Reduction of Cisplatin Dosage by ZD 1839

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Abstract. Cisplatin (CDDP) is the main chemotherapeutic drug in the treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (SCCHN), but its nephrotoxicity often limits the treatment. ZD 1839 is an orally-applicable, selective EGFR tyrosine kinase inhibitor. This investigation explored whether the cisplatin dose can be reduced by the additional application of ZD 1839. Four different SCCHN cell lines were treated with descending doses of CDDP alone or in combination with ZD 1839. Proliferation was measured by the MTT assay; tumor cell toxicity was measured by using the lactate dehydrogenase approach. ZD 1839 augments CDDP-dependent antiproliferative effects. By adding ZD 1839 to the treatment regimen, the CDDP dose could be reduced by up to 25% of the CDDP IC50 dose without compromising the antiproliferative effect. Adding ZD 1839 to CDDP may therefore enable CDDP treatment at a lower dose without compromises antiproliferative effects.

Squamous cell carcinoma of the head and neck (SCCHN) often presents as a locally advanced disease. Chemotherapeutic treatment protocols are often platinum-based, while the toxicity of cisplatin (CDDP) limits the treatment of those patients. Phase III randomized trials in patients with recurrent or metastatic SCCHN have demonstrated single-agent response rates to chemotherapy of between 10% and 15% and median survivals of 6 to 8 months (1-6). Therefore, the goal in treatment of advanced SCCHN is the reduction of toxicity.

Since the first description of the epidermal growth factor receptor (EGFR) in 1980 (7), interest has grown on targeting this protein in cancer therapy. The expression of EGFR has been linked to carcinogenesis, metastasis and survival in SCCHN patients (8). Phosphorylation of EGFR cytoplasmic tyrosine residues initiates a cascade of signals including activation of the mitogen-activated protein kinase pathway (8). The mitogen-activated protein kinase pathway culminates in activation and nuclear translocation of the extracellular signal-regulated kinases (ERK) 1 and 2 and transcription of their target genes (9). Preclinical studies have confirmed that interruption of EGFR phosphorylation can inhibit these downstream activation events, leading to cell cycle arrest and compromising tumor growth (10-12). ZD 1839 (gefitinib, Iressa) is an orally-active, low-molecular-weight anilino-quinazoline that reversibly inhibits EGFR tyrosine kinase activity. It has demonstrated an acceptable toxicity profile in phase I trials, with predictable pharmacokinetics concerning dose, schedule and dose-limiting toxicity (13). Our investigation explored whether adding ZD 1839 to the treatment regimen can reduce the CDDP dose in order to minimize CDDP-mediated toxicity.

Materials and Methods

ZD 1839 (Iressa) was kindly provided by Astra Zeneca, Macclesfield, UK).

MTT assay. Cells, 1x10^5 /well in a 96-well plate, after incubation for 24 h, were treated with different concentrations of CDDP (12.6; 3.15 Ìmol/L) and the IC50 of ZD 1839 (82.4; 164.8 Ìmol/L) for 24, 48, 72 and 96 h, respectively. Ten ÌL of MTT (5 g/l) (Sigma Chemical Co., St. Louis, USA) was added to the medium triplicate at each dose and incubated for 4 h at 37°C. The culture media were discarded, followed by addition of 0.2 mL DMSO and vibration for 10 min. Absorbance (A) was measured at 570 nm using a microplate reader.

