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Cellular accumulation of cisplatin is mediated by the ubiquitously expressed sodium-dependent human ASCT1-amino acid transporter

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Cellular accumulation of cisplatin (DDP) is not well understood, however, the involvement of an active transport system has long been suggested. We have shown previously that L-cysteine (Cys) strongly and competitively inhibited the uptake of DDP in renal tubule epithelial cells and that pre-complexation of DDP with Cys was not responsible for this effect (Cancer Chemother Pharmacol 45: 43, 2000). These data implicate competitive DDP transport through the ubiquitously expressed ASC-amino acid transport system (named after substrates Ala, Ser, Cys). Human ASCT1 is a sodium-dependent transporter of the ASC-system (J Biol Chem 268: 15329, 1993). To demonstrate the involvement of the ASC-system in DDP accumulation we micro-injected Xenopus laevis oocytes with *in vitro*-synthesized ASCT1-cRNA derived from a pOTV-ASCT1 clone and compared their subsequent DDP accumulation rates with those of control oocytes either injected with dH2O or ASCT1-antisense-cRNA. Functional expression of ASCT1 was verified by measuring the transport of marker amino acid L-[3H]-alanine. During a 20min exposure to 400microM DDP the expression of ASCT1 accelerated DDP accumulation by over 110% compared to basal DDP uptake in control groups. A 10-fold excess of Cys completely inhibited DDP transport in ASCT1-expressing oocytes but not in control oocytes. Due to the low endogenous expression of amino acid transporters in oocytes these data suggest that in ASCT1-expressing oocytes approx. 50% of DDP uptake occurs through passive diffusion and 50% through ASCT1-mediated transport and agree with existing data on DDP uptake in cancer cells. RT-PCR analysis established expression of ASCT1-RNA in a number of clinically important tumors including cervix, ovary, uterus, lung, breast, and renal cell lines, thus supporting the rationale of targeting the ASC-system for tumor treatment. A 30min pre-incubation with 100nM phorbol 12-myristate 13-acetate (PMA), a PKC-dependent activator of amino acid transport, significantly sensitized A2780 ovarian carcinoma cells to DDP. To further support the concept of competitive binding, we compared lowest-energy structures of DDP and Cys using 3-dimensional models with electron density overlay. The distances between the amino group present in each molecule and the electronegative centers Cl- in DDP and S- in Cys differ by only 0.59Å. This structural similarity suggests that DDP can bind to a Cys-binding site. This study demonstrates that expression of the human ASCT1 can modulate the transport of DDP and suggests a clinically relevant role of the ASC-amino acid transport system in the accumulation of DDP in human cancer cells.

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New hydrazones, a novel class of experimental antitumor agents

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Bicyclic hydrazones are compounds exhibiting antitumor activity (Easmon et al., Int. J. Cancer, 94, 89-96, 2001). Several of the novel compounds exhibit above-average inhibition of leukemias, melanomas, colon, non-small cell lung and renal carcinoma cells in the NCI panel of cell lines. Compounds EPH101 (4-acetylpyrimidine 2'-benzothiazolylhydrazone), EPH136 (3-acetylisoquinoline 2'-bezoxazolylhydrazone) and showed positive results in the NCI hollow fiber assay. A COMPARE analysis showed that the mechanism of action of the novel compounds is not related to any of the standard antitumor drugs. A bioinformatical approach, based on the IC_{50} values and the levels of gene expression of 9700 genes in the NCI panel of cell lines showed that genes belonging to the following groups are important for the antiproliferative activity: (i) DNA-, ATP-, and GTP-binding proteins, ATPases and GTPases, (ii) genes involved in tumor proliferation, (iii) proteins involved in cell adhesion, cell migration and neuronal development, (iv) kinases and (v) calcium-binding proteins. Following treatment with 2-fold IC_{50} concentrations of EPH136 alterations in the expression of 5000 genes were determined by gene array. The genes found to be overexpressed more than 2-fold belong to the same groups as that found by the bioinformatical approach. In addition, RNA-binding proteins, transcription factors (belonging to the DNA-binding proteins), phosphatases, the GABA and benzodiazepine receptor, protein-degrading enzymes and protease inhibitors were overexpressed. In conclusion, the novel hydrazones exhibit antitumor activity *in vitro* and *in vivo* by a new mechanism of action which is unknown at present. Financial support was provided in part by the Austrian Science Foundation (FWF), project No. P12384-MOB

