reductase activity was measured by the method based on cytochrome c reduction in the presence of NADPH. Antioxidative enzymes in tissue and circulation (SOD, CAT, GR, and GST) were analyzed by standard procedures; enzymes, markers of tissue necrosis, were determined by commercial assays. Apo-direct assay was used for apoptosis detection.

Results: Proanthocyanidins did not prevent tumour development, but they influenced tumour growth dynamics. Mice treated with PRO/DOX combination developed neither ascitic nor solid tumours. PRO significantly prolonged survival of mice treated with DOX/PRO combination. Proanthocyanidins inhibited *in vitro* ESR signal of DOX semiquinone radical, and competitively inhibited activity of NADPH:cytochrome P450 reductase. Proanthocyanidins modified antioxidative enzymes activity and modulated their activity in rat heart and circulation 48 hours after DOX treatment. Proanthocyanidins synergistically increased DOX-induced apoptosis in Ehrlich ascitic tumour cells.

Conclusions: Obtained results indicated that effects of PRO are multiple and complex and PRO should not be treated only as free radical scavengers. Proanthocyanidins decreased DOX cytotoxicity but did not compromise its antitumour activity. The underlying molecular mechanism of this effect was inhibition of P450 reductase by PRO that resulted in decreased metabolic transformation of DOX followed by smaller production of reactive oxygen species. These results indicated that, for the PRO effects observed in our model system, activity in decreasing production of DOX metabolites and ROS reactive oxygen species was even more important than direct free radical scavenging activity of PRO.

239 15-deoxy-delta-12,14-Prostaglandin-J2 inhibits multidrug resistance genes and induces apoptosis in doxorubicin-resistant ovarian carcinoma cells

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Background: Development of chemoresistance in tumour cells is one of the biggest problems in cancer therapies. Multidrug resistance (*mdr1*) upregulation is known to be related to the activation of NF-κB pathway in tumour cells. Recently, we have reported that 15-deoxy-delta-12,14-Prostaglandin-J_2 (15d-PGJ_2), a peroxisome proliferator-activated receptor-gamma (PPAR-γ) agonist, induces cell death in non-resistant tumour cells by inhibiting at least partly the NF-κB activity and exhibited antitumour effects *in vivo*. In the present study, we investigated whether 15d-PGJ_2 could also inhibit NF-κB pathway in doxorubicin-resistant ovarian carcinoma cells and thereby induce apoptosis and inhibit *mdr1* expression.

Material and Methods: Human ovarian carcinoma cell lines, A2780 (non-resistant) and A2780/AD (doxorubicin-resistant), were used for this study. The cell viability was evaluated using Alamar blue assay by incubating with 15d-PGJ_2 and other compounds for 48h in serum-free medium. To determine the NF-κB and caspase-3/7 activities, a plasmid-based Luciferase reporter assay and a luminescence assay were used, respectively. Gene expression levels were determined through quantitative real-time PCR.

Results: Doxorubicin-resistant A2780/AD cells had significantly higher expression of *mdr1* and enhanced NF-kB activity compared to the non-resistant A2780 cells. Treatment with 15d-PGJ_2 induced cell death in both A2780 and A2780/AD cells with a similar potency (IC_{50}=5.0 μM). The effects were found to be PPARγ-independent as an irreversible PPARγ antagonist GW9662 could not block these effects. Also, 15d-PGJ2 significantly enhanced the caspase-3/7 enzyme activity in both cell types indicating its caspase-mediated apoptotic activity. Furthermore, we found that 15d-PGJ_2 significnalty inhibited the NF-kB activity either inherent or induced with TNF-alpha in these cells. At a low dose (2.5 μM), 15d-PGJ_2 significantly inhibited the expression of drug resistant-related genes (*mdr1* and *sirt1*) and antiapoptotic genes (*bcl-2* and *bcl-xl*) in A2780/AD cells. Treatment with a specific NF-κB inhibitor BAY11–7082 only inhibited *mdr1* and *sirt1* gene expression but not of *bcl-2* and *bcl-xl*. These data suggest that inhibition of the drug resistance regulating genes by 15d-PGJ_2 is mediated through the blockade of the NF-κB pathway.

Conclusion: The present study demonstrates that 15d-PGJ_2 can induce apoptosis in doxorubicin-resistant ovarian tumour cells and also inhibits multidrug resistance-regulating genes. Therefore, 15d-PGJ_2 is in potential a promising therapeutic agent for the treatment of chemoresistant tumours.

240 Inhibition of cell survival, tumour growth and histone deacetylase (HDAC) activity by the dietary flavonoid luteolin in human epithelioid cancer cells

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Phytochemical compounds and histone deacetylase (HDAC) inhibitors are emerging as a new generation of anticancer agents with limited toxicity in cancer patients. The present study investigates the impact of luteolin, a dietary flavonoid, on survival, motility, invasion of cancer cells, and tumour growth *in vivo*. We found that luteolin (25–200 mM) decreased the viability of human cancer cell lines originating from the lung (LNM35), colon (HT29), liver (HepG2) and breast (MCF7). Luteolin effectively increased the sub-G1 fraction of apoptotic cells through caspases 3 and 7-dependent pathways. The effect of luteolin on cell-cell adhesion, motility and invasion was investigated using slow aggregation, wound healing, collagen type I and chick heart invasion assays, no effect on intercellular adhesion and motility was observed at non toxic concentration. However, the effect of luteolin on cell invasion seem to be highly tissue and cell type-dependent since luteolin was ineffective to reverse the invasive potential of LNM35, but inhibit the invasiveness of MCF-7/6 cells. Moreover, we demonstrated that luteolin is a potent HDAC inhibitor and that it potentiates the cytotoxicity of cisplatin in cultured LNM35 cells and decreased the growth of LNM35 tumour xenografts after intra-peritoneal injection (20 mg/kg) in athymic mice. Taken together, our data indicate that luteolin is a promising HDAC inhibitor for the treatment of lung cancer in combination with standard anticancer drugs such as cisplatin.

241 L-2-oxothiazolidine-4-carboxylate reverses the growth-promoting effect of growth factors, improving the oxaliplatin antitumour response in WiDr colon cancer cells

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Background: A common cause of treatment failure in colorectal cancer is chemoresistance, which may be related to the redox state of cancer cells and the tumour microenvironment, where growth factors (GFs) play an important role. Glutathione (GSH), a key regulator of the redox balance, is involved in GF signalling systems and may also protect against drug-induced cellular injury.

Materials and Methods: The effect of L-2-oxothiazolidine-4-carboxylate (OTZ, a GSH modulator) on oxaliplatin activity in the WiDr colon cancer cell line was studied. Cell proliferation and GSH content were assessed. Cells were exposed to the OTZ before treatment with oxaliplatin in the presence of either hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF) or epidermal growth factor (EGF).

Results: Exposure to GFs significantly increased GSH levels and induced a pro-tumour effect. During the first 48 h of incubation, GFs induced a near 20% reduction in oxaliplatin antitumour activity. Treatment with OTZ abrogated the growth-promoting effects of GFs and increased the antitumour effect of oxaliplatin. Moreover, the enhancement of oxaliplatin-induced growth inhibition produced by OTZ pretreatment was observed at 24 h with approximately a 30% increase being noted with respect to drug activity in the presence of GFs. In fact, this combination resulted in a dose modification factor (DMF) of 2, 1.3 and 1.5, in the presence of HGF, VEGF and EGF, respectively at 24 h, indicating an apparent synergistic effect ($P < 0.001$). At 72 h the combined therapy resulted in a 2.5-fold reduction ($P < 0.001$) in the proliferation rate compared with controls (DMF of nearly 0.9).

Conclusion: GSH manipulation by OTZ could yield a therapeutic gain for chemotherapy with oxaliplatin in the presence of GFs.

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