Experimental Study

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Cetuximab alone has a dose-dependent antitumor effect in oral cavity cancer cells: an in vitro study

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Abstract

Objective: To evaluate the antitumor effect of cetuximab as a single agent for the treatment of oral cavity cancers and to clarify the dose-dependent growth inhibitory effect in oral cavity squamous cell carcinoma cell line (OCSCCCL).

Methods: The OCSCCCL (UPCI-SCC131) were cultured and continuously monitored using the xCELLigence RTCA SP instrument. Thereafter, they were divided into seven groups as: (i) negative control: medium+OCSCCCL, (ii) positive control: medium+OCSCCCL+cisplatin 10 µM/ml, (iii) medium+OCSCCCL+cetuximab 25 µg/ml, (iv) medium+OCSCCCL+cetuximab 50 µg/ml, (v) medium+OCSCCCL+cetuximab 100 µg/ml, (vi) medium+OCSCCCL+cetuximab 200 µg/ml, (vii) medium+OCSCCCL+cetuximab 400 µg/ml. The cell index and viability were statistically analyzed and compared between groups.

Results: The distribution of cell index (mean value) and percentage of viability in groups were as follows: (i) 2.66 (100%), (ii) 0.17 (6.08%), (iii) 2.28 (85.71%), (iv) 2.31 (86.84%), (v) 1.92 (72.18%), (vi) 1.79 (67.29%), (vii) 0.28 (10.53%). The change trend in drug concentration was statistically different in all study groups to which cetuximab was administered (Pillai's trace; p<0.0001). The antitumor effect of cetuximab was initially detected at a dose of 100 µg/mL, when compared with negative control (p=0.01). However, a dose of 400 µg/mL was required in order to have a statistically similar antitumor effect of cisplatin at a dose of 10 µM.

Conclusion: Cetuximab alone is a potentially effective chemotherapeutic agent and has a concentration-dependent growth inhibitory effect in OCSCCCL. The antitumor activity of cetuximab was initially detected at a dose of 100 µg/mL. However, significant antitumor effect was determined at a dose of 400 µg/mL.

Keywords: Antitumor, cetuximab, oral cavity, cancer.

Özet: Tek bir ajan olarak setuksimab oral kavite kanser hücrelerinde doza bağımlı etkiye sahiptir: Bir in vitro çalışma

Amaç: Tek bir ajan olarak setuksimabın oral kavite kanserlerinin tedavisindeki antitümöral etkisini değerlendirmek ve oral kavite yassı epitel hücreli karsinom dizininde (OCSCCCL) doza bağımlı büyümeyi inhibe etme etkisini açıklığa kavuşturmak.

Yöntem: OCSCCCL (UPCI-SCC131) kültürü elde edildi ve xCELLigence RTCA SP cihazı kullanılarak izlendi. Daha sonra yedi gruba bölüştürüldü: (i) negatif kontrol: besiyeri+OCSCCCL, (ii) pozitif kontrol: besiyeri+OCSCCCL+sisplatin 10 µM/ml, (iii) besiyeri+OCSCCCL+setuksimab 25 µg/ml, (iv) besiyeri+OCSCCCL+setuksimab 50 µg/ml, (v) besiyeri+OCSCCCL+setuksimab 100 µg/ml, (vi) besiyeri+OCSCCCL+setuksimab 200 µg/ml, (vii) besiyeri+OCSCCCL+setuksimab 400 µg/ml. Hücre indeksi ve viyabilite istatistiksel açıdan incelendi ve gruplar arasında karşılaştırıldı.

Bulgular: Hücre indeksinin (ortalama değer) ve viyabilite yüzdesinin dağılımı şu şekilde bulundu: (i) 2.66 (%100), (ii) 0.17 (%6.08), (iii) 2.28 (%85.71), (iv) 2.31 (%86.84), (v) 1.92 (%72.18), (vi) 1.79 (%67.29), (vii) 0.28 (%10.53). İlaç konsantrasyonundaki değişiklik eğilimi setuksimabın uygulandığı çalışma gruplarının tümünde istatistiksel açıdan anlamlı idi (Pillai trasesi; p<0.0001). Negatif kontrolle karşılaştırıldığında setuksimabın antitümöral etkisi ilk olarak 100 µg/mL dozda saptandı (p=0.01). Ancak 10 µM sisplatinin etkisine istatistiksel açıdan benzer antitümöral etki için 400 µg/mL doz gerekliydi.

