SYNERGISTIC CYTOTOXICITY, INHIBITION OF AKT AND C-KIT PHOSPHORYLATION, MODULATION OF GENE EXPRESSION BY SORAFENIB AND GEMCITABINE IN HUMAN PANCREATIC CANCER CELLS

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Introduction: pancreatic cancer is one of the most lethal tumours and, although gemcitabine produces a clinical meaningful response, there has been little improvement in prognosis. Therefore, research effort has focused on target-specific agents, such as sorafenib, which blocks both the RAF/MEK/ERK signaling pathways and receptors involved in neovascularization and tumour progression, including VEGFR-2 and c-Kit. We investigated whether sorafenib would be synergistic with gemcitabine against pancreatic cancer cell lines. **Material and Methods**: cells were treated with sorafenib and gemcitabine, alone or in combination and pharmacologic interaction was studied using the combination index (CI) method. Cell cycle was investigated with flow cytometry. Moreover, the effects of drugs on Akt (S473) and c-Kit (Y823) phosphorylation, and on apoptosis induction were studied with ELISA and fluorescence microscopy respectively. Finally, quantitative PCR analysis was performed to assess whether sorafenib modulated the expression of the gemcitabine activating enzyme deoxycytidine kinase (dCK) and the drug target ribonucleotide reductase (RR). **Results**: A dose dependent inhibition of cell growth was observed after gemcitabine and sorafenib treatment; the CI analysis showed that both schedules of two drugs exhibited synergism in all cell lines. Flow cytrometric studies demonstrated that gemcitabine enhanced cellular population in the S phase (from 8.9% in PANC-1 to 14.8% in MIA PaCa-2 cells), whereas sorafenib was not able to significantly modulate cell cycle distribution. Cell exposure to gemcitabine resulted in a significant Akt phosphorylation inhibition, whereas sorafenib exposure reduced c-Kit phosphorylation in all cell lines. Fluorescence microscopy demonstrated that cells treated with drugs and their combinations presented typical apoptotic morphology; in particular, drug combinations significantly increased ($P<0.05$) apoptotic index with respect to single agents in Capan-1, MIA PaCa-2 and PANC-1 cells, in PANC-2 cells the most effective induction of apoptosis was observed after gemcitabine exposure. PCR showed that sorafenib reduced the expression of RRM1 and RRM2 in MIA PaCa-2, Capan-1 and PANC-1 cells, enhancing the dCK/(RRM1xRRM2) ratio. **Conclusions:** these data demonstrate that sorafenib and gemcitabine synergistically interact against pancreatic tumour cells, through suppression of Akt and c-Kit phosphorylation, induction of apoptosis and reduction of RRM1 and RRM2 gene expression, thus providing the experimental basis for developing this combination for the treatment of pancreas cancer.