# SYNERGISTIC CYTOTOXICITY OF OXALIPLATIN AND PEMETREXED IN COLON CANCER CELLS: CELLULAR AND MOLECULAR MECHANISMS

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Oxaliplatin is active in the treatment of colorectal cancer and its effect is improved upon combination with thymidylate synthase (TS) inhibitors. Since the novel folate analogue pemetrexed blocks folate metabolism and DNA synthesis by inhibiting TS, dihydrofolate reductase (DHFR) and glycinamide ribonucleotide formyltransferase (GARFT), the present study investigated the in vitro cytotoxicity of oxaliplatin and pemetrexed associations. Human HT29, WiDr, SW620 and LS174T colon cancer cells were treated with oxaliplatin and pemetrexed, alone or in combination. Drug interaction was studied using the combination index method, while cell cycle was investigated with flow cytometry. Moreover, the effects of drugs on Akt phosphorylation and apoptosis were studied with ELISA and fluorescence microscopy, respectively. RT-PCR analysis was performed to assess whether oxaliplatin and pemetrexed modulated the expression of pemetrexed targets, folylpolyglutamate synthase (FPGS) and excision repair cross complementing group 1 and 2 (ERCC1/2). Finally, the basal gene expression analyses were related to pemetrexed sensitivity. A dose-dependent inhibition of cell growth was observed after drug exposure and simultaneous and sequential combinations showed synergism. Oxaliplatin significantly enhanced cellular population in the S phase in HT29 (from 26.2% to 40.9%), SW620 (from 44.9% to 57.1%) and LS174T (from 33.5% to 50.0%). Drug combinations increased apoptotic index with respect to single agents. Oxaliplatin and pemetrexed were able to reduce the phosphorylated Akt ($P<0.05$), with pemetrexed being more potent than oxaliplatin. Finally, RT-PCR showed that pemetrexed significantly reduced ERCC1 and ERCC2 gene expression (i.e. -21.8±7.6% and -59.8±5.0% in HT29 cells, respectively). Basal mRNA levels of FPGS, TS, DHFR and GARFT were related to pemetrexed sensitivity: a good correlation was found between the FPGS/(TS*DHFR*GARFT) ratio and $IC_{50}$ values of pemetrexed ($R^2=0.945$; $P=0.028$). Oxaliplatin modulated gene expression of pemetrexed targets in WiDr, SW620 and LS174T cells ($P<0.05$). TS expression was significantly decreased up to -25.7±4.0% and -39.0±6.0% in SW620 and LS174T cells, respectively, while DHFR and GARFT expression was strongly reduced in LS174T cells (-78.5±3.5% and -72.2±4.4%, respectively) and slightly modulated in WiDr and SW620 cells. These data demonstrate that oxaliplatin and pemetrexed synergistically interact against colon cancer cells through modulation of cell cycle, inhibition of Akt phosphorylation, induction of apoptosis and modulation of gene expression.