Sonuç: Tek başına setuksimab potansiyel olarak etkili bir kemoterapötik ajan olup OCSCCL'de konsantrasyona bağımlı büyümeyi inhibe edici etkiye sahiptir. Setuksimabın antitümöral aktivitesi başlangıçta 100 µg/mL dozda saptanmıştır. Ancak 400 µg/mL dozda anlamlı bir antitümöral etki belirlenmiştir.

Anahtar sözcükler: Antitümöral etki, setuksimab, oral kavite, karsinom.

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Epidermal growth factor receptor (EGFR) or ErbB1/human epidermal growth factor receptor-1, a transmembrane glycoprotein, is one of the members of ErbB/HER family of receptor tyrosine kinase.[1] The ErbB/HER receptors are normally inactive monomers which are dimerized just after the binding of their ligands. The dimerization of the receptors leads to activation of intracellular tyrosine kinase. Epidermal growth factor receptor and its ligands play an essential role in embryological development of several tissue and organs such as eye, mammary gland, lung and gut.[2–4] Unfortunately, they also promote mechanisms of cancer by activating intracellular signaling pathways such as Ras/mitogen activated protein (MAP or ERK) kinase cascade, phosphatidylinsositol-3-kinase (PI3K)/protein kinase B (AKT) and mTOR pathways. Thereby trigger cellular proliferation, differentiation, survival, invasion, angiogenesis and metastasis.[5,6] Recently, the active role of EGFR and its ligands have been demonstrated in several cancers such as breast, colorectal, lung, esophageal, bladder, gastric, ovarian, head and neck.[7] Therefore, the idea of EGFR targeting strategy was evolved in in the armamentarium of cancer treatment and several therapeutic agents have been introduced thereafter. Currently, there are two main categories of EGFR targeting therapies: (i) monoclonal antibodies that affect by targeting the extracellular domain of EGFR; thereby preventing the activation of ligand-dependent EGFR tyrosine kinase, and (ii) tyrosine kinase inhibitors which exert by blocking the intracellular tyrosine kinase domain of EGFR.[8] Although several members of both categories have been applied in treatment of different cancers; cetuximab, an anti-EGFR monoclonal antibody, is the first and only targered chemotherapeutic agent that has already been approved by Food and Drug Administration (FDA) for the application in head and neck cancers.

Cetuximab, a chimeric monoclonal antibody of EGFR, competitively binds to the extracellular domain of EGFR. Its affinity is 5–10 times higher when compared with the ligands of EGFR.[9–11] As cetuximab binds to EGFR, antibody-receptor complex is internalized and degraded before activation and/or phosphorylation of the receptor.[12] Thereby, the amount of EGFR is down-regulated and EGFR related signaling pathways active in cancer cells can be blocked.[13]

In 2000, initial clinical trial in which cetuximab was applied as a single or adjunctive therapeutic agent reported promising outcomes in patients with several recurrent or metastatic cancers such as head and neck, lung, prostate, breast, pancreas, ovarian, kidney and bladder cancers.[14] Thereafter, a phase Ib clinical trial, particularly focused on head and neck cancers, which examined the effectiveness of cetuximab in selected patients with recurrent and/or metastatic squamous cell carcinoma (SCC) and whose tumor had EGFR overexpression, was conducted. This study demonstrated significant responses with high tolerability and mild to moderate degrees of skin reactions.[15] As a result of this study, authors recommended a loading dose of cetuximab at 400 mg/m^2 with a maintenance dose at 250 mg/m^2. In 2006, FDA approved cetuximab as a single agent in patients with cisplatin-resistant head and neck cancers and as a combination agent with radiotherapy in patients with locally advanced head and neck cancers. Even though, clinical effectiveness of cetuximab in different regions of head and neck cancers has been demonstrated in literature, the efficacy and effective dose of cetuximab in oral cavity cancers has not been reported yet. Therefore, the aim of this in vitro study is to investigate the effectiveness and antitumor dose of cetuximab alone in oral cavity squamous cell carcinoma cell line (OCSCCCL).