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CT-32228: a lysophosphatidic acid acyltransferase-beta (LPAAT-b) inhibitor, induces apoptosis in a variety of solid tumor, leukemia and lymphoma cell lines, but not in normal cells

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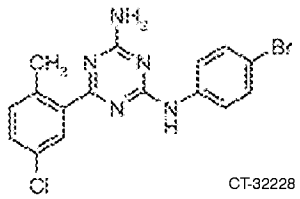
LPAAT-b is a member of a family of 5 intrinsic membrane enzymes that catalyze the biosynthesis of phosphatidic acid (PA). PA is associated with the activation of raf and mTOR. LPAAT-b is highly expressed in a large proportion of lung, ovary, prostate, bladder, cervix, and brain tumors, while its expression in normal tissues is primarily limited to endothelial, smooth muscle and inflammatory cells. CT-32228 (N-(4-bromophenyl)-6-(5-chloro-2-methylphenyl)-[1,3,5]triazine-2,4-diamine), was derived following medicinal chemistry optimization of a diversity library screen to identify compounds that inhibited LPAAT-b, but not the related housekeeping enzyme LPAAT-alpha. CT-32228 had a Ki of 47 nM in a high through-put cell free screening assay against LPAAT-b expressed in SF-9 membranes. Subsequently, CT-32228 was tested in standard 72 h *in vitro* proliferation and cytotoxicity assays using a panel of 12 hematologic malignancy cell lines and 16 solid tumor cell lines. In the proliferation assays, CT-32228 had an average IC_{50} of <100 nM in the leukemia and lymphoma cell lines, without a clear correlation between disease type (i.e. genetic alteration(s)) or total LPAAT enzyme levels. In intact IM-9 cells, CT-32228 inhibited the acylation of lysophophatidylmethanol, an alternate substrate for LPAAT-b, with an IC_{50} of 22 nM. The anti-proliferative IC_{50} on the solid tumor cells on average was 1.5 times higher than that for the hematologic malignancies. Again, no correlation was found between the IC_{50} and genetic alterations.



CT-32228

CT-32228 also showed a broad spectrum of activity in the NCI's *in vitro* antitumor screen consisting of 60 human tumor cell lines and is currently being tested in the hollow fiber assay. The anti-proliferative effect of CT-32228 on tumor cells was irreversible after 72 h exposure and was due to induction of apoptosis, which was already detectable after 8 h. Cell cycle analysis showed that CT-32228 caused an arrest in G2/M. In contrast to the effect on tumor cells, CT-32228 at the same concentrations had no or very little effect on normal human bone marrow progenitors or murine T-lymphocytes. In the human HT-29 (colon) and DU-145 (prostate) xenograft tumor models in nude mice, repeated IV treatment (BID × 10) with CT-32228 showed antitumor efficacy in a dose dependent manner and was well tolerated. These data suggest that inhibition of LPAAT-b activity may represent a novel and selective target for cancer therapy

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Diverse and unique cellular effects of SK-7068, a novel histone deacetylase inhibitor

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Epigenetic gene silencing is a common molecular alteration in cancer cells and recruitment of HDAC-containing complexes to promoter regions leads to the transcriptional silencing of various target genes such as tumor suppressor genes. Accordingly, inhibition of HDAC enzymatic activity is expected to induce re-expression of aberrantly repressed genes and might