogens [4, 14, 45–47]. AEBS specifically bind anti- estrogens, but not estrogens, and have been characterized as physically distinct from ER [9, 15, 23, 44, 45]. The development of resistance to TAM is associated with changes in AEBS [30, 39] and with an alteration in the ability of TAM and cDDP to interact synergistically. Selection for TAM resistance in the human melanoma cell line T289 resulted in a decrease in the number of AEBS but produced no change in their affinity for TAM [32]. There was a highly synergistic cytotoxic interaction between TAM and cDDP in the parental T289 cells that could not be accounted for by any effect of TAM on the biochemical pharmacology of cDDP [31, 34]. However, the synergy was completely absent in the TAM-resistant subline [32]. Further evaluation of this interaction showed that in addition to being capable of interacting synergistically, TAM

delayed the development of resistance during in vitro selection with cDDP [33]. Whether this effect is also mediated via AEBS is not yet known.

cDDP is one of the drugs successfully used in head and neck carcinoma [19]. The current treatment of this malignancy utilizes three major modalities of therapy: chemotherapy, radiation therapy (XRT), and surgery. Further improvement in the treatment of this disease is likely to lie with the use of chemotherapy either before or after XRT and/or surgery. However, the development of resistance to cDDP is a common problem and constitutes a major obstacle to the cure of even those head and neck carcinomas that are initially sensitive to cDDP [27]. It is known that in vitro and in vivo resistance to cDDP develops rapidly, and clinically significant degrees of resistance are present after as few as three exposures to the drug [1].

The ability of TAM to interact synergistically with cDDP and, thus, potentially to overcome cDDP resistance holds some promise for future clinical application [31, 34]. First, however, it must be determined whether this interaction is specific to a particular cell line or tumor type. Herein we report on studies on the nature of the cytotoxic interaction between cDDP and TAM and on the ability of TAM to suppress the development of cDDP resistance in two human head and neck carcinoma cell lines.

Materials and methods

Cell lines and clonogenic assay

The UM-SCC-10B and UM-SCC-5 cell lines were derived from human squamous-cell carcinomas of the head and neck region [17, 24]. Cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2 in 150-cm^2 flasks (Corning, Corning, N.Y.) with RPMI 1640 medium (Mediatech Inc., Herndon, Va.) containing 10% fetal bovine serum (Gemini Bioproducts Inc., Calabasas, Calif.), 2 m*M* L-glutamine, 100 units of penicillin/ml, and 100 µg of streptomycin sulfate/ml. The sensitivity of the UM-SCC-10B and UM-SCC-5 cell lines and their cDDP-resistant variants to cDDP, TAM, and the combination of both agents was tested by colony-forming assays (CFA). Single-cell suspensions were plated into 60-mm tissue-culture dishes (Corning) at 300 cells/dish. Following drug exposure, the cultures were washed twice with phosphate-buffered saline (PBS) to remove cDDP, 5 ml of fresh medium was added, and the cultures were incubated for 13–15 days to allow formation of colonies. Colonies were fixed with methanol, stained with Giemsa stain for 60 min, and counted manually and compared with the control.

Drugs

cDDP was obtained from Bristol Myers Squibb (Syracuse, N.Y.). TAM was obtained from Zeneca (Macclesfied, England).

cDDP accumulation

The uptake of cDDP was investigated using the cDDP analogue [^{3}H]-*cis*-dichloro(ethylenediamine)platinum(II) ([^{3}H]-DEP) as described by Kimura et al. [22]. UM-SCC-10B and UM-SCC-5 cells were seeded into 60-mm tissue-culture dishes, allowed to attach, and incubated with TAM (1 µ*M*) 24 h before cells were treated with 5 µ*M* [^{3}H]-DEP (5 µCi/ml). After DEP exposure the medium was aspirated and the cells were washed rapidly four times with PBS at 4 °C. Cells were digested in 1 *N* NaOH overnight and radioactivity was determined by liquid scintillation counting. An aliquot was removed for the determination of protein content [22].

