

SYNERGISTIC CYTOTOXICITY AND PHARMACOGENETICS OF GEMCITABINE AND ZD6474 IN BLADDER CANCER CELL LINES

Mey Valentina, Giovannetti Elisa, Nannizzi Sara, Ricciardi Simona, Danesi Romano, Del Tacca Mario

Division of Pharmacology and Chemotherapy, Department of Internal Medicine, University of Pisa

Introduction. The deoxycytidine analog gemcitabine is an active agent against a variety of solid tumours, including genito-urinary malignancies. However, a great deal of interest has been focused on research into new drugs and new drug combinations for chemotherapy of these diseases. In particular, several studies are investigating the emerging role of biological agents targeting molecular pathways involved in tumour growth and angiogenesis, such as ZD6474, a new inhibitor of the kinase domain-containing VEGFR2 and EGFR. Therefore, the present study was performed in bladder cell lines to analyze the ability of ZD6474 to synergistically interact with gemcitabine, and to study cellular and genetic mechanisms underlying these effects. **Methods.** Cells were treated with gemcitabine and ZD6474, alone or in sequence. The cytotoxicity was assessed by the CellTiter 96 Non-radioactive cell proliferation kit; pharmacologic interaction was studied using the combination index (CI) method. The effects of drugs on cell cycle, Akt (S473), EGFR (Y992 and Y1173) phosphorylation and apoptosis were investigated by flow cytometry, ELISA and fluorescence microscopy, respectively. Finally, quantitative PCR was used to study target gene expression profile and its modulation by single drugs. **Results.** A dose-dependent inhibition of cell growth was observed with gemcitabine and ZD6474, with IC_{50} values of 0.92 ± 0.05 and 1.35 ± 0.16 μ M (T24), and 4.37 ± 0.83 and 1.68 ± 0.29 μ M (J82), respectively. The combination index analysis showed synergism for both drug sequences ($CI < 1$). Flow cytometric studies demonstrated that gemcitabine enhanced cellular population in the S phase with respect to control in T24 (+19.41), while the percentage of T24 and J82 cells in the G1 phase increased significantly ($P < 0.05$) after treatment with ZD6474 for 48 hours. ZD6474 significantly decreased the amount of activated Akt and cells exposed to both drugs presented typical apoptotic morphology. Furthermore, gemcitabine significantly enhanced the content of pY1173 EGFR levels in all cell lines, while ZD6474 decreased EGFR phosphorylation at both tyrosine residues. PCR analysis showed that ZD6474 increased the gene expression ratio between the gemcitabine activating enzyme deoxycytidine kinase and the gemcitabine target ribonucleotide reductase in T24 cells. **Conclusions.** Several factors, including modulation of Akt and EGFR phosphorylation, induction of apoptosis and expression of genes involved in drug activity, may contribute to the synergistic effect between gemcitabine and ZD6474, and these data provide experimental basis for the rational development of this combination against bladder cancer.