

Does Dexamethasone Inhibit the Antineoplastic Effect of Cisplatin and Docetaxel in Head and Neck Cancer Cells?

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Abstract. *Background/Aim:* Comedication with glucocorticoids such as dexamethasone is frequently given to head and neck cancer patients treated with chemotherapy. However, an increasing body of evidence suggests that dexamethasone may induce resistance to antineoplastic agents. The present study was the first to investigate the effect of dexamethasone on the antiproliferative activity of cisplatin and docetaxel in vitro in squamous cell carcinoma of the head and neck (SCCHN) cell lines. *Materials and Methods:* The cytotoxic effect of cisplatin and docetaxel on eight SCCHN cell lines was determined for each drug alone or with increasing concentrations of dexamethasone. Cell growth inhibition and viability were measured quantitatively after 24, 48, 72 hours of treatment using water-soluble-tetrazolium-test and lactate dehydrogenase assays. Absolute tumor cell numbers were determined by cell counting in a Rosenthal chamber. *Results:* Cisplatin and docetaxel alone inhibited the growth of all eight SCCHN cell lines significantly ($p=0.012$). The antiproliferative activity of both agents was not decreased by the addition of dexamethasone in any of the cell lines ($p>0.05$). *Conclusion:* Dexamethasone does not interfere with the cytotoxic action of cisplatin or docetaxel in the investigated SCCHN cell lines.

Cancer of the head and neck is among the most common neoplastic diseases worldwide (1-3). In Germany alone, 7,600 males and 2,800 females are newly diagnosed with the disease every year. Although treatment modalities and surgical

techniques have become more sophisticated in the last 50 years, the prognosis of this malignancy remains poor. The 5-year survival rates in Germany are approximately 50% (4).

Head and neck cancer most frequently affects the upper aerodigestive region including the oral cavity, pharynx, larynx and salivary glands. Most head and neck carcinomas are of squamous cell origin (SCCHN). The disease stage and performance status of the patients at the time of diagnosis are strong determinants of treatment outcome and survival (5, 6). The treatment armamentarium is limited for metastatic and/or recurrent SCCHN and more effective and well tolerated new therapies are urgently required (7).

For nearly 50 years, physicians have used glucocorticoids (steroid hormones normally secreted by the body in response to stress) to treat several types of neoplastic disease. Currently, glucocorticoids such as dexamethasone are widely used as adjunctive agents in the treatment of head and neck cancer, for example to improve appetite or decrease weight loss, fatigue or nausea and allergic reactions in patients with advanced disease. However, despite their widespread use in supportive care, no prospective clinical studies have systematically assessed the effect of glucocorticoids on cancer growth and treatment outcome. Preclinical and, to some extent, clinical studies have suggested that glucocorticoids may induce treatment resistance in some solid tumors (8, 9).

Only recently, Herr and Pfizenmaier (10) hypothesized that glucocorticoids such as dexamethasone may inhibit the antineoplastic effect of various cytotoxic agents. They advised physicians to pay greater attention to this potential problem in daily oncology practice to avoid any compromise for patient outcome.

The development of drug resistance in patients receiving chemotherapy represents a major problem in cancer treatment. In several treatment protocols containing cisplatin and/or docetaxel, dexamethasone is given as an antiemetic.

Thus, it should be excluded that dexamethasone given during cisplatin and docetaxel-based chemotherapy may

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induce resistance to these agents in the treatment of SCCHN. The present work was the first to investigate the effect of dexamethasone on the antineoplastic activity of cisplatin and docetaxel in SCCHN cell lines.

Materials and Methods

The following eight SCCHN cell lines were used in the study: PE/CA-PJ-15, PE/CA-PJ-34, PE/CA-PJ-41 and PE/CA-PJ-49 cells were obtained from the ECACC (European Collection of Cell Cultures, Salisbury, Wiltshire, GB), and Cal-27 and Kyse-140 cells were purchased from DSMZ GmbH (Braunschweig, Germany). CLS-354 and UM-SCC-14C were obtained from CLS Cell Line Service (Eppelheim, Germany). Cisplatin was obtained from Hexal AG (Holzkirchen, Germany), docetaxel from Sanofi-Aventis Deutschland (Frankfurt, Germany), and dexamethasone from Sigma-Aldrich (Munich, Germany).

