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Concurrent cetuximab, cisplatin, and radiation for squamous cell carcinoma of the head and neck in vitro

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ABSTRACT

Background and purpose: For locoregionally advanced HNSCC, chemoradiotherapy with cisplatin or another platinum compound is considered as one of the standard treatment regimes. Cisplatin has improved the loco-regional control, but also increased especially the acute side effects. Cetuximab blocks ligand binding and receptor activation by binding to the extracellular domain of the EGFR. The blockade of EGFR signaling in combination with cytotoxic drugs or with radiotherapy could be a novel effective management with a relatively favourable toxicity for HNSCC. In the present study we have examined in vitro a potentially novel effective management for HNSCC, cetuximab combined with cisplatin and radiotherapy.

Materials and methods: Seven head and neck SCC cell lines were studied. Cetuximab concentrations of 0.22–8.20 nM and cisplatin concentrations of 0.038–0.220 μg/ml were used. In order to test the concurrent use of cetuximab, cisplatin and radiation, the cells were treated with the desired drug concentrations immediately after irradiation, plated into 96-well culture plates, and incubated for 4 weeks. The number of positive wells was counted. The PE was calculated and fraction survival data were fitted to the LQ model. AUC value was obtained with numerical integration. The types of interaction were analyzed.

Results: Cetuximab and cisplatin constantly induced an additive or supra-additive effect when combined with irradiation in the seven HNSCC cell lines tested.

Conclusions: We evaluated concurrent cetuximab, cisplatin, and radiation for HNSCC cell lines. Preliminary efficacy results are encouraging, and further development of this targeted combined modality paradigm is warranted.

For locoregionally advanced squamous cell carcinoma of the head and neck (HNSCC), surgery and radiotherapy are the corner stones in the treatment [1]. Considering organ preservation and survival, concurrent chemoradiotherapy has gradually been established during the past 20 years [2,3]. At present, due to the improvement of the loco-regional control, chemoradiotherapy with cisplatin or another platinum compound is considered to be one of the standard treatment regimes for patients with locoregionally advanced HNSCC. However, only 50–60% of the patients treated with this combination are cured from their cancer. Additionally, using this method especially the acute side effects in terms of their onset and duration are increased. Thus, a novel and more effective management with a relatively favourable toxicity for HNSCC is clearly needed.

The epidermal growth factor receptor (EGFR/ErbB1/HER1) is a member of the ErbB/HER family of receptor tyrosine kinases that includes ErbB2, ErbB3, and ErbB4. EGFR, a transmembrane glycoprotein, is expressed at high levels in a number of tumor types and in a large proportion of HNSCC [4,5]. Overexpression of the EGFR is correlated with a poor prognosis in HNSCC [6,7]. Furthermore, EGFR has been implicated as an important mediator of radioresistance in HNSCC [8–11]. For example, EGFR has been suggested to protect cells from immediate radiation-induced DNA damage, as well as to mediate cellular survival and repopulation via activation of phosphoinositide 3-kinase/Akt and Ras/mitogen-activated protein kinase (MAPK) pathways after radiotherapy [12].

Cetuximab (Erbitux, IMC-C225) is an IgG1 monoclonal antibody that targets at EGFR. Cetuximab blocks ligand binding and induces

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receptor internalization and degradation, and hence down-regulates EGFR expression. As a result, cetuximab leads to inhibition of cancer cell growth and survival [13,14]. Moreover, cetuximab sensitizes cancer cells to radiotherapy [15–18]. Most studies of cetuximab have shown significant activity and safety not only as a single drug, but also in combination with irradiation in head and neck cancers [16–21]. Importantly, concurrent use of cetuximab with radiotherapy improves survival in HNSCC compared to that of radiotherapy alone without significant increase in side effects [19].

Increasing evidence suggests that the efficacy of standard chemoradiotherapy may be enhanced by simultaneous blockage of EGFR [22]. The aim of the present study was to examine interactions between cetuximab, cisplatin and radiotherapy in seven HNSCC cell lines with known ErbB family receptor status. This concurrent combination induces a supra-additive effect in two cell lines and an additive effect in five cell lines.

Materials and methods

Cell lines

In this study, seven head and neck SCC cell lines were used. The cell lines were established in the University of Turku as described previously [23]. UT-SCC-8, UT-SCC-9, UT-SCC-29, UT-SCC-34 and UT-SCC-38 are SCCs of laryngeal origin, whereas UT-SCC-24A and UT-SCC-40 are SCCs from tongue. UT-SCC-8, UT-SCC-29, UT-SCC-34, UT-SCC-38, UT-SCC-24A and UT-SCC-40 were established from primary tumors and UT-SCC-9 from a neck metastasis. For a description of the characteristics of the cell lines used in the experiment, see Table 1.