Materials and Methods

Cell culture

The human oral cavity squamous cell carcinoma cells (UPCI-SCC131; DSMZ, Braunschweig, Germany), were cultured in MEM Earle's medium (Biochrom GmbH, Berlin, Germany) supplemented with 10% fetal bovine serum (Biochrom GmbH), 2 mM L-glutamine (Biochrom GmbH), penicillin/streptomycin 100 IU/100 µg/ml (Biochrom GmbH) at 37°C in a 5% CO_2 cell incubator (Thermo Fisher Scientific, Waltham, MA, USA).

Real-time cell growth and cytotoxicity analysis

Real-time assessment of cell growth and cytotoxicity were performed using the xCELLigence Real-Time Cell Analyzer (RTCA) Single-Plate (SP) instrument (Roche Diagnostics GmbH, Freiburg i. B., Germany). The basic principle of the RTCA system consisting of RTCA Analyzer, RTCA SP station and capillary gold electrodes coated E-plate 96, is to detect the impedance changes by the interaction of adherent cells and the gold microelectrodes. The cell number and viability of adherent cells will affect the level of electrode impedance, which is presented as the cell index (CI). Cells (10^4 cells/well) were cultured on an electrode-containing 96-well plate for 24 hours. Test compounds were added to the growth medium after 24 hours of seeding and monitored for 72 hours to see the effect of chemotherapeutics.

Study groups are formed as follows:

i. Negative control: Medium + UPCI-SCC 131 cell line.
ii. Positive control: Medium + UPCI-SCC 131 cell line + cisplatin 10 µM
iii. Study group I: Medium + UPCI-SCC 131 cell line + cetuximab 25 µg/ml
iv. Study group II: Medium + UPCI-SCC 131 cell line + cetuximab 50 µg/ml
v. Study group III: Medium + UPCI-SCC 131 cell line + cetuximab 100 µg/ml
vi. Study group IV: Medium + UPCI-SCC 131 cell line + cetuximab 200 µg/ml
vii. Study group V: Medium + UPCI-SCC 131 cell line + cetuximab 400 µg/ml

After the administration of chemotherapeutic agents, impedance was measured automatically for the following 48-hour in every 60 minutes. The alterations on impedance signal were analyzed by normalizing the data of each well to the first read after starting the treatment. Graphical result were represented as normalized CI.

Statistical analysis

Data were collected by software provided with the RTCA system and were analyzed using SPSS v.20.0 (SPSS Inc., Chicago, IL, USA). The changing trends of cells in all groups were analyzed by Mauchly's sphericity test and multivariate analysis was performed by Pillai's trace test. The change in CI according to cetuximab and cetuximab-cisplatin relationship was analyzed using Mann-Whitney U test.

Results

Cetuximab alone has an antitumor activity on OCSCCCL

The analysis of cell viability demonstrated a decrease in CI in all study groups after the application of cetuximab (Figs. 1a and b; Table 1). However, when a comparison between all study groups and negative control was performed according to the growth inhibitory effect of cetuximab, the minimum concentration of cetuximab at which statistically significant difference detected was 100 µg/ml (72.18%, p=0.01).

Cetuximab has a dose-dependent antitumor effect on OCSCCCL

The growth inhibitory effect of cetuximab was initially determined in study group III (cetuximab, 100 µg/ml). However, when study group III was compared with positive control (cisplatin, 10 µM) according to CI, a statistically significant difference in the favor of positive control was determined (p=0.024). Thereby, when all study groups were compared between positive controls according to the inhibition of cell proliferation, similar antitumor effects were detected at a concentration of 400 µg/ml (Figs. 1a and b; Table 1).