Median-effect analysis

Median-effect analysis was used to assess the interaction between cDDP and TAM using a schedule of 1-h exposure to cDDP and continuous exposure to TAM [7]. After seeding of the cells into 60-mm tissue-culture dishes at 300 cells/dish in fresh medium followed by a 5-h interval to allow them to attach, TAM was added. For the combined treatment, cDDP was added to the dishes 24 h after seeding of the cells. Drugs were combined in a fixed ratio equivalent to the ratio of the drug concentration inhibiting colony formation by 50% (IC_{50}) values for each drug as determined by CFA. After incubation, the dishes were washed twice with phosphate-buffered saline (PBS) and fresh medium containing the appropriate concentration of TAM was added. In synergy experiments, dose-response curves for the single agents were initially generated. The extent of the combined treatment was then analyzed by the isobole method [6] for a combination of cDDP and TAM from the equation

$$cDDP_c/cDDP_i + TAM_c/TAM_i = CI,$$

where $cDDP_c$ and TAM_c correspond to the doses of drugs used in the combination treatment and $cDDP_i$ and TAM_i correspond to the concentrations of drugs capable of giving the same magnitude of effect if used individually. If CI (combination index) <1, the effect of the combination was synergistic, whereas if CI = 1 or CI >1, the effect was additive or antagonistic, respectively.

Selection of cDDP-resistant variants

cDDP-resistant cells were selected by chronic exposure to cDDP in the absence or presence of TAM. The cDDP levels during the first three selections for the UM-SCC-10B and UM-SCC-5 cell lines were 0.25 and 0.5 µ*M*, respectively, which allowed a quadrupling of the cell number in 1 week (the doubling time of both cell lines was approximately 48 h). For each subsequent set of three selections the cDDP dose was increased by approximately 20%. When selections were done in the presence of TAM, the concentration of TAM was always 4-fold that of cDDP. Cells were cultured in drug-free medium for 2 weeks prior to being tested for drug sensitivity.

Estrogen- and progesterone receptor assays

The estrogen-receptor (ER) and progesterone-receptor (PR) content of UM-SCC-10B and UM-SCC-5 cells were determined by the quantitative dextran-coated charcoal method [36].

Assay for AEBS

Quantitation of AEBS content was based on the method of Miller and Katzenellenbogen [37]. UM-SCC-10B and UM-SCC-5 cells were cultured in 225-cm^2 flasks and allowed to grow to confluence. For each assay, three flasks were used and cells were harvested by scraping. The cells were centrifuged for 10 min at 800 *g*, resuspended in 4 ml of 10 m*M* TRIS-Cl (pH 7.4) containing 1.5 m*M* ethylenediaminetetraacetic acid (EDTA, pH 8; TE buffer), and homogenized with a Dounce homogenizer (100 strokes with pestle A). The cell homogenates were centrifuged for 30 min at 12,000 *g* and the supernatant was collected and diluted to a total volume of 6 ml with TE buffer. The supernatants were mixed well and 250 µl was aliquoted into 20 tubes. To each tube was added 5 µl of 150 n*M* [^{3}H]-TAM (84 Ci/mmol; Amersham, Amersham, UK) in dimethylsulfoxide and 5 µl of ethanol or 100 µ*M* estradiol (Sigma) in 5 µl of ethanol. After an incubation period of 30 min at 4 °C, 235 µl of TE buffer was added together with

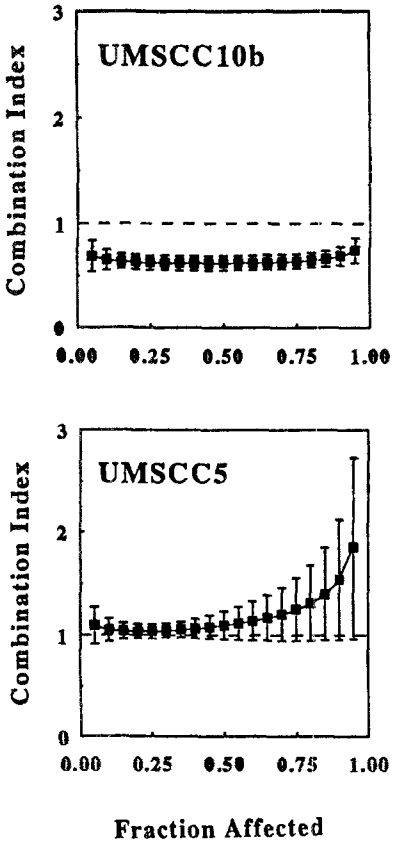

Fig. 1 Combination index plots for the interaction between cDDP and TAM for UM-SCC-10B (*top*) and UM-SCC-5 cells (*bottom*). Each point represents the mean value ± SD for three independent experiments performed in triplicate

