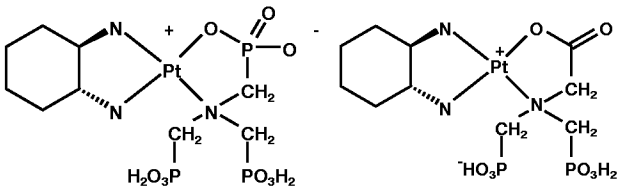
fects within this class of compounds involve modifications of both the leaving phosphonate-containing group, which is responsible for the osteotropic properties, and the non-leaving amine group, which is decisive for the cellular processing of DNA adducts. Structure-activity relationships have been investigated *in vitro* within a series of complexes of the general formula cis-[PtA2X2], where A2 is either two ammine ligands or one bidentate ethanediamine or isomerically pure cis-, trans-R,R- or trans-S,S-1,2-diaminocyclohexane (DACH) and X2 is one bidentate aminopolymethylenephosphonate, either aminotris(methylenephosphonate) (ATMP) or bis(phosphonomethyl)aminoacetate (BPMAA). In the cisplatin-sensitive human ovarian tumor cell line CH1 the complexes of ATMP display a 2-20fold higher potency than their BPMAA-containing counterparts. Within the series of ATMP complexes potency decreases depending on the amine ligand in the following order: trans-R,R-DACH > trans-S,S-DACH > cis-DACH ≈ diammine > ethanediamine. Within the BPMAA-containing series the order of decreasing potency is somewhat different: trans-R,R-DACH > trans-S,S-DACH ≈ ethanediamine > cis-DACH.



Thus, in both series the complexes of trans-R,R-DACH (Fig. 1) prove to be superior to those of other isomers of DACH and those of ethanediamine, which is consistent with published findings for oxaliplatin and other DACH-containing platinum compounds. As complexes of this latter type usually exhibit low levels of cross-resistance with diammine platinum drugs like cisplatin and carboplatin, we expect that the activity of the phosphonate-containing derivatives is retained in cells resistant to cisplatin.

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Covalent binding of the acronycine derivative S23906-1 to glutathione prevents DNA alkylation and reduces cytotoxicity

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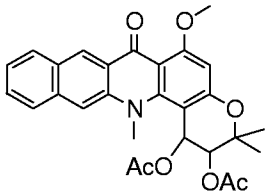
The benzoacronycine derivative S23906-1 has been recently identified as a potent anticancer drug active against a variety of human tumor xenograft models in mice and has been selected for advanced preclinical evaluation (1). This promising new anticancer agent derives from the alkaloid acronycine isolated from a plant distributed in Australia. The parent tetracyclic alkaloid is weakly cytotoxic to a wide range of tumor cells *in vitro* and displays moderate antitumor activities *in vivo*. Clinical testing of acronycine itself showed insufficient antitumor responses and the development of this compound was discontinued. Nevertheless, the antitumor potential of this compound has stimulated the synthesis of more potent and more active analogues, such as S23906-1 which is the lead synthetic compound in these new series.



From the mechanistic point of view, S23906-1 was recently characterized as a DNA alkylating agent reacting irreversibly with guanine residues in double stranded DNA (2). The covalent binding to DNA is thought to be responsible for the cytotoxic action and the capacity of the drug to trigger apoptosis in tumor cells (3). However, covalent binding to other intracellular reactive nucleophilic species may also occur. In the course of our ongoing studies aimed at characterizing the interaction of S23906-1 with biologically significant molecules, the binding and bonding to glutathione (GSH) was examined. Direct measurements by mass spectrometry as well as competition experiments with DNA demonstrated that S23906-1 forms covalent adducts with GSH, but not with its glutathione disulfide (GSSG). However, the drug binds non covalently to GSSG. Circular dichroism measurements revealed that S23906-1 form very stable complexes with both GSH and GSSG. A range of GSH derivatives was use to delineate the portion of the GSH molecule responsible for the binding and bonding interaction with S23906-1. The cytotoxicity of the GSH-S23906-1 covalent adducts was evaluated using human KB epidermoid carcinoma cells sensitive and resistant to S23906-1 (KB-3-1 and KB/S23-500, respectively). The formation of covalent complexes between GSH and S23906-1 decreases the formation of potentially lethal DNA cross-links, thereby modulating the cytotoxic action of the drug.

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Gamma-Gutamyltransferase-dependent extracellular detoxification of cisplatin by human kidney proximal tubule cells

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Elevated nephrotoxicity is the main limiting factor for utilization of the anticancer agent cisplatin. *In vivo*, the administration of the cysteine-containing tripeptide GSH has been found to reduce nephrotoxicity, but the precise biochemical mechanism of this protective action is not fully understood. The aim of the present study was to gain insights into the mechanism by which GSH prevents cisplatin nephrotoxicity, and in particular whether the protective action of GSH is mediated by products of the extracellular breakdown of GSH operated by gamma-glutamyl transpeptidase (GGT), an enzyme activity highly expressed in kidney tubular cells. HK-2 cells, derived from immortalization of human kidney proximal tubule cells, were challenged with cisplatin in the presence of extracellular GSH, in conditions capable of enhancing or inhibiting GGT anzyme activity. Cisplatin cytotoxicity was judged by its antiproliferative action as assessed by WST-1 reduction test. HK-2 cells exhibited a high GGT activity, corresponding to that normally found in the proximal convolute tubule. The antiproliferative effect of cisplatin was only little affected by addition of GSH. However, when the antiproliferative assay was performed in the presence of glycyl-glycine, to serve as transpeptidation acceptor and thus to stimulate GGT-mediated GSH catabolism, cisplatin-induced growth inhibition was prevented to a large extent. This effect was not mediated through an increase of intracellular GSH levels, which were not affected by glycyl-glycine supplementation. The thiol dipeptide cysteinyl-glycine, i.e. the GSH catabolite generated by GGT activity, showed a higher reactivity against cisplatin *in vitro* than GSH, as shown by the quicker oxidation of its ?SH groups. Neither the cisplatin/GSH nor the cisplatin/cysteinyl-glycine adducts displayed an antiproliferative effect. However, 2h pre-complexing with GSH in the presence of GGT, or directly with the GSH catabolite cysteinyl-glycine decreased the antiproliferative effect of cisplatin and drug-induced DNA platination to a greater extent than pre-complexing with GSH alone. The results support that extracellular metabolism of GSH by GGT plays a role in modulating cisplatin nephrotoxicity. A better understanding of these reactions might help to devise strategies o reduce cisplatin nephrotoxicity without impairing its terapeutic efficacy.

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Enhanced antitumor activity of irofulven in combination with gemcitabine against the MV522 human lung carcinoma xenograft

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Effective therapy for cancer often requires a multi-modal approach combining therapies with differing mechanisms of action to enhance antitumor activity. The novel antitumor agent, irofulven (HMAF, MGI 114), has demonstrated both preclinical and clinical antitumor activity as monotherapy. Its activity has been shown to be independent of resistance mechanisms such as p53 and p21 mutations, MDR or MRP expression, and bcl-2