Discussion

In head and neck cancers, monoclonal antibody therapies targeting EGFR, such as cetuximab, has been popularized recently. However, evidence regarding the effectiveness and efficacy of cetuximab in oral cavity cancers is inadequate; although the dose-dependent pharmacokinetic effect of cetuximab has been demonstrated previously in different cancer cell lines.[14,16,17] This in vitro study obviously demonstrated that cetuximab alone has a concentration dependent antitumor effect on OCSCCCL. The minimum dose of cetuximab that provided a statistically significant difference in growth inhibitory effect, was 100 µg/mL (72.8%, p=0.001); even though an antitumor effect was detected in every study group. Similarly, Zhang et al. reported the antitumor effect of cetuximab in two different OCSCCCLs in vitro, although they did not examine the growth inhibitory and/or cytotoxic effect of cetuximab as a single agent.[18] In their study, a supra-additive effect was determined, when a combination of chemoradiotherapy (radiation and cisplatin) with cetuximab was administered. In addition, Bussu et al. examined the effectiveness of cetuximab as a single agent and combination with cisplatin in Hep-2 laryngeal cancer cell line and similarly demonstrated the time-dependent effect of cetuximab.[19] They also mentioned the growth inhibitory effect of cetuximab as a single agent at a concentration of 100 µg/mL; however, they were not able to detect its cytotoxic effect. On the other hand, a synergistic effect in growth

Table 1. The cell index and viability of control and study groups at 48th hour, and statistical comparison between study groups and negative and positive controls.

Group	Cell index (median) (min–max)	Cell viability (%)	Negative control (p value)	Positive control (p value)
Negative control	2.74 (2.15–3.04)	100	-	0.004
Positive control	0.17 (0.17–0.18)	6.08	0.004	-
Study group I	2.29 (2.24–2.33)	85.71	0.233	0.100
Study group II	2.33 (2.11–2.48)	86.84	0.053	0.024
Study group III	1.91 (1.35–2.53)	72.18	0.010	0.024
Study group IV	1.79 (1.75–1.82)	67.29	<0.0001	0.024
Study group V	0.28 (0.16–0.41)	10.53	<0.0001	1.000