the appropriate concentration of TAM (1 n*M*–500 μ*M*). The tubes were incubated for 18 h at 4 °C. To remove nonbinding [^{3}H]-TAM, 88 μl of dextran-coated charcoal was added, followed by an incubation period of 10 min at 4 °C and centrifugation for 10 min at 12,000 *g*. The radioactivity of 400 μl of the supernatant mixed in 10 ml of EcoLite scintillation fluid (ICN, Costa Mesa, Calif.) was counted by liquid scintillation. Cell homogenates were assayed for protein content by the method of Bradford [3]. The data were subjected to Scatchard analysis.

Statistical analysis

All data are reported as mean values ± SD. The significance of differences was assessed by Student's *t*-test, and the test results were regarded as significant if $P < 0.05$.

Results

Median-effect analysis

Median-effect analysis is a statistical technique that allows formal evaluation of the nature of the interaction between two cytotoxic drugs [7]. It permits calculation of the combination index (CI), which is a measure of the extent of interaction. A CI value of < 1 indicates synergy, whereas a value of 1 indicates additivity, and values of > 1 indicate antagonism. The value of the CI at 50% cell kill is abbreviated as the CI_{50}. Median-effect analysis was used to examine the nature of the interaction between cDDP and TAM in the head and neck carcinoma cell lines UM-SCC-10B and UM-SCC-5 using a schedule of drug exposure that mimicked the clinical conditions [34]. The IC_{50} values recorded for continuous TAM exposure for the parental UM-SCC-10B and UM-SCC-5 cells were 3.56 ± 0.41 and 3.22 ± 0.54 μ*M*, respectively. The IC_{50} values noted for a 1-h exposure to cDDP were 6.5 ± 0.6 and 7.2 ± 1.5 μ*M*, respectively. The median-effect analysis was done using a molar cDDP:TAM ratio of 1.8 and 2.2, the IC_{50} ratios for cDDP and TAM in the UM-SCC-10B and UM-SCC-5 lines, respectively. When UM-SCC-10B cells were exposed for 1 h to cDDP and continuously to TAM, the CI plot indicated a high degree of synergy with a CI_{50} value of 0.62 ± 0.02 (Fig. 1). However, the two drugs were not synergistic against the UM-SCC-5 cell line; the CI_{50} was 1.10 ± 0.14, indicating only an additive or a weakly antagonistic effect between the two drugs (Fig. 1).

Effect of TAM on the development of cDDP resistance

The UM-SCC-10B and UM-SCC-5 cells were selected for resistance to cDDP by continuous exposure to cDDP in the presence or absence of TAM. The rate of development of resistance to cDDP was monitored by periodically determining the sensitivity of the cells to 1 h of cDDP exposure using a clonogenic assay. Repeated exposures to cDDP alone led to the development of detectable levels of resistance at as early as the third selection, and the level of resistance continued to increase with subsequent cDDP selections as shown in Fig. 2. The rate of development of cDDP was more rapid in the UM-SCC-10B cells than in the UM-SCC-5 cells. After the 15th selection, the IC_{50} values for the UM-SCC-10B and UM-SCC-5 cells were 40.1 ± 1.2 and 20.1 ± 1.2 μ*M*, respectively. The presence of TAM during the selection delayed the emergence of cDDP resistance significantly in UM-SCC-10B cells. The IC_{50} values in the cDDP+TAM-selected cells were significant lower than those in the cells selected in cDDP alone at each point during the selection process. In UM-SCC-5 cells, however, there was no effect of TAM on the rate of development of resistance to cDDP.

