**In vitro** studies on gemcitabine combinations with other antiblastics

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The use of gemcitabine in combination with chemotherapeutic agents, including cisplatin, pemetrexed and taxanes, is characterized by the enhancement of their anticancer activity. The analysis of the underlying pharmacodynamics has revealed that modulation of nucleotide pools, drug metabolism, and cellular DNA repair capability are the most common factors to explain the additive to synergistic interaction between gemcitabine and anticancer agents in several human cancers in vitro and in vivo.

**Key words:** gemcitabine, metabolism, drug interaction

**background**

Gemcitabine is an antimetabolite cytotoxic prodrug that undergoes complex cellular uptake and metabolism. The drug is transported into the cells mostly by the human equilibrative nucleoside transporter 1 (hENT1) and is rapidly activated by the enzyme deoxycytidine kinase (dCK) to gemcitabine monophosphate (dFdCMP), that is phosphorylated to the active compounds diphosphate (dFdCDP) and triphosphate (dFdCTP) (Figure 1). Gemcitabine triphosphate is the major drug metabolite, while relatively small concentrations of either dFdCMP or dFdCDP are present in human cells. This indicates that phosphorylation of gemcitabine to dFdCMP by dCK is the rate-limiting step of drug activation. Gemcitabine is irreversibly deaminated by deoxycytidine deaminase (CDA) to difluoro-deoxyuridine (dFdU), while dFdCMP is reversibly dephosphorylated by purine endo-5′-nucleotidase (5′-NT). In proliferating cells, ribonucleotide reductase is a key enzyme of de novo nucleotide synthesis pathway and is an heterodimer composed by regulatory (RRM1) and catalytic (RRM2) subunits that converts ribonucleotides to deoxynucleotides to be used for DNA replication. In contrast, the salvage pathway, initiated by dCK, accounts for the majority of deoxycytidine nucleotide generated for repairing DNA, while only a small portion of the dCTP derived from the salvage pathway serves as a substrate for DNA replication. These findings suggest a model characterized by a deoxynucleotide triphosphate pool being derived from de novo pathway that is directed to replicating DNA and a second larger pool derived from the salvage pathway, which is available for DNA repair. The efficiency by which nucleoside analogues may be enhanced by combination with folate antagonists, including pemetrexed, which depletes nucleoside pools and is likely to generate higher dFdCTP concentrations; this would increase the chances of the drug being incorporated into the DNA. Indeed, the inhibition of hENT1 by nitrobenzylmercaptopurine ribonucleoside (NBMPR) in cells treated with fluorinated pyrimidines and gemcitabine, protects cells from drug cytotoxicity [1]. The increase in dCK activity may improve the efficacy of gemcitabine [2], while high expression of 5′-NT and CDA [3, 4] and RRM1 and RRM2 [5, 6] (Figure 1) has been found in cell lines resistant to gemcitabine.

**in vitro** studies on gemcitabine combinations

Recent data indicate that modulation of cellular enzymes of gemcitabine metabolism and uptake may influence drug activity against human tumor cell lines, underlying the need for a rational choice of combination treatments [7]. Simultaneous and sequential topotecan>gemcitabine treatments were synergistic in non-small cell lung cancer (NSCLC) cells, while the reverse sequence was antagonistic. DNA fragmentation, nuclear condensation and enhanced caspase-3 activity demonstrated that the drug combination markedly increased apoptosis in comparison with either single agent, while cell cycle analysis showed that topotecan increased cells in S phase. Furthermore, topotecan treatment significantly decreased the amount of phosphorylated Akt, and enhanced the expression of dCK in A549 and Calu-6 cells, potentially facilitating gemcitabine activity. On the contrary, the gemcitabine>topotecan sequence was antagonistic [8]. The combination of gemcitabine with the multitargeted antifolate pemetrexed in MIA PaCa-2, PANC-1, and Capan-1 pancreatic cancer cell lines proved to be synergistic and enhanced apoptosis most efficiently with the sequence pemetrexed>gemcitabine. Pemetrexed treatment...
accumulated cells in the S phase, the most sensitive to gemcitabine, and a positive correlation was found between the expression ratio of dCK:RR and gemcitabine sensitivity. Indeed, pemetrexed significantly enhanced dCK gene expression in MIA PaCa-2, PANC-1, and Capan-1 cells, and the crucial role of this enzyme was confirmed by impairment of gemcitabine cytotoxicity after dCK inhibition with 2′-deoxycytidine, thus demonstrating that gemcitabine and pemetrexed combination displays schedule-dependent synergistic cytotoxic activity, favorably modulates cell cycle, induces apoptosis, and enhances dCK expression in pancreatic cancer cells [7]. The same combination was tested against A549, Calu-1, and Calu-6 NSCLC cells. Synergistic cytotoxicity was demonstrated, and pemetrexed significantly decreased the amount of phosphorylated Akt, enhanced apoptosis, and upregulated dCK in A549 and Calu-6 cells, as well as hENT1 in all cell lines. Gemcitabine sensitivity was related to dCK expression, whereas transcription levels of TS, DHFR, and GARFT, the cellular targets of pemetrexed (Figure 1), were predictive of drug chemosensitivity, being higher in resistant cells. These results suggest that gemcitabine and pemetrexed synergistically interact against NSCLC cells through modulation of nucleotide pools, suppression of Akt phosphorylation and induction of apoptosis and pemetrexed enhances dCK and hENT1 expression, thus underlying the role of gene-expression modulation for rational development of chemotherapy combinations [9].