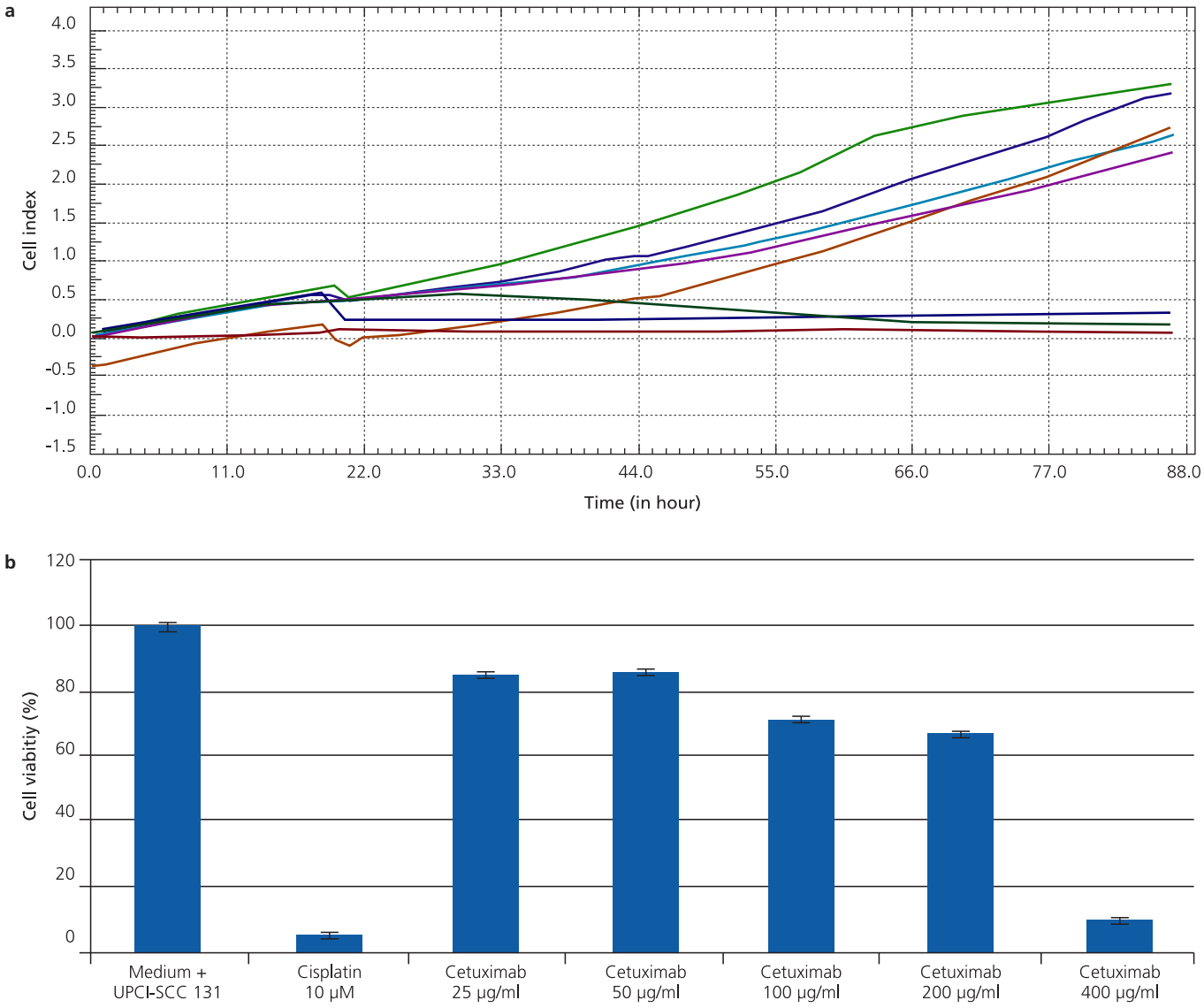

Fig. 1. The growth inhibition curves of both negative and positive controls, and all study groups were presented in panel **a**. UPCI-SCC 131 cell lines were plated onto a 96-well E-Plate and allowed to grow for 24 hours. Thereafter, test compounds were added to the culture. Cells were grown in media alone (**light green**), media alone without cell (**red**) or were treated with cisplatin, 10 µM (**dark green**), cetuximab, 25 µg/ml (**orange**), 50 µg/ml (**purple**), 100 µg/ml (**blue**), 200 µg/ml (**pink**) and 400 µg/ml (**dark blue**). The change in cell index was measured every 60 min for the following 48 hours until the end of the experiment using the RTCA system. The cell viability (%) in all study groups and negative and positive controls were presented in panel **b**.

inhibition and cytotoxicity was determined when a combination of cetuximab and cisplatin was administered.

To date, the antitumor activity of cetuximab, either as a single agent or in combination with cytotoxic or chemotherapeutic agents and/or radiation, has been demonstrated in a variety of head and neck cancer cell lines and xenografts in several in vitro and in vivo studies. However, we were unable to detect a study in which the similar growth inhibitory and/or cytotoxic effects between cisplatin and cetuximab was investigated for OCSSSCL. Therefore, this is the first study which demonstrated that cetuximab at a concentration of 400 µg/mL (0.28, 10.53%) had a similar antitumor and growth inhibitory effect when compared with cisplatin, a well-known cytotoxic agent, at

a dose of 10 µM (0.17, 6.08%). However, further preclinical and clinical studies are required in order to identify the cetuximab related tumor control and toxicities in oral cavity cancers.

Conclusion

Cetuximab has a dose-dependent antitumor activity in OCSCCCL. Cetuximab alone provides a growth inhibitory effect at a dose of 100 µg/mL, eventhough significant antitumor effect was determined at a dose of 400 µg/mL.

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Conflict of Interest: No conflicts declared.

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