The rate of development of cDDP resistance was determined by linear regression analysis of the change in IC_{50} values over time, and the results are presented in Fig. 3. It is clear that the emergence of resistance in the UM-SCC-10B cells occurred more slowly in the presence of TAM. The slope of the regression line was 0.36 in the absence of TAM, and this was reduced by more than 40% to 0.21 in the presence of TAM. In contrast, the presence of TAM had no effect on the slope of the regression line for the emergence of resistance to cDDP in the UM-SCC-5 lines where the slopes were 0.14 and 0.13, respectively. These results indicate that in the cell line in which there was synergy between cDDP and TAM with respect to cytotoxicity, TAM delayed the emergence of resistance, whereas under circumstances in which there was no synergy between the

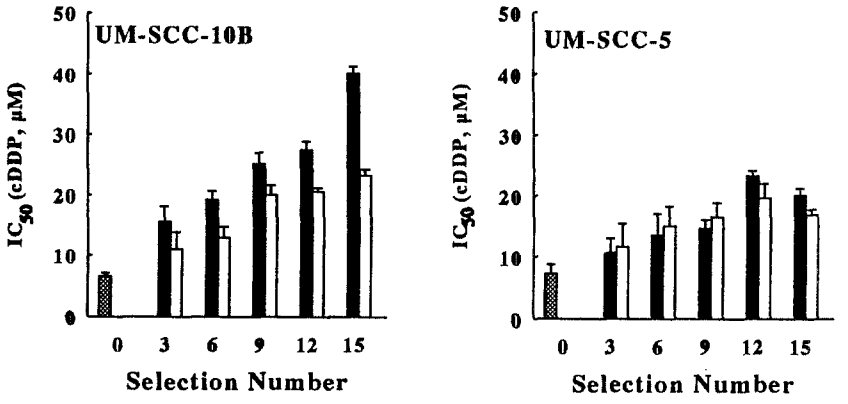

Fig. 2 IC_{50} values recorded for cDDP during selection for resistance in UM-SCC-10B (*left*) and UM-SCC-5 (*right*) cells in the absence (*solid bars*) or presence (*open bars*) of TAM. *Vertical bars* represent the SD of three to five independent experiments performed in triplicate

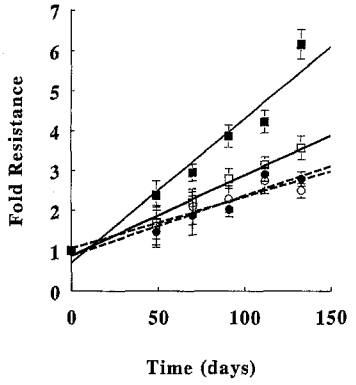

Fig. 3 Emergence of cDDP resistance as a function of time in the UM-SCC-10B (*solid lines with squares*) and UM-SCC-5 (*dotted lines with circles*) cell lines treated with cDDP alone (*solid symbols*) or cDDP plus TAM (*open symbols*). Each point represents the mean value ± SD for two independent cDDP selections performed in triplicate. The resistance in the selected lines is compared with that in the unselected cell line and expressed as magnitude of resistance

drugs, TAM had no effect on the rate of resistance development.

[3H]-DEP accumulation

Given the observation of clear synergy between cDDP and TAM against one cell line but not the other, we sought to identify differences between the lines that could account for this effect. Using the cDDP analogue [^{3}H]-DEP, we determined the cellular accumulation of [^{3}H]-DEP. No difference in accumulation was detected between the UM-SCC-10B and UM-SCC-5 cell lines. The [^{3}H]-DEP concentrations were 85.4 ± 3.7 and 81.6 ± 2.4 pmol/mg protein, respectively. In the presence of TAM a slight change in [^{3}H]-DEP accumulation occurred. The [^{3}H]-DEP concentration in the UM-SCC-10B cells decreased slightly in the presence of TAM from 85.4 ± 3.7 to 74.9 ± 1 pmol/mg protein, whereas in the UM-SCC-5 cells a slight increase could be detected from 81.6 ± 2.4 pmol [^{3}H]-DEP/mg protein for [^{3}H]-DEP alone to 97.2 ± 8.3 pmol [^{3}H]-DEP/mg protein for the combination of [^{3}H]-DEP and TAM. These small differences in [^{3}H]-DEP accumulation cannot account for the difference in synergy observed between cDDP and TAM in these two cell lines.