The cell lines were cultivated according to the instructions of the suppliers at 37°C in cell-type specific medium Quantum 263 with L-glutamine (PAA Laboratories GmbH, Pasching, Austria) and antibiotics. The cells were seeded in 96-multiwell plates (1×100,000 cells/well), and after incubation for 24 h, the cells were treated with cisplatin or docetaxel alone or in combination with dexamethasone for 24, 48, 76 h, respectively.

In all the experiments, cisplatin and docetaxel were used at a cell line specific, fixed concentration which had resulted in maximum tumor cell decline in previous investigations (Table I). Dexamethasone was added at four concentrations ranging from 1 to 10 µmol/L; these concentrations were similar to those administered in tissues in the clinical setting (11). The number of cells was determined in a Rosenthal chamber after 24, 48 and 72 h of treatment. Cell viability and cell killing were determined by a water-soluble-tetrazolium-test (WST) and lactate dehydrogenase (LDH) assay, respectively. For the WST assay, 1×10⁵ cells per well were cultivated in a 96-well plate for 24 h and then treated with cisplatin or docetaxel plus dexamethasone at the concentrations specified above for 24, 48 or 72 h, respectively. Ten µl of WST at 5 g/L (Roche Diagnostics, Mannheim, Germany) were added to the medium in triplicate at each dose and incubated for 1 h at 37°C. Absorbance was measured at 450 nm using a microplate reader. LDH activity in the culture medium was measured with a Cytotoxicity Detection Kit plus purchased from Roche Mannheim, Germany. Briefly, cells were incubated in a 96-well microplate (Falcon, Franklin Lakes, NJ, USA), with 5,000 cells in 200 µl seeded per well with Quantum 263 PAA. After 24 h, the medium was removed and replaced either by the same medium containing cisplatin or docetaxel with or without dexamethasone at the concentrations specified above, or drug-free medium (low controls) or medium containing 1% Triton X-100 (Sigma Chemical Company) to determine the total cellular LDH (high controls). After 24, 48 or 72 h of treatment, 5 µl of lysis buffer was added to each high control well followed by incubation for 15 min. Then 100 µl of the LDH assay reaction mixture was added to each well, and the cells were subsequently incubated for 30 min at room temperature. During the incubation period, the microplates were protected from light. The optical density of each well was determined using a microplate reader (Dynatech Laboratories, Chantilly, VA, USA) at a wavelength of 490 nm with a reference wavelength of 630 nm. Each experiment was conducted in triplicate. For statistical analysis, a Wilcoxon test for matched pairs (dependent samples) was performed using SPSS 13.0 software for Windows (SPSS GmbH, Munich, Germany).

Table I. Tumor cell lines investigated in this study and the cell line specific cisplatin and docetaxel doses administered.

Tumor cell line	Cisplatin dose (µM)	Docetaxel dose (nM)
CLS-354	3.125	1.25
UM-SCC-14C	1.560	2.50
PE/CA-PJ-49	3.125	5.00
PE/CA-PJ-34	1.560	5.00
PE/CA-PJ-41	0.390	15.00
PE/CA-PJ-15	0.780	10.00
Cal-27	0.097	2.50
Kyse-140	3.125	20.00

Results

Cisplatin and docetaxel alone had a highly significant ($p=0.012$) antiproliferative effect compared with controls at all the time points (24, 48 and 72 h) in all eight SCCHN cell lines.

To determine the effect of dexamethasone on the cytotoxic activity of cisplatin and docetaxel, both agents were combined with dexamethasone at four different concentrations. At each of the four concentrations and after incubation for 24, 48, or 72 h, the growth-inhibitory effects of cisplatin and docetaxel remained significant ($p=0.012$ at 24 h, $p=0.012$ at 48 h and $p=0.012$ at 72 h *versus* untreated controls) and comparable in magnitude to the experiments without dexamethasone. Although at some concentrations of dexamethasone the number of tumor cells was higher than with the cytotoxic agent alone, growth inhibition was significant compared with the untreated control group. ($p>0.05$, for differences in tumor cell number after administration of cisplatin and docetaxel either alone or with dexamethasone added at several concentrations).

