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Effect of silybin and docetaxel on LNCap prostate cell growth and total PSA levels

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Silybin, a flavonoid obtained from *Silybum marianum L.*, and its phospholipid complex IdB 1016 (silipide) have been shown to have antitumor activity and to potentiate the effects of cisplatin (Giacomelli et al., Life Sci. 70: 1447, 2002), and doxorubicin in mice implanted with DU145 human prostate tumor xenograft (Tyagi et al., Clin. Cancer Res. 8: 3512, 2002). The current study was performed to assess the effects of silybin on the growth of human prostate LNCap cells and their secretion of prostate specific antigen (PSA) *in vitro*. LNCap cells were plated at different densities in 96 well coated (poly-D-lysine) plates (Becton-Dickenson Biocoat) and incubated for 24h prior to the addition of various concentrations of silybin (0.2–200uM) or docetaxel (0.1–100nM). After 5 days, tumor cell mass was assessed using sulphorhodamine B assay and total secreted PSA was measured using a kit supplied by United Biotech. The concentration of agent to decrease tumor cell growth by 50% (IC50) was 55uM for silybin and 0.36nM for docetaxel. When human LNCap prostate tumor cells were treated with subtoxic concentrations of silybin (10–50uM) and docetaxel (0.1–0.3nM), dose dependent decreases in both cell number and PSA level were noted. However, when secreted PSA levels were normalized per cell number for docetaxel treated cells, there was no dose dependent change noted. This suggests that silybin not only inhibits LNCap cell growth but also directly decreases PSA secretion, supporting its potential use as a treatment for prostate cancer in man.

Prodrugs

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MVA-FCU1: a highly potent gene-based chemotherapy providing 5-FU local delivery

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Background: Direct transfer of pro-drug activation systems into tumours was demonstrated to be an attractive method for the selective *in vivo* elimination of tumour cells. Besides its local cytotoxic impact, this strategy was further demonstrated to enhance the host anti-tumour immune response through the local release of cellular debris that can be presented by the antigen presenting cells.
Material and methods: We describe a novel and highly potent suicide gene derived from the *Saccharomyces cerevisiae* cytosine deaminase (*FCY1*) and uracil phosphoribosyltransferase genes (*FUR1*). This suicide gene, designated *FCU1*, encodes a bifunctional chimeric protein that combines the enzymatic activities of *FCY1* and *FUR1* and efficiently catalyses the direct conversion of 5-fluorocytosine (5-FC), a non-toxic pro-drug, into the cytotoxic metabolites 5-fluorouracil (5-FU) and 5-fluorouridine-5′-monophosphate (5-FUMP). Interestingly, the cytosine deaminase activity is 10-fold higher in the chimeric protein compared to the natural protein.
Results: In this study we demonstrate that a MVA (Modified Vaccinia Virus of Ankara) engineered to express the *FCU1* gene significantly enhances the sensitivity of numerous human tumour cells to 5-FC (LD_{50} 5-FC = 1 μM in the FCU1 treated cells compared to LD_{50} 5-FC = 10mM in the CDase treated cells; $p<0.01$). Moreover, passive diffusion of the 5-FU ensures an impressive bystander effect with the ability to kill 100% of a *in vitro* tumour cell population with only 1% FCU1-transduced cells.
Intratumoral injections of MVA-FCU1 into human tumour-bearing mice, with concomitant systemic administration of 5-FC, led to a sustained control of tumour growth. The FCU1-induced tumour growth suppression was observed in different human colorectal tumour models whereas 5-FU administered IP at the maximum tolerated dose did not show any anti-tumor effect in the same model.
Finally, a 10-fold higher concentration of 5-FU is detected inside the tumour compared to a systemic administration of 5-FU while no detectable 5-FU is found in the circulation, ensuring a higher safety profile with no systemic toxicity.
Conclusions: The FCU1 suicide gene is a **unique combination** of an **innovative approach** and a **validate and secure chemotherapy** that makes it a novel and powerful candidate for treating all 5-FU sensitive tumours. A Phase I clinical trial is scheduled early 2005 in metastatic colorectal cancer patients.