ER and PR assay

Since it has been reported that the ER can mediate the antitumor effect of TAM, we determined the ER and PR content of both cell lines. The dextran-coated charcoal method indicated an ER content of <3 fmol/mg and a PR content of <5 fmol/mg in both the UM-SCC-10B and the UM- SCC-5 cells. Such levels are characteristic of estrogen-nonresponsive tumors, suggesting that it was unlikely that TAM was acting via the ER or PR in these cell lines.

AEBS analysis

In addition to binding to ER, TAM binds to a partially characterized group of proteins referred to as antiestrogen binding sites, or AEBS [9, 15]. The role of these binding sites in mediating the action of TAM is unknown, but they do mediate the effects of several other antiestrogen compounds [21]. Both the number and the affinity of the AEBS in each cell line were determined using the MCF-7 breast-carcinoma cell line as a positive control. MCF-7 cells contained 404.8 ± 21.5 fmol AEBS/mg protein, which was similar to the values previously reported for these cells [37]. The numbers of AEBS detected in the microsomal fraction of the UM-SCC-10B and UM-SCC-5 cells were similar to those found in the MCF-7 cell line, i.e., 404.2 ± 85.4 and 352.5 ± 24.5 fmol AEBS/mg protein, respectively, and these levels were not significantly different from each other. The affinity of TAM for the AEBS was determined in each of the lines by measuring the equilibrium dissociation constant determined from Scatchard plots (Fig. 4). The values recorded for UM-SCC-10B and UM-SCC-5 cells were 1.9 ± 0.3 and 1.7 ± 0.5 n*M*, respectively. Thus, neither the numbers nor the affinity of AEBS were sufficiently different to account for the difference in the degree of synergy between cDDP and TAM observed in the two cell lines.