Results of all the tumor cell lines investigated for cell growth are shown in Figure 1, for the LDH assay in Figure 2 and for WST assay in Figure 3.

Discussion

There was no evidence of adverse interactions between dexamethasone and cisplatin or docetaxel in the investigated head and neck cancer cell lines. Moreover, dexamethasone did not exert an anti-apoptotic effect in any of the eight SCCHN cell lines. A study recently reported by Zhang *et al.* (12) found the opposite, demonstrating glucocorticoid-induced resistance to chemotherapy in the majority of cell lines derived from various malignancies including brain, breast and cervical cancer, melanoma and neuroblastoma. Gassler *et al.* (8) reported that glucocorticoids exerted an anti-apoptotic effect in tissue samples from lung cancer. Glucocorticoids have also been suggested to induce resistance and accelerate tumor growth in patients with prostate, renal cell, bladder and testicular cancer (13). There is no

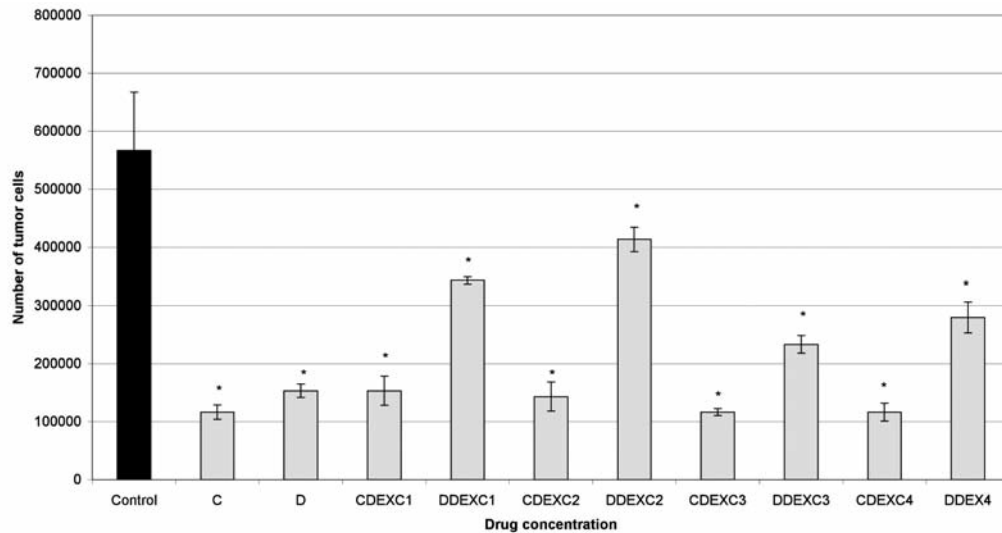


Figure 1. Effect of cisplatin and docetaxel with or without dexamethasone at various concentrations on the growth of the SCCHN cell line UM-SCC-14C, after incubation for 48 h. Control, untreated; C, cisplatin; D, docetaxel; DEX1, dexamethasone 1.0 μ M; DEX2, 2.5 μ M; DEX3, 5.0 μ M; DEX4, 10.0 μ M. Mean values of three independent experiments with standard deviation are shown: *significant difference versus untreated control ($p > 0.05$, for differences in tumor cell number after administration of cisplatin and docetaxel either alone or with dexamethasone added at several concentrations). Similar results were achieved at 24 h and 76 h.