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Bio-reductive prodrug approach to target angiogenesis in tumours

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Angiogenesis, the development of new blood vessels from existing ones, is a natural process occurring at many stages of life. A tumour cannot grow beyond a size of 1–2mm without being supplied by oxygen and nutrients. To overcome this problem, cancerous cells in hypoxic areas of tumours have the ability to trigger angiogenesis by a complex mechanism involving up and down regulations of transduction modulators. The subsequent vasculature of the tumours enables its growth and the formation of metastases.
In the last decade, many therapeutic compounds have been developed to inhibit angiogenesis. This work is focused on improving the delivery of such compound to hypoxic areas of tumours, using the unique properties of the tumour micro-vasculature. Endothelial cells within tumours are known to over-express reducing enzymes such as NQO1, NQO2 and cytochrome P450 reductase. We aim to design "bio-reductive prodrugs" which will, upon reduction by one of these enzymes, release the active anti-angiogenic drug. This project is particularly focused on the design and evaluation of nitroaromatic delivery systems.
Five nitroaromatic systems (**Figure 1**) were attached to a fluorescent compound (4-methyl-7-hydroxycoumarin) thus forming five "pro-fluorescent" prodrugs. These model-compounds enable the comparison by fluorescence spectroscopy of the behaviour of the delivery systems under chemical reductive conditions. The same delivery systems were then attached to two well-known anti-angiogenic compounds: the isoflavone biochanin A and an oxindole (SU5416®).

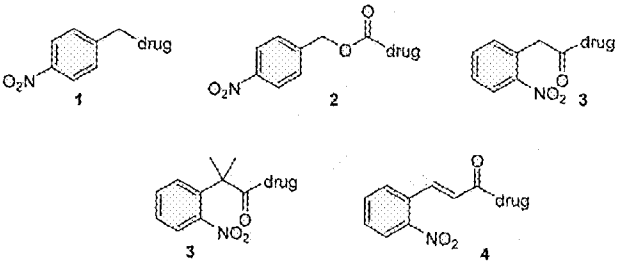

Figure 1. Delivery system–drug complexes.

The prodrugs prepared have been tested *in vitro* on a VEGF stimulated angiogenesis model. Endothelial cells in culture, in presence of VEGF, form a "vessel-like" structure. The intracellular enzymatic reduction of the nitro group into the hydroxylamine/amine is expected to trigger the release of the drug, which will inhibit the formation of this vessel-like structure.
While the prodrugs **1** and **5** did not lead to the delivery of the free drug upon chemical reduction and prodrug **2** was easily hydrolysed in aqueous conditions, prodrugs **3** and **4** seemed to be good systems under chemical reduction and *in vitro*, leading to very good inhibition of angiogenesis at concentrations of 10 and 100 μM.
Further research needs to be completed in quantifying the enzymes involved and optimising the delivery system–drug combination.

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PSA-activated prodrugs for the treatment of prostate cancer

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The construction and characterization of prodrugs activated by prostate specific antigen (PSA) – a protease – is described. A panel of recombinant, PSA-sensitive molecules was engineered from a template Type II ribosome-inactivating protein. The resultant prodrugs are latent toxins comprised of: an A chain with ribosome-inactivating N-glycosidase activity, and a B chain with lectin-binding activity and an interchain peptide linker. In previous studies, we successfully created prodrugs that could be activated by MMPs associated with solid tumors. In the current study, linkers were introduced to the protein template so as to regulate cytotoxicity by PSA cleavage. Linkers varied in length from 8 to 14 amino acids and contained variations of an hexapeptide PSA recognition sequence. Using Western blot analysis, prodrugs with longer linkers (10 to 14 aa) were efficiently cleaved by recombinant PSA; whereas no cleavage of prodrugs having 8 or 9 aa linkers was detected. Cleavage and activation of the prodrugs was PSA-specific insofar as the molecules could not be cleaved by tumor-associated MMPs or other selected proteases. The cytotoxicity of the PSA variants was measured using control cell line (COS-1) and two prostate cell lines (DU145 and LNCaP), which are PSA-negative and PSA-positive,