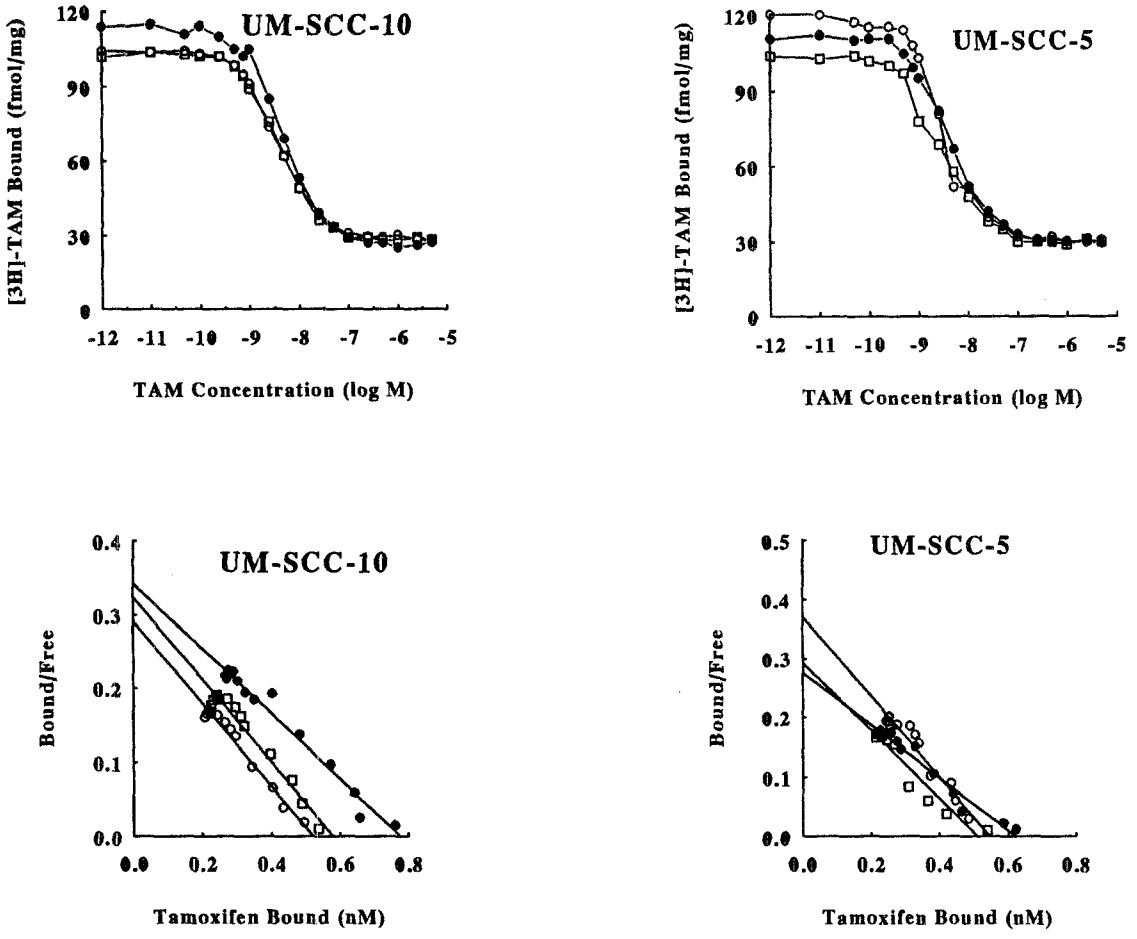

Fig. 4 Competitive binding assay for AEBS (3 independent experiments). A 12,000-*g* subcellular fraction, containing AEBS, of the UM-SCC-10B (*upper left*) and UM-SCC-5 cell lines (*upper right*) was incubated with [^{3}H]-TAM and increasing concentrations of unlabeled TAM. [^{3}H]-TAM values were normalized for the protein concentration of the cell homogenates. The K_d value as well as the number of sites were determined by Scatchard-plot analysis (*lower left* for UM-SCC-10B and *lower right* for UM-SCC-5)

Discussion

Development of resistance to cDDP and its analogues occurs commonly during treatment and is a major obstacle to the cure of head and neck cancers. Modulation of cellular sensitivity to the platinum-containing resistance, therefore, has become an important aspect of clinical strategies directed at improving cDDP-based chemotherapy. TAM, a nonsteroidal triphenylethylene compound, was recently shown to be synergistic with cDDP in at least three different cell lines of human origin, including a small-cell carcinoma of the lung, a melanoma, and an ovarian carcinoma cell line [31–34]. The observed synergy was not due to an effect of TAM on the known mechanisms of cDDP resistance, including drug accumulation, which was confirmed in this study and a clinical study in head and neck patients [28]; intracellular glutathione or metallothionein levels; DNA intrastrand adduct formation and repair. There is also no evidence that the effect of TAM is being mediated via binding to ER or inhibition of either protein kinase C or calmodulin [18, 34]. In addition to the cytotoxic synergy between cDDP and TAM, McClay et al. [33] also demonstrated that TAM could enhance the efficacy of cDDP treatment by delaying the development of cDDP resistance in an ovarian carcinoma and in melanoma cell lines.

In the present study we demonstrated synergy between cDDP and TAM in one of the two head and neck cancer cell lines tested (UM-SCC-10B) tested. Concordant with the synergistic effect observed between cDDP and TAM in the UM-SCC-10B cells, the rate of development of resistance to cDDP was delayed when selections were performed in the presence of TAM. However, in the UM-SCC-5 cell line TAM was neither synergistic nor did it delay the development of cDDP resistance. It is important to emphasize that the delay in resistance development could not be attributed simply to greater degrees of cell kill by the cDDP/TAM combination in the UM-SCC-10B cells, since cultures of both cell lines were allowed to grow to equal cell numbers following each round of selection.

In the case of the head and neck carcinoma cell lines, the differences observed with respect to synergistic interaction with TAM and the effect of TAM on resistance development were not related to any difference in sensitivity to the cytotoxic effect of either drug alone, indicating that the key

determinants of synergy were not ones that impacted on overall drug sensitivity. This appears to be different from what has been observed in the T289 melanoma cell line, where McClay et al. [34] have shown that whereas selection for resistance to cDDP did not alter the degree of synergy between cDDP and TAM, selection for resistance to TAM completely eliminated the synergistic interaction. It is noteworthy that in the case of the UM-SCC-10B cell line, synergy was present over a full 2 logs of tumor cell kill. This reflects a broader range of synergy than has been observed between cDDP and some other drugs. For example, methotrexate and edatrexate demonstrate synergy with cDDP only at drug levels producing >60% inhibition of cell growth [8]. In the case of the UM-SCC-5 cells, there was a suggestion that the combination became somewhat antagonistic with increasing cell kill; however, this effect must be interpreted with caution, since the error associated with this part of the curve increases as well.

