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ORIGINAL RESEARCH

Comparison of the effects of the oral anticancer platinum(IV) complexes oxoplatin and metabolite cis-diammine-tetrachlorido-platinum(IV) on global gene expression of NCI-H526 cells

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Abstract: Platinum(IV) coordination complexes like oxoplatin (*cis,cis,trans*-diammine-dichlorido-dihydroxido-platinum[IV]) show high stability and therefore can be utilized orally for outpatient care. Although oxoplatin is capable of binding directly to DNA after prolonged incubation, platinum(IV) agents are considered to be largely inert prodrugs that are converted to highly cytotoxic platinum(II) compounds by reducing substances, enzymes, or microenvironmental conditions. Reaction of oxoplatin with 0.1 M hydrogen chloride mimicking gastric acid yields *cis*-diammine-tetrachlorido-platinum(IV) (DATCP[IV]), which exhibits two-fold increased activity. The presence of chlorides as ligands in the axial position results in a high reduction potential that favors transformation to platinum(II) complexes. In this study, the intracellular effect of the highly reactive tetrachlorido derivative was investigated in comparison with an equipotent dose of cisplatin. Genome-wide expression profiling of NCI-H526 small cell lung cancer cells treated with these platinum species revealed clear differences in the expression pattern of affected genes and concerned cellular pathways between DATCP(IV) and cisplatin. Application of DATCP(IV) resulted in extensive downregulation of protein and ATP synthesis, cell cycle regulation, and glycolysis, in contrast to cisplatin, which preferentially targeted glutathione conjugation, pyruvate metabolism, citric acid cycle, and the metabolism of amino acids and a range of carbohydrates. Thus, the oxoplatin metabolite DATCP(IV) constitutes a potent cytotoxic derivative that may be produced by gastric acid or acidic areas prevailing in larger solid tumors, depending on the respective pharmaceutical formulation of oxoplatin. Furthermore, DATCP(IV) exhibits intracellular effects that are clearly different from the expected reduced product cisplatin(II). In conclusion, activation of the platinum(IV) complex oxoplatin seems to involve the generation of a cytotoxic six-coordinate species, dependent on prevailing conditions, and its effects need to be considered in addition to the effects of the potential final platinum(II) product.

Keywords: platinum, oxoplatin, metabolites, small cell lung cancer, cell line, gene expression, microarray

Introduction

Cisplatin (*cis*-diammine-dichlorido-platinum[II]) was established as a drug that is active against a range of malignancies, including testicular, ovarian, head and neck, bladder, esophageal, and small cell lung cancer (SCLC).[1,2] However, tumors like colon and breast cancer show limited sensitivity, and cisplatin-induced resistance and severe side effects are frequently observed.[3] Second-generation platinum(II)-based drugs include carboplatin, which has similar anticancer activity but fewer side effects than

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cisplatin, and oxaliplatin, which exhibits cytotoxicity against cisplatin-refractory cancer types like colorectal tumors.[4] In an attempt to develop platinum drugs with enhanced stability that are suitable for oral application, axial ligands were introduced, yielding platinum(IV) coordination complexes with increased kinetic inertness and reduced reactivity, resulting in decreased degradation in the bloodstream, lower toxicity, and partial efficacy in cisplatin-resistant tumor cell lines.[5,6] Thus, pharmacokinetic properties of these agents can be fine-tuned by modification of the axial substituents. Satraplatin (bis-acetato-ammine-dichlorido-cyclohexylamine-platinum[IV]; JM 216), an orally applicable cisplatin analog, constitutes one of the first third-generation platinum complexes that has undergone clinical trials with limited success.[7]

Because it is generally accepted that reduction of the platinum(IV) central atom has to occur prior to binding to target DNA, these molecules are believed to represent prodrugs.[8,9] The following reduction produces platinum(II) species that bind to DNA and lead to the formation of intra- and/or interstrand adducts, which results in cell cycle arrest in the G2M phase and cell death.[9,10] Cellular reducing substances such as ascorbic acid and thiol-containing species like metallothioneins and glutathione are regarded as activators of platinum(IV) prodrugs.[11,12]

A further orally applicable platinum(IV) anticancer drug that is currently under development is oxoplatin, which was synthesized for the first time by Chugaev and Khlopin in the Russian Federation in 1927 (Figure 1).[13] Its cytotoxic activity was not demonstrated in rat tumor models until 1977.[14] Presnov et al compared antitumor and pharmacokinetic properties of oxoplatin with those of cisplatin. Therapeutic and maximum tolerated doses were 10-fold higher for oxoplatin than for cisplatin. Additionally, oxoplatin exhibited a prolonged therapeutic effect, antimetastatic activity, and inhibition of tumor growth similar to, or even better than, cisplatin. Oxoplatin can bind directly to DNA; however, this process is so slow that it is of minimal biological relevance.[15] The in vitro cytotoxicity of oxoplatin and its possible activation by reduction through reaction with hydrogen chloride (HCl) and ascorbic acid were investigated in a previous study.[16] Because oxoplatin may represent a prodrug of cisplatin, the effects of both platinum drugs on gene expression patterns of a sensitive cell line were compared using microarrays for genome-wide expression analysis.[16]

