and DNA-PK and act as a radiosensitizer, potentiates the cytotoxicity of etoposide, a topoisomerase II poison.
Materials and methods: Cell lines competent or defective in non-homologous end-joining (ATM, Ku86, DNA-PKcs) or in the zeta isoform of protein kinase C (PKCzeta), were exposed to graded concentrations of etoposide without or with pre-treatment with wortmannin. Cell survival, topoisomerase II decatenation activity, DNA double-strand break rejoining and phosphorylation of effectors downstream, on the one hand from ATM or ATR (Chk2, Chk1), on the other hand from PI3K (Akt/PKB, PKCzeta) were taken as an endpoint.
Results: Wortmannin stimulated the decatenating activity of topoisomerase II, promoted accumulation of DNA double-strand breaks and potentiated the lethal effect of etoposide through two pathways. Sensitization to high, micromolar amounts of etoposide required DNA-PK integrity. In contrast, wortmannin dramatically enhanced the susceptibility to low, nanomolar amounts of etoposide in a large fraction of the cell population irrespective of the status of ATM, Ku86 and DNA-PKcs, and shifted the specificity for cell killing by etoposide from S to G1 phase of the cell cycle. To determine whether PKCzeta was involved in this process, U937 cells bearing stable expression of a dominant-negative, kinase dead mutant of PKCzeta were exposed to submicromolar amounts of etoposide. PKCzeta-defective cells actually reproduced the hypersensitivity pattern induced by wortmannin. In addition, transient hyperphosphorylation of PKCzeta was observed in PKCzeta-competent cells at 2 h interval from contact with etoposide and was abolished by wortmannin.
Conclusions: It is proposed that wortmannin acts through disruption of a phosphorylation cascade involving PI3K, type I phosphoinositide-dependent protein kinase (PDK1) and PKCzeta in sequence, resulting in loss of topoisomerase II Ser phosphorylation. After Plo et al. (J Biol Chem 277: 31407–31415, 2002) it is suggested that potentiation of the cytotoxic response to submicromolar concentrations of etoposide induced by wortmannin or PKCzeta knockout, is due to increased activity of the beta isoform of topoisomerase II.

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AQ4N mediated potentiation of chemoradiotherapy in human lung tumour xenografts

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Background: AQ4N (1,4-bis[[2-(dimethylamino)ethyl] amino]-5,8-dihydroxy-anthracene-9,10-dione bis-N-oxide) is a bioreductive topoisomerase II inhibitor that is currently in a Phase I clinical trial in combination with radiation. The purpose of this study was to provide data to support the use of AQ4N in chemo (cisplatin)-radiation protocols in carcinoma of the lung.
Materials and Methods: Calu-6 xenografts (established from the ID implant of 2×10^6 cells in 0.1ml of a 1:1 matrigel:serum free RPMI mix) were treated at a size of 240–280mm^3. Radiotherapy was given as 2 Gy fractions (five days on, two days off) to maximum doses of 20 or 30 Gy. AQ4N (60mg/kg) was given weekly 30 minutes prior to the first radiation dose. Cisplatin (2mg/kg) was given once 6 hours after the first fraction. The growth delay for tumours to quadruple in size was taken as the experimental endpoint. To disclose the hypoxic fraction of Calu-6 xenografts, pimonidazole was administered at 60mg/kg. AQ4N metabolism was evaluated using HPLC.
Results: Pimonidazole binding revealed that Calu-6 xenografts have a clinically relevant hypoxic fraction of 1–10%. AQ4N potentiated the response of Calu-6 xenografts to chemoradiotherapy *in vivo*. When combined with 10×2 Gy and cisplatin, AQ4N afforded an additional 7-day growth delay compared with cisplatin-radiotherapy alone (44 ± 1 versus 37 ± 4 days). This enhancement equated to that which would be achieved using an additional 4 fractions of radiation. In support of previous data AQ4N significantly enhanced the outcome of radiotherapy alone yielding a growth delay of 37 ± 2 days compared with 26 ± 5 days ($p=0.05$). Metabolism studies revealed that nitric oxide synthase (NOS), an enzyme that is commonly over-expressed in human tumours, can reduce AQ4N to form the $2e^-$ intermediate AQ4M whereas cytochrome p450s may have a preferential role in the conversion of AQ4M to the cytotoxic AQ4 moiety.
Conclusions: These data support the future clinical evaluation of AQ4N in cisplatin based chemoradiotherapy protocols in carcinoma of the lung and indicate that tumour specific expression of NOS may facilitate reductive metabolism of this agent.

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Alterations of topoisomerase IIalpha, associated with in vivo resistance to tafluposide, are partially reversible

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Earlier studies identified tafluposide as a novel dual inhibitor of topoisomerases I and II, with different mechanistic properties from most specific inhibitors of topoisomerases. Indeed, tafluposide appears to act by preventing the binding of topoisomerase I or II to DNA through a preferential interaction with the enzyme, but it is also capable of trapping topoisomerase II in an ATP-independent noncovalent salt-stable complex. Subsequent studies provided evidence that tafluposide is also a potent inhibitor of nucleotide excision repair. This compound has marked antitumor activity in a series of experimental tumor models and is now ongoing Phase I clinical trials. In the attempt to identify *in vivo* the targets involved in the antitumor activity of tafluposide, a P388 leukemia subline resistant to tafluposide was established *in vivo*. The results showed that resistance to tafluposide was mainly associated with alterations of topoisomerase IIα and the endonuclease XPG, involved in NER, whilst only minor modifications of topoisomerases I and IIβ were recorded. P388/tafluposide resistant cells exhibited a marked reduction in topoisomerase IIα protein level (87%), and a K155N mutation of this enzyme. Nucleotide excision repair activity was decreased, which was more specifically associated with a decreased level of XPG. To further study the implication of topoisomerase IIα in tafluposide antitumor activity, the stability of the resistance to tafluposide was investigated. P388/tafluposide tumor cells were maintained in mice without treatment with tafluposide for 6 months. The resulting P388/tafluposide-6m cells did not exhibit further K155N mutation, whilst they still exhibited a reduction of the level of topoisomerase II, although it was less important (75%). These P388/tafluposide-6m cells partially recovered sensitivity to tafluposide treatment *in vivo*, as reflected by an optimal increase of lifespan of 100%, as compared to 0% for the P388/tafluposide resistant subline and 300% for the parental sensitive P388 cells. These data suggest that resistance to tafluposide is partially reversible and further support the implication of topoisomerase IIα in tafluposide antitumor activity.

Tubulin-interacting agents

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Preclinical evaluation of the second generation vascular disrupting agent OXI 4503

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Vascular disrupting agents (VDAs) for the treatment of cancer are designed to cause a rapid and selective shutdown of tumor vasculature, which leads to secondary ischemic tumor cell death. The lead drug combretastatin A4 disodium phosphate (CA4DP) are currently being examined in various clinical trials with encouraging results. Efforts are ongoing to develop new agents with improved activity and therapeutic windows. In the present studies, we studied the efficacy of a novel analog of combretastatin, OXI 4503.
The vascular and antitumor effects of OXI 4503 were assessed in the KHT murine sarcoma model. Tumor blood perfusion was estimated by Hoechst 33342 fluorescent labeling. Significant reduction in functional vessel was observed as early as * hr after a dose of 10 mg/kg or 25 mg/kg of OXI 4503. Histologic and morphometric assessments carried out using image analysis system on tumor sections showed the viable tissue remaining in these tumors 24 hr after treatment with a 25 mg/kg dose of OXI 4503 to be less than 6%. There was extensive necrosis induced within the tumor. This pattern extended to the very edge to the tumor, stopping at the surrounding soft tissue at many locations; while at other sites there remained a rim that was few cells thick. Tumor vascular status prior to and post treatment with OXI 4503 was also assess with non-invasive magnetic resonance imaging (MRI). The results of MRI GdDTPA inflow measurement revealed that OXI 4503 treatments significantly reduced perfusion in KHT sarcomas. The proportion of perfused tumor tissue was notably less after OXI 4503 treatment compared to CA4DP.
Administration of OXI 4503 to tumor bearing mice resulted in a dose-dependent increase in tumor cell killing. OXI 4503 was more potent than CA4P on a per dose basis. For example, compared to CA4DP, 4-fold lower doses of OXI 4503 resulted in equivalent reduction in KHT sarcoma clonogenic cell survival. Administration of OXI 4503 to the animals also