Although the mechanism of action of TAM is not fully understood, there is evidence that TAM competes with estrogen at the ER [10, 13, 26, 41]. However, we could not identify a role for the ER in the synergistic interaction between cDDP and TAM. Both cell lines were ER- and PR-negative as determined by a standard dextran-charcoal assay [36]. In general it is accepted that the presence of ER in a tumor enhances the likelihood that it will respond to TAM [20]. However, TAM can also produce responses in a small fraction of patients with ER-negative tumors [5, 38], suggesting that TAM has at least one other mechanism of action. In addition, McClay et al. [34] demonstrated synergy between cDDP and TAM in a melanoma cell line that was ER-negative, further suggesting that a synergistic interaction between cDDP and TAM does not require the presence of ER. However, a possible role for type II ER is not excluded. Scambia et al. [42] found synergy between TAM and cDDP in four different ER-negative ovarian carcinomas expressing type II ER.

Synergy between TAM and doxorubicin has been reported in leukemia [40] and breast-cancer [12] cell lines by other investigators. It has been speculated that in addition to binding to the ER, TAM might interact with a lipid domain in the cell membrane due to its lipophilic tricyclic structure and that this might change doxorubicin uptake [40]. In the human ovarian cancer cell line BG-1, TAM was found to interact synergistically with both cDDP and doxorubicin, suggesting a possible similarity in mechanism of interaction between the cDDP/TAM and doxorubicin/TAM combinations [16].

Although antiestrogens clearly exert some of their effects through the ER [10, 13, 25, 41], they also bind to other sites within the cell, including AEBS, which are a group of receptors distinct from the ER [4, 14, 37, 43, 44, 46]. In the present study, the questions to be answered were whether the AEBS could mediate the interaction between cDDP and TAM and whether the difference in interaction between TAM and cDDP observed in the two head and neck cancer cell lines was due a difference in the number or affinity of AEBS. Whereas both cell lines turned out to have abundant AEBS as compared with the positive control MCF-7 cells, the number of such sites and their affinities were similar in the two head and neck carcinoma cell lines. Therefore, the observed difference in interaction between cDDP and TAM in the UM-SCC-10B and UM-SCC-5 cell lines cannot be explained by either the number of AEBS nor their affinity for TAM. These results do not formally exclude a role of AEBS in this drug interaction, but they do indicate that some other determinant is likely to account for the observed difference between these two head and neck carcinoma cell lines.

The ability of TAM to delay the emergence of resistance to cDDP, and to do so only in the cell line that also demonstrated a synergistic interaction between cDDP and TAM, was a particularly intriguing observation in this study. This suggests that the determinants of synergy are also determinants of the ability of TAM to suppress the emergence of resistance. McClay et al. [33] were the first investigators to demonstrate that TAM could delay the emergence of cDDP resistance in human melanoma and ovarian carcinoma cells. In a clinical study, the same authors demonstrated that in a subset of patients with metastatic melanoma, the addition of TAM to a cDDP regimen could overcome established cDDP resistance [35]. Although the mechanism of this interaction remains unknown, if it can be substantiated in in vivo models, it suggests that every patient being treated with cDDP should also be receiving TAM.

In conclusion, in the present study we demonstrated that in spite of the highly significant interaction between cDDP and TAM observed in a number of cell lines, synergy between cDDP and TAM was present in only one of the two head and neck cancer cell lines tested. Recent studies have suggested a role for AEBS in the synergistic interaction between TAM and cDDP in the T-289 melanoma cell line [32]. Our data, however, suggest that a role for AEBS is questionable for the head and neck carcinoma cell lines since both cell lines had the same number of AEBS with an equal and high affinity for TAM. Much more importantly, we confirmed in a cell line representative of yet another tumor type that TAM can delay the emergence of resistance to cDDP and that in the head and neck cell lines this effect is linked to the nature of the interaction between cDDP and TAM.

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