The isobologram analysis of cells treated with rapamycin in vitro alone and in combination with various anticancer drugs, showed synergistic interactions in combinations with paclitaxel, carboplatin, and vinorelbine. Additive effects were observed in combinations with doxorubicin and gemcitabine. Rapamycin dramatically enhanced paclitaxel- and carboplatin-induced apoptosis. This effect was sequence-dependent and mediated, at least in part, through caspase activation. Furthermore, rapamycin enhanced chemosensitivity to paclitaxel and carboplatin in HER2/neu-overexpressing cells [11].

Gemcitabine and paclitaxel dose-dependently inhibited the proliferation of bladder cancer cell lines and the combination yielded schedule-dependent cytotoxicity since the simultaneous treatment with gemcitabine/paclitaxel was associated with superior cytotoxicity after 48 h and 72 h, while sequential treatment showed similar results when gemcitabine was given 24 h before paclitaxel. However, when paclitaxel was given before gemcitabine, antagonism was observed. These results can be explained on the basis of cell cycle effect of drugs, since a shift towards the S-phase after gemcitabine and a progressive G2/M block after paclitaxel treatment were demonstrated. The results provide evidence that the combination of gemcitabine and paclitaxel in vitro yields superior cytotoxic efficacy, if given simultaneously or with gemcitabine first [12].

The cytotoxicity of gemcitabine on renal cancer cells was superior to vinblastine; pre-treatment with interferon (IFN)-α enhanced growth inhibition caused by gemcitabine and vinblastine, and was more effective than IFN-γ. Pre-exposure to IFN-α sensitized SN12C and ACHN renal cancer cells to gemcitabine. A-498 cells achieved a decreased sensitivity to gemcitabine and vinblastine after pre-exposure to IFN-γ. The data demonstrate the efficacy of gemcitabine in human renal cancer at clinically achievable doses and the favorable combination with IFN-α in vitro [13]. Gemcitabine and/or vinorelbine showed single-agent activity against NCI-H460 cells and targeted different phases of the cell cycle. When both drugs were used in combination, they showed schedule-dependent interaction. The optimum combination schedule was sequential exposure to vinorelbine followed by gemcitabine 24 h later, while an antagonistic effect was observed with simultaneous exposure to the two drugs [16]. The combination of gemcitabine and cisplatin (CDDP) or carboplatin on a panel of endometrial carcinoma cell lines revealed a dose-dependent decrease in cell growth by both gemcitabine its combination with platinum agents. The half-maximal inhibitory concentration of gemcitabine with CDDP was significantly lower than that of gemcitabine alone for all cell lines, including a platinum-resistant line [14]. These findings are in agreement with the evidence that inhibition of the repair of CDDP-induced DNA lesions plays a critical role in gemcitabine-mediated cytotoxic synergism with CDDP. The nucleotide excision repair (NER) process, which is responsible for the cellular removal of CDDP-DNA adducts, may be a target for the mechanism of the cytotoxic synergism of gemcitabine and CDDP. In a NER-proficient, MMR-deficient human colon carcinoma cell line gemcitabine inhibited the incorporation of dATP and dCTP, suggesting that the repair inhibition by gemcitabine does not result simply from competition for the incorporation site but rather is also due to prevention of chain elongation during the DNA resynthesis process. Finally, suppression of ERCC1 expression and impairment of cellular repair capability by ERCC1 antisense RNA significantly correlated with the reduction of the cytotoxic synergism of the two agents [15].

**conclusions**

The in vitro studies provide evidence of a remarkable versatility of gemcitabine which is able to synergize with several anticancer agents, particularly cisplatin and pemetrexed. All these effects are explained on the basis of the ability of gemcitabine to affect cell cycle, cooperate in the DNA damage of combined anticancer drugs or being modulated at the metabolism level. Information obtained from preclinical studies will help in the optimal
development of gemcitabine combinations for the treatment of several types of cancer.

disclosures
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references