IN VITRON STUDY OF THE INTERACTION BETWEEN GEMCITABINE AND SORAFENIB IN NON SMALL CELL LUNG CANCER (NSCLC) A549 CELL LINE

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

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

Background: gemcitabine is a new cytotoxic agent approved for the treatment of advanced NSCLC as a first line, whereas sorafenib is a small molecule that blocks both the Raf/mitogen extracellular kinase/extracellular signal-related kinase (RAF/MEK/ERK) signaling pathway and receptors involved in neovascularization and tumour progression such as B-RAF, C-RAF, platelet-derived growth factor receptor (PDGFR-β), vascular endothelial growth factor receptor (VEGFR-2) and stem cells factor receptor (c-KIT). There are few studies that demonstrate the activity of sorafenib in NSCLC cell lines. The aim of this study was to evaluate the interaction between gemcitabine and sorafenib in a cancer cell line, A549, and to study the biological effects of their combined administration. **Methods:** Cells (A549) were treated for evaluate IC50, combination index (CI), AKT and c-KIT phosphorylation, cell-cycle modulation, apoptosis, gene expression, at the following concentrations: 1) gemcitabine 0.1 nM to 1 µM for 24 h; 2) sorafenib 0.1 nM to 1 µM for 72 h; 3) gemcitabine for 24 h, followed by a 24-h washout in drug-free medium then sorafenib for 72 h; 4) sorafenib for 72 h followed by a 24-h washout in drug-free medium then gemcitabine for 24 h. To establish a correlation between drug effect and modulation of gene expression, we treated cell line at IC50 values of gemcitabine, sorafenib, and their combinations. **Results:** A dose-dependent inhibition of cells growth was observed with gemcitabine and sorafenib, with IC_{50} values of 0.10± 0,05 and 1.15± 0.49 µg/ml, respectively. Combination studies were performed at fixed concentration ratios (1:10 gemcitabine/sorafenib). The sequential exposures of cell line to sorafenib followed by gemcitabine and reverse sequence reduced the IC_{50} values up to 10-100 folds. The calculation of the CI value showed that both schedules of gemcitabine and sorafenib demonstrated synergism. Gemcitabine and drug combination treatments significantly increased the apoptotic index, 15-32%, with respect to control cells, 3%. Gemcitabine was able to significantly reduce the amount of phosphorylated Akt whereas sorafenib reduced c-KIT phosphorylation. **Conclusion:** Study results suggest that the combinations of sorafenib and gemcitabine are synergic, as demonstrated by cytotoxic assays, and may represent a suitable model for developing this combination for the treatment of NSCLC.