Drug resistance and pre-clinical drug development

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Inhibition of cisplatin-induced apoptosis by dexamethasone in cervical and bronchial carcinoma cells

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Cisplatin is one of the most frequently used cytotoxic drug in the chemotherapy of human cancers. Its therapeutic effectiveness, however, is restricted by toxic side effects on normal tissues and increasing resistance of the tumor cells. Nausea and hyperemesis are almost regularly observed in patients receiving cisplatin and require antiemetic medication. Various clinical trials suggest that the efficacy of the most antiemetic drugs used to prevent cisplatin-induced nausea is significantly enhanced by dexamethasone. For this reason, dexamethasone is almost regularly given to patients during cisplatin-based chemotherapy protocols. The potential interference of dexamethasone with the cytotoxic activity of cisplatin, however, has not been assessed so far.

In experiments described here, we observed that dexamethasone significantly reduces the cytotoxic activity of cisplatin in various human cervical and bronchial cancer cell lines. DNA staining and subsequent flow cytometric analyses of cisplatin-treated carcinoma cell lines revealed the interference of the cisplatin-mediated induction of apoptosis by dexamethasone. In order to analyze the molecular mechanism for the enhanced resistance to cisplatin treatment, we investigated the dexamethasone-mediated modulation of genes which are involved either in the regulation of resistance to cisplatin or in the induction of apoptosis upon genotoxic stress in the cervical carcinoma cell line SW756 and the squamous cell lung carcinoma cell line P693. Our data suggest that different molecular mechanisms are responsible for the enhanced cisplatin resistance of dexamethasone treated cancer cells depending on the individual genotype.

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The $\alpha v\beta 3$ integrin as a molecular target for anticancer drug design

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Rationale: Upregulation of the $\alpha v\beta 3$ integrin is associated with increasing metastatic potential in malignant melanoma. Integrins are now known to be involved in cell functions other than adhesion. Blockade of the $\alpha v\beta 3$ integrin using monoclonal antibodies has previously been shown to induce apoptosis in melanoma cell lines (Montgomery, et al., PNAS 91: 8856–60, 1994). A common ligand binding motif for integrins is the amino acid sequence RGD. Cyclisation of RGD containing peptides can enhance binding affinity for integrins.

Aims of Study: To characterise the biological effects of a cyclic RGD peptide on a panel of melanoma cell lines derived from different stages of tumour progression.

Methods: Integrin profiles were determined using FACS analysis and Western Blotting. The activity of cyclic RGDfV was investigated on melanoma cell lines growing on various extracellular matrix proteins and on cells growing in 3-dimensional type-1 collagen gels. Morphological and biochemical parameters associated with programmed cell death were investigated using standard assays. Phosphorylation of paxillin and Focal Adhesion Kinase were examined using Western Blotting.

Results: All of the cell lines examined expressed high levels of $\alpha v\beta 3$ with one of the cell lines also expressing low levels of $\alpha v\beta 5$. Our studies indicate that cRGDfV has potent activity against melanoma cell lines, the main effects being as follows; cRGDfV (i) inhibits $\alpha v\beta 3$ mediated cell adhesion, (ii) prevents colony formation in 3-D collagen gels, (iii) induces programmed cell death of cells growing in 3-D collagen gels, (iv) induces a rapid dismantling of Focal Adhesion Complexes.

Conclusion: Blockade of the $\alpha v\beta 3$ integrin with a cyclic RGD containing peptide has a profound effect on melanoma cell morphology and survival. Our observations suggest that the $\alpha v\beta 3$ integrin is a useful molecular target for anticancer drug design.

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Expression of *r*-glutamylcysteine gene in human urothelial cancer: Relation to chemotherapy response

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Purpose: We evaluated the possible correlation between intra cellular glutathione (GSH) production and drug sensitivity of urothelial cancer.

Methods: Tumor specimens from 19 patients with metastatic or advanced urothelial cancer were studied for GSH content, and expression of *r*-glutamylcysteine synthetase (*r*-GCS). The intracellular GSH was assayed with HPLC (Hewelett Packard 1050 series). The messenger RNA expression of *r*-GCS was quantitated by RT-PCR assay with gene specific primers All patients were then treated with methothrexate, epirubicin and cisplatin (MEC) combination chemotherapy for at least two cycles and were classified accordingly by clinical response criteria.

Results: Three patients had complete response (CR), 8 had partial response (PR) and 8 had no response (NR). The results were shown in following table:

Clinical Response	GSH levels μM/mg protein	*r*-GCS RNA Expression
CR+PR (11)	2 88 ± 2 00	2.75 ± 1 92
NR (8)	9 38 ± 5.90	10.36 ± 4.04
P value	0.0012	0.0001

The tumor tissue of patients with CR and PR contained significantly lower level of GSH and expression of *r*-GCS RNA. The higher GSH content in poor responders was correlated to the increase gene expression of *r*-GCS (Spearman rank correlation coefficients 0.6965, $P = 0.001$).

Conclusion: Increase expression of *r*-GCS in urothelial cancer is responsible for overproduction of GSH which may play an important role for intrinsic resistance of urothelial cancer to chemotherapy.

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Pharmacokinetics (PK) and pharmacodynamics (PD) of SDZ PSC 833, a novel multidrug resistance reversing agent, in phase 1 trials with chemotherapeutic agents

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Purpose: SDZ PSC 833, a multidrug-resistance (MDR) reversing agent, has progressed through clinical trials. As P-glycoprotein is expressed in normal tissues with excretory and/or barrier protective functions, PK interactions between MDR-reversing agents and P-glycoprotein substrates are to be expected. We therefore characterized the PK and PK/PD relationships of SDZ PSC 833.

Methods: Patients with advanced cancer were first given chemotherapy alone. Subsequently, patients were given SDZ PSC 833 alone to characterize SDZ PSC 833 PK, followed by a combination of SDZ PSC 833 with chemotherapy.

Results: SDZ PSC 833 PK are linear and predictable. SDZ PSC 833 PK are correlated with dose-limiting toxicity – only patients with concentrations above 3000 ng/ml developed reversible Grade III ataxia. SDZ PSC 833 causes an increase in the area under the curve (up to 80%) and decrease in the clearance (up to 60%) of chemotherapy, necessitating dose reduction for equitoxicity.

Conclusions: SDZ PSC 833 can safely be administered to cancer patients. Necessary dose reductions in chemotherapy when given with SDZ PSC 833 produce equitoxicity while offering the chance for resistance reversal.