Cell culture

Before the start of the experiments, the cells were maintained in logarithmic growth in T25 culture flasks by passing weekly in Dulbecco's modified Eagle's minimal essential medium (DMEM) containing 2 mM L-glutamine, 1% non-essential amino acids, 100 U ml^{-1} streptomycin, 100 U ml^{-1} penicillin and 10% fetal bovine serum (FBS). Cells in mid-logarithmic growth (40–60% confluency) were used for the experiments and fed with fresh medium on the day before plating for the experiments.

Drug preparation

Cetuximab (Erbitux®, kindly provided by Merck KgaA, Darmstadt, Germany) was received as an infusion concentration of 2.0 mg/ml and was diluted with NaCl to give a stock solution of 1.0 μM. Final cetuximab dilutions of 0.22–8.20 nM were used, and new stock solutions were made for each experiment.

Cisplatin (Platinol®, 1 mg/ml, Bristol-Myers Squibb) was diluted with NaCl to give a stock solution of 100 μg/ml. Final cisplatin dilutions of 0.038–0.220 μg/ml were used, and new stock solutions were made for each experiment.

Clonogenic growth assay and irradiation

The 96-well plate clonogenic assay was described earlier in detail [24,25]. In short, the cells were harvested with trypsin–EDTA, counted, and suspended in Ham's F-12 medium containing 15% FBS. The stock solution containing 4167 cells/ml was used, and 120 μl of this solution was diluted in 50 ml of growth medium. The concentration of two cells per well was achieved by applying 200 μl of this suspension to each well using an octapipette. The number of cells plated per well was adjusted according to the plating efficiency (PE) of the cell line. The cells were irradiated in suspension using a linear accelerator (Clinac 4/100; Varian, CA) with 4 MeV photon irradiation at a dose rate of 2.0 Gy/min [26]. To prevent hypoxia due to sedimentation of the cells during irradiation, the tubes were inverted after each dose. The irradiation was given as a single fraction at room temperature. Immediately after irradiation the cells were plated into 96-well plates in duplicates using an octapipette (Costar) by applying 200 μl/well. To test the concurrent use of cetuximab, cisplatin and irradiation, the cells were treated with the drugs immediately after irradiation. The desired drug concentrations were added in 50 ml growth medium, which was applied in 96-well plates (200 μl/well) using an octapipette.

Finally, the plates were incubated at +37 °C for 4 weeks, whereafter the positive number of wells containing coherent, living colonies, consisting of 32 cells or more, was counted using an inverted phase-contrast microscope. The drugs were present in the medium throughout the whole incubation period.

For each cell line, four different sets of plates with three or four repeats were used. The first set was used as control without drugs, whereas in the three other sets, cisplatin was added in concentrations corresponding to the IC70 values of the respective cell line based on the previous experiments. There was no cetuximab added in the first and the second set. In the third and the fourth set, cetuximab was added in concentrations corresponding to the IC70 and IC50 values of the respective cell line based on the previous experiments. In the first and the second set two control plates were used, and in the third and the fourth set four control plates were used containing different drugs, namely, cetuximab without cisplatin or with cisplatin. Each set consisted of two or four control plates and two plates with following radiation doses: 0.75 Gy, 1.25 Gy, 2.5 Gy, 5.0 Gy and 7.5 Gy. Thus, in the first and the second set, 12 plates were used for each experiment; in the third and the fourth set, 14 plates were used for each experiment. The whole study with one cell line included 52 plates.

Data analysis

The PE was calculated using the formula −ln(number of negative wells/total number of wells)/number of cells plated per well.

Table 1
Characteristics of the cell lines.

Cell line	Gender	Primary tumor location	TNM	Specimen site	Type of lesion	Histologic grade
UT-SCC-8	Male	Supraglottic larynx	$T_2N_0M_0$	Larynx	Primary	1
UT-SCC-9	Male	Glottic larynx	$T_2N_1M_0$	Neck	Metastasis	1
UT-SCC-24A	Male	Tongue	$T_2N_0M_0$	Tongue	Primary	2
UT-SCC-29	Male	Glottic larynx	$T_2N_0M_0$	Larynx	Primary	1
UT-SCC-34	Male	Supraglottic larynx	$T_4N_0M_0$	Supraglottic Larynx	Primary	1
UT-SCC-38	Male	Glottic larynx	$T_2N_0M_0$	Larynx	Primary	2
UT-SCC-40	Male	Tongue	$T_3N_0M_0$	Tongue	Primary	1

TNM status of primary tumors according to the International Union against Cancer (1997).
Histologic grade: 1, well differentiated; 2, moderately differentiated; 3, poorly differentiated.