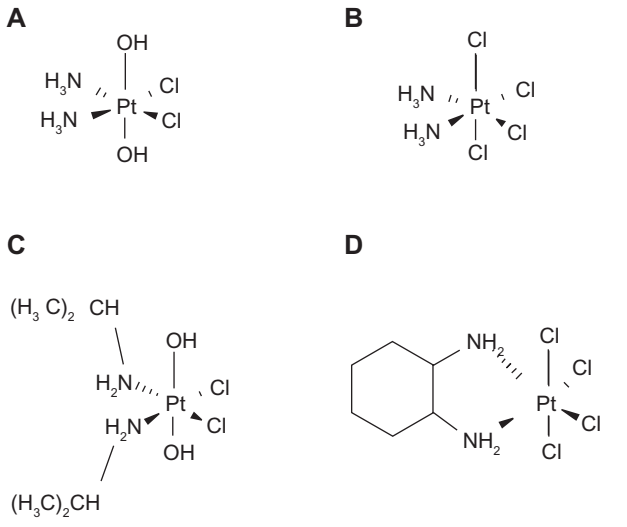

Figure 1 Chemical structures of the platinum compounds used or discussed (iproplatin and ormaplatin) in the present study. The full chemical formulas are *cis, cis,trans*-diammine-dichlorido-dihydroxido-platinum(IV) for oxoplatin, *cis*-diammine-tetrachlorido-platinum(IV) for DATCP(IV), *cis*-dichloro-*trans*-dihydroxy-bis-isopropylamine-platinum(IV) for iproplatin, and tetrachloro-(D,L-*trans*)-l,2-diamino-cyclohexane-platinum(IV) for ormaplatin, respectively.

The antiproliferative activity of cisplatin was not affected by previous incubation with 0.1 M HCl; however, these highly acidic conditions resulted in two-fold enhanced cytotoxicity for oxoplatin due to its conversion to *cis*-diammine-tetrachlorido-platinum(IV) (DATCP[IV]) (Figure 1). Similar platinum(IV) complexes, namely iproplatin and ormaplatin (also termed tetraplatin) (Figure 1), had been investigated in clinical trials that were abandoned because of high toxicity of ormaplatin and low activity of iproplatin.[17,18] Both agents are prodrugs that are converted to platinum(II) species with increased activity via reduction that takes place rapidly for ormaplatin and slowly for iproplatin.[19] A breakthrough was achieved with bis(carboxylato)-platinum(IV) analogs, showing reduction potentials situated between drugs with either chloride or hydroxide axial ligands.[20] Two bis(carboxylato)-platinum(IV) compounds, namely satraplatin and LA-12 (bis[acetato]-adamantylamine-[ammine]-dichlorido-platinum[IV]), have been investigated in clinical trials.[21] Satraplatin has two acetate moieties and needs to be hydrolyzed and subsequently reduced in order to exert an anticancer effect.

The presence of axial chloride or acetate ligands results in a slightly higher lipophilicity compared with the platinum(II) analog, whereas hydroxide substituents lead to significantly lower lipophilicity.[22,23] According to these data, the tetrachlorido metabolite of oxoplatin, DATCP(IV), is expected to be reduced immediately in the cytoplasm and its actual cytotoxic effects to be caused by the main resulting reduction product cisplatin. In order to test this assumption, the effects of cisplatin and DATCP(IV) on global gene expression of the platinum-sensitive SCLC cell line NCI-H526 were investigated by microarrays in the present study.

Materials and methods

Chemicals and cell line

Unless otherwise noted, all chemicals and solutions were obtained from Sigma-Aldrich (St Louis, MO). Oxoplatin and DATCP(IV) were synthesized according to standard procedures by Chiracon (Luckenwalde, Germany) and kindly provided by IPSS (Berlin, Germany). The NCI-H526 cell line was obtained from the American Tissue Culture Collection (ATCC, Manassas, VA). Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (Seromed, Berlin, Germany), 4 mM glutamine, and antibiotics.

All compounds were prepared as stock solutions of 2 mg/mL in DMSO and aliquots stored at –20°C.

Cell proliferation assay

Cells were harvested, counted, and distributed into the wells of flat-bottomed 96-well microtiter plates at a density of 1×10^4 cells/well in 100 µL medium. A total of 100 µL of appropriate dilutions of test compounds were added to each well, and the plates were incubated under tissue culture conditions for 4 days. Stock solutions of the compounds were diluted more than 100-fold for use in assays. Solvent control wells were included in all tests. Dose-response curves were obtained by assessment of cell growth at two-fold drug dilutions in triplicate and used for calculation of the IC_{50} values. Cell proliferation was quantified using a modified tetrazolium dye assay (MTT; EZ4U, Biomedica, Vienna, Austria).

Genome-wide gene expression analysis

Lysates of 30×10^6 cells (extraction buffer: 4 M guanidine isothiocyanate, 0.5% sodium N-lauroyl sarcosinate, 10 mM EDTA, 5 mM sodium citrate, 100 µM β-mercaptoethanol; 30 minutes, 4°C) were added to cesium trifluoroacetate and centrifuged (46,000 rpm, 15°C, 20 hours). Supernatant containing DNA was removed and RNA precipitated with ice-cold 96% ethanol. Pellets were washed and, following removal of ethanol, resuspended in sterile water. RNA content was measured photometrically.

Gene expression analysis was performed using the Applied Biosystems Human Genome Survey Microarray V2.0 (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Therefore, 2–5 µg mRNA (20–50 µg total RNA) was reversely transcribed (RT) to first-strand cDNA (MyCycler thermocycler, BioRad, Hercules, CA). The RT mixture was labeled on ice and purified according to the manufