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Biological activity of thiocoraline, a novel marine depsipeptide

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Introduction: Thiocoraline (THIO) is a cyclic octapeptide isolated from *Micromonospora marina*. THIO exhibits potent activity against human NSCL, colon, and melanoma cell lines with IC50's of 2.50E-09M. This profile is expanded to include *in vivo* activity and levels of effect.

Methods: The antitumor (AT) activity of THIO was evaluated against colon, NSCL and mammary adenocarcinomas implanted *sc* in nude mice. In addition, THIO was evaluated against an *iv* administered melanoma. The drug was given *ip* at 1/4, 1/2 or MTD (120 mg/kg) using a Q4D × 3 schedule. Macrosynthesis assays were performed in a cell-free system using purified enzymes. Cell cycle studies utilized LoVo colon tumor cells and a Pgp overexpressing adriamycin-resistant subline (LoVo/DX) treated for 1 or 24 hrs. Phase distribution was evaluated using biparametric BrdU/DNA flow cytometric analysis.

Results: THIO is a very effective AT agent against A549 lung (31% T/C) and MX-1 mammary (22% T/C) human tumors at 1/2 and 1/4 MTD's, respectively, but not against CX-1 colon human tumors. THIO is also very potent against an *iv* implanted murine B16 melanoma with a 248% ILS (348% T/C) at the MTD and 133% ILS (233% T/C) at 1/2 MTD. THIO strongly inhibits RNA synthesis, binds DNA, but does not cause any detectable alkylation nor inhibits DNA topoisomerases. THIO blocks progression of cells from G_1 to S phase and slows progression of cells from S to G_2M. The effect is greater on LoVo/DX cells and the inhibitory effects are reversible.

Conclusion: THIO has significant AT activity, a good therapeutic index, and is currently under preclinical evaluation as a potential candidate for clinical development.

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Malignant mesothelioma: In vitro responses to new chemotherapeutic agents, and correlation to GSTM1 and NAT2 gene polymorphism

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Purpose: To assess the responses of 3 mesothelioma cell lines to 5 new chemotherapeutic agents and interleukin-4, and to correlate them to the known GSTM1 and NAT2 genotypes of the cell lines.

Methods: The 3 cell lines will be tested with Gemcitabine, docetaxel, paclitaxel, vinorelbine and CPT-11 and with interleukin-4 (IL-4) as single agents and in combination. Anti-proliferative effects will be assayed by vital dye exclusion. Subsequently a clinical trial will be designed using the most effective combination chemotherapy and IL-4.

Results: Gemcitabine and paclitaxel have already been tested in the three cell lines, two of which have the GSTM1-null/NAT2 slow acetylator genotype and one which has the normal GSTM1/NAT2 fast acetylator genotype. All three cell lines responded in a dose-dependent manner to Gemcitabine, but the cell line with the normal genotype was 50 times less sensitive. The two "negative" cell lines were very sensitive to Gemcitabine with only 56 and 61% of living cell surviving, compared to controls, in 5×10^{-8} M Gemcitabine. The response to paclitaxel was not dose-dependent and there was no difference in sensitivity between the cell lines.

Conclusion: These initial findings suggest that the sensitivity of mesothelioma cell lines may be related to their GSTM1/NAT2 genotype, and this may have important implications for mesothelioma chemotherapy.

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Antitumoral effects of octadecylphosphocholine analogous

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A new class of alkylphosphocholines has been synthesized which shows stronger antitumoral properties than the well known hexadecylphosphocholine. The strong antitumoral effects of the enantiomeric octadecylphosphocholines substituted in position 2 with amino, hydroxyl, acetic ester or acetamide was demonstrated in the leukemic HL-60 and in the breast cancer cell line MDA 468. To differentiate between specific effects on enantioselective phospholipases and unspecific membrane alterations based on the biophysical properties of the new compounds, the R- and the S-(unnatural)-configurated compounds were tested. PLA_2 and lysophosphatidyl acyltransferase have been identified as targets for the new compounds. Structures similar to 1-O-phosphocholine-2-hydroxy-octadecane have been reported as lysophosphatidyl transferase inhibitors and some of the new compounds are potent inhibitors of PLA_2-II *in vitro*. Our studies indicate that the inhibition of cellular phospholipases by specific inhibitors is a very promising approach to inhibit tumor progression.

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Antitumour activity of acid labile transferrin and albumin doxorubicin conjugates in *in vitro* and *in vivo* human tumour xenograft models

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Purpose: Site-specific delivery of anticancer agents is an important goal in cancer chemotherapy. We therefore assessed the *in vitro* and *in vivo* efficacy of acid labile doxorubicin conjugates with the carrier proteins transferrin and albumin, which both exhibit a significant uptake in tumour tissue (the link between drug and protein was realised through a carboxyl hydrazone bond).

Methods: *In vitro* activity of the protein conjugates and free doxorubicin was evaluated in 5–10 human tumor xenografts using a clonogenic assay and furthermore in xenograft mamma carcinoma models in nude mice (s.c. MCF-7 and MDA-MB-435) – route of application: i.v.; treatment: twice, interval of 7 days; dose: 4, 8 and 12 mg/Kg.

Results: *In vitro* activity of the conjugates was comparable or exceeded that of doxorubicin. *In vivo* data show a significantly reduced toxicity (reduced lethality and gastrointestinal side effects) with a concomitantly stable or improved antitumour activity of the protein conjugates compared to free doxorubicin.

Conclusion: Acid labile transferrin and albumin conjugates of doxorubicin are suitable candidates for further biological studies in *in vivo* systems.

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Design of an antisense oligonucleotide strategy to investigate the mode of action of the multidrug resistance-associated protein

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Purpose: To date, the search for blockers of the multidrug resistance-associated protein (MRP) has yielded agents of limited specificity and high cellular toxicity. An overexpression of MRP has been implicated in multidrug resistance in human cancer, drug-derived human cancer cell lines and MRP-transfected cultured cells. The presence of the protein at the cell membrane is frequently associated with a reduced intracellular drug concentration and/or altered drug distribution within the cell. While it appears likely that substrate drugs or drug derivatives (particularly glutathione-S conjugates) are translocated by MRP across cell membranes, direct evidence for translocation and substrate-binding sites has not been found. In this study, a strategy has been devised to exploit inhibition of MRP translation through an mRNA-targeted antisense oligonucleotide (ODN).

Methods: Delivery of ODNs to COR-L23/AR, a human large cell lung cancer line that overexpresses MRP, has been optimised using a series of ODNs targeted to different regions within the MRP mRNA. The effects of the ODNs are assessed by measurement of the accumulation of the fluorescent marker calcein.

Results: Cationic liposome-mediated delivery was shown to yield vastly increased levels of intracellular ODN when compared to levels in cells bathed in ODN-containing medium. Notably, nuclear concentrations were particularly increased. Differential effects of ODN on calcein uptake are discussed.

Conclusions: Antisense ODNs against MRP may provide a means to investigate the precise role of MRP in multidrug resistance.