MODULATION OF BCL-2 EXPRESSION BY GEMCITABINE AND NIMESULIDE IN HUMAN PANCREATIC TUMOR CELL LINES

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Gemcitabine (Gtb) is an inhibitor of ribonucleotide reductase and DNA synthesis and is an effective antitumoral agent used for the treatment of pancreas cancer. The majority of human primary pancreatic carcinoma overexpress the prostaglandin endoperoxide synthase Cox-2 (inducible form) in contrast to the benign pancreatic tumor. Cox-2 activity produces prostaglandin E2 and induces the production of angiogenic factors, which can favour tumorigenesis through neoangiogenesis.
The present study analysed the effects of gemcitabine and nimesulide (Nms), a selective Cox-2 inhibitor, on the expression of the anti-apoptotic Bcl-2 protein in two pancreatic tumor cell lines BxPc3 and MiaPaCa, characterized by high and low Cox-2 expression respectively. We defined two pharmacological doses, for each drug, able to induce a nontoxic stimuli. We assayed the mitochondrial activity of 30x10^4 living cell treated with different concentractions of the drugs (Nms 15-30-60-120 µM and Gtb 15-30-60-120 nM) for 24, 48, 72h. The cytotoxicity test shows that Bxpc3 are more sensitive than MiaPaCa to both drugs and the lowest toxicity effect corresponds to Nms 30 µM and Gtb 15 nM. Cell lines were also exposed to parallel treatments for 24, 48, 72h: Gtb 15 nM; Nms 30 µM, Gtb 15 nM +Nms 30 µM. Total proteins were extracted and the Bcl-2 expression level was assayed by westhern-blot.
BXPC3 showed: at 24h an increase of Bcl-2 upon Gtb and Gtb+Nms treatment, while Nms alone did not modify the Bcl-2 level; at 48h Nms induced an increase of Bcl-2 higher than that induced by Gtb or Gtb+Nms; at 72h a similar high level of Bcl-2 was observed for each treatment.
The Bcl-2 levels in MiaPaCa-2 at 24h and 48h were increased only upon Gtb+Nms treatment. On the contrary at 72h a decrease in Bcl-2 level upon each treatment was observed.
The increase of the antiapoptotic Bcl-2 protein upon chronic treatment of BxPC3 and Miapaca-2 with gemcitabine 15nM may be part of a resistance mechanism of cancer cell to this chemotherapeutic agent. The decrease of Bcl-2 observed in MiaPaCa-2 (low Cox2 expression) upon Nms 30 µM may be explained by inhibition of antiapoptotic cox-2-dependent pathway. High Cox-2 expression in BxPc3 may be responsible for the lack of inhibition of the antiapoptotic pathway by nimesulide.
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