Lactate dehydrogenase (LDH) assay. The detection of medium lactate dehydrogenase (LDH) activities was performed by the Cytotoxicity Detection Kit purchased from Boehringer Mannheim (Mannheim, Germany) and was based on the detection of the LDH activity in the culture medium. Briefly, cells were incubated in 96-well microplate (Falcon, Franklin Lakes, NJ, USA) as 5,103 cells / 200 Ìl/well with RPMI 1640 supplemented with 10% fetal calf serum (FCS). After 48 h, the media were removed and replaced either by a medium containing different concentrations of the drug or by a drug-free medium (low control condition). The wells for the high control...
condition were added with the media containing 1% Triton X-100 (Sigma Chemical Co.) to determine total cellular LDH. After 24, 48, 72 and 96 h treatment, 100 µl/well supernatants were removed and transferred into corresponding wells. To determine the LDH activity of the supernatants, 100 µl reaction mixture were added to each well and incubated with the cells for 20 min at room temperature. During this 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.

Cell lines. All cell lines derived from SCCHN. Detroit 562 and SCC 1624 were obtained commercially from ATCC. UM-SCC-33 and -5 were established by Thomas Carey, University of Michigan, USA.

Statistical analysis. Wilcoxon matched pairs and Mann-Whitney U-tests were performed using SPSS 11 software for Mac Os X.

Results

Single agent effects. As seen in Figures 1-4, all cell lines were significantly growth inhibited by either CDDP or ZD 1839 compared to the control group. Release of LDH did not differ significantly between CDDP monotreatment (3.6 µmol and 12.5 µmol) and the control (spontaneous release, SRD) of the cell lines UM-SCC 5 and SCC 1624 (Figures 2 and 4). In contrast (Figures 1 and 3), CDDP monotreatment enhanced LDH release compared to the control (SRD). ZD 1839 did not increase LDH release of any cell line (Figures 1-4).

Combination effects. To elucidate the combination effects of CDDP and ZD 1839, the CDDP dose without significant LDH increase (i.e. 25% of the IC₅₀ CDDP dose) was applied together with the IC₅₀ dose of ZD 1839 to each cell line (Figures 1-4). The inhibition of proliferation through the combination treatment was comparable to the highest CDDP dose, used as monotreatment. Furthermore, LDH release did not increase in the combination treatment group. These results were comparable in all investigated squamous cell carcinoma cell lines (Figures 1-4). Combination treatment was partially more effective than monotreatment with CDDP or ZD 1839. In the case of Detroit 562 and UM-SCC 33 cells (Figures 1 and 3), the strongest inhibition of proliferation was obtained with the combination treatment of low-dose cisplatin and ZD 1839. ZD 1839 alone inhibited proliferation significantly (p<0.05) more than CDDP (both concentrations). Combination of low-dose CDDP with ZD 1839 led to comparable growth inhibition as observed in high-dose cisplatin monotreatment.

Discussion

Our preclinical investigations demonstrate that combination effects of CDDP and ZD 1839 could lead to a reduction of the CDDP dose in future treatment plans, resulting in a lower toxicity profile. CDDP and its analogs...
are known to be mainly nephrotoxic, which in most cases limits their therapeutic use. Furthermore, CDDP may cause immunosuppression, hearing loss, hematopoietic disorders and cardiovascular complications.

However, CDDP is the most common substance in the chemotherapeutic treatment of SCCHN (1-6). ZD 1839 (gefitinib) is an orally applicable, low-molecular-weight anilinoquinazoline that reversibly inhibits EGFR tyrosine kinase activity. It demonstrated an acceptable toxicity profile in phase I trials with predictable pharmacokinetics concerning dose, schedule and dose-limiting toxicity (13). ZD 1839 seems to make the tumor cell vulnerable to CDDP treatment. Referring to this observation, we hypothesized that the CDDP dosage could be reduced if the cells were stimulated with ZD 1839 in order to obtain a comparable treatment efficacy.

Compared to the control group, all cell lines were significantly inhibited in proliferation by either CDDP or ZD 1839. The combination of CDDP and ZD 1839 can lead to an additive growth inhibition (Figures 1-4). Upon reducing the CDDP dose, the antiproliferative effect is not affected. Therefore, the combination of low-dose cisplatin with ZD 1839 led to comparable growth inhibition as observed in high-dose cisplatin monotherapy.

**References**


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