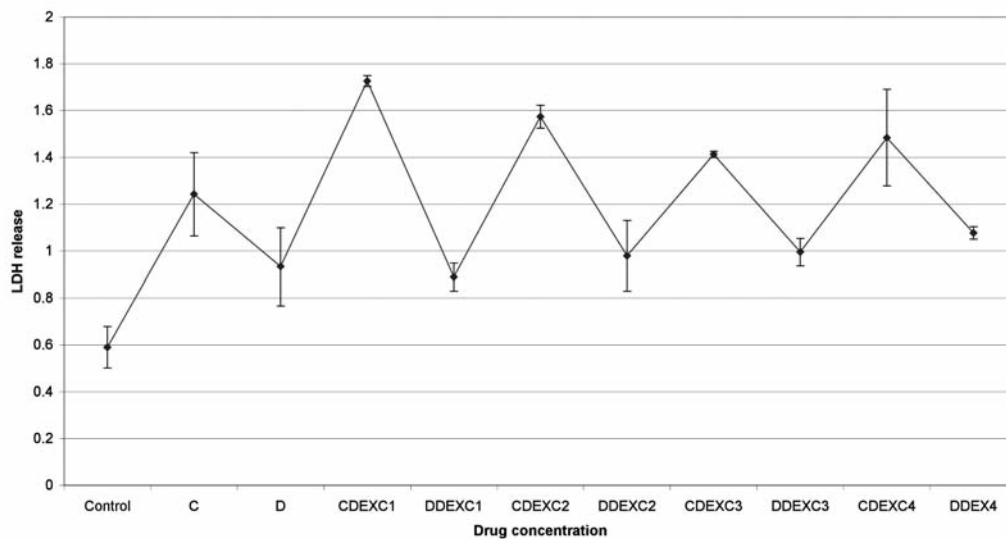


Figure 2. LDH assay in the SCCHN cell line UM-SCC-14C treated with cisplatin and docetaxel with or without dexamethasone at various concentrations, after incubation for 48 h. Mean values of three independent experiments with standard deviation. Control, untreated; C, cisplatin; D, docetaxel; DEX1, dexamethasone 1.0 μ M; DEX2, 2.5 μ M; DEX3, 5.0 μ M; DEX4, 10.0 μ M. Mean values of three independent experiments with standard deviation. Control, untreated; C, cisplatin; D, docetaxel; DEX1, dexamethasone 1.0 μ M; DEX2, 2.5 μ M; DEX3, 5.0 μ M; DEX4, 10.0 μ M.

straightforward explanation for the present discrepant findings in SCCHN cell lines. It may well be the case that intrinsic properties of tumor cells play a role, suggesting a cell-type specific effect of glucocorticoids. This would be consistent with the complexity of tumor cell biology, as indicated by the well-known pro-apoptotic and anti-proliferative effects of

glucocorticoids in lymphoid cells, as demonstrated by other research groups (14, 15). The mechanism of the cytotoxic effect of glucocorticoids in hematological cells has been investigated in depth. These drugs bind to the cytosolic glucocorticoid receptor that is able to translocate to the nucleus where it gives rise to several transcriptional

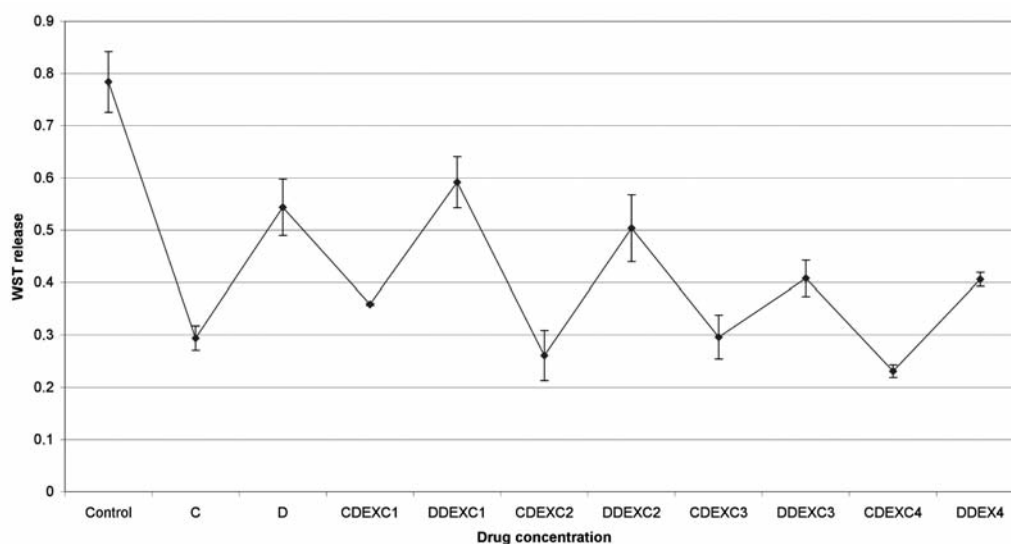


Figure 3. WST assay in the SCCHN cell line UM-SCC-14C treated with cisplatin and docetaxel with or without dexamethasone at various concentrations, after incubation for 48 h. Mean values of three independent experiments with standard deviation. Control, untreated; C, cisplatin; D, docetaxel; DEX1, dexamethasone 1.0 μ M; DEX2, 2.5 μ M; DEX3, 5.0 μ M; DEX4, 10.0 μ M.

modifications. This results in the regulation of multiple caspases, ultimately leading to apoptotic cell death (14, 16, 17). In established human lung and breast cancer cell lines, the mechanisms underlying the glucocorticoid action in lymphoid cells were shown to be blocked, resulting in the inhibition of chemotherapy and radiation-induced apoptosis. It still remains unclear how glucocorticoids mediate these cell-type specific effects that are known to be related to a functional glucocorticoid receptor (15, 18). There is convincing evidence, however, that the use of systemic glucocorticoids can be associated with adverse clinical effects, *e.g.* an increased metastatic potential in breast cancer patients (19). Moreover, a growing body of evidence from *in vitro* investigations in specific cell lines strongly suggests the induction of glucocorticoid-mediated cellular resistance to cancer therapy, *e.g.* by enhancing DNA repair capacity, blocking apoptosis, suppressing host anti-tumor immune responses and causing biophysical resistance to drug access by triggering diabetes (20, 21).

Studies in animal models have demonstrated that the administration of dexamethasone results in immunosuppression which, in turn, may exacerbate metastatic spread and accelerate tumor growth (22). This could explain why glucocorticoids inhibited the effect of conventional chemotherapy with cisplatin and docetaxel in some solid tumor models, while they were shown to be beneficial as an adjunct to bortezomib in the treatment of hematological malignancies (23). Therefore it remains to be clarified whether glucocorticoid-mediated resistance in solid tumor models is the rule or an occasional event occurring in a few cell lines only. The present results indicate that the data obtained with glucocorticoids in

carcinomas of the prostate, bladder, kidney, bone, brain, breast and cervix cannot be extrapolated to solid tumors in general. The cell-type specific expression of glucocorticoid receptor coactivators and corepressors may be one explanation for the divergent effects of dexamethasone in different solid tumor models, which has been proposed to explain the opposite effects of tamoxifen on mammary and endometrial tissues (24). Other findings have strongly suggested that tissue-specific differences in dexamethasone-induced apoptosis and survival outcomes may be due to cell-type specific transcriptional regulation.

Lastly, the lack of any effect of dexamethasone on the cytotoxic activity of cisplatin and docetaxel in the present *in vitro* study of SCCHN cell lines could be explained by the process of signal transduction itself. It cannot be excluded that both the pro-apoptotic and anti-apoptotic effects of dexamethasone in solid tumor models are mediated by an interaction of the glucocorticoid with the signal transduction pathway of the antineoplastic agents. It is conceivable that the effects of dexamethasone are governed by cell differentiation features and proliferation properties as well as complex resistance mechanisms of different tumor cell lines, resulting from the subspecialisation of the cancer cells in these models. Considering the present unique findings that contrast with most other data reported in the literature, one may conclude that the effect of dexamethasone varies both with the type of antineoplastic agent used and the type of cancer. This complex interaction is consistent with the heterogeneous biology of malignant diseases.

In contrast to other solid tumor models, glucocorticoids do not modulate the cytotoxic activity of cisplatin and docetaxel in SCCHN cell lines *in vitro*. Thus, patients with

head and neck cancer might benefit from the multiple favorable effects of glucocorticoids without any compromise in survival. However, since experimental findings cannot be translated easily into clinical practice, *in vivo* trials on a mouse model are planned to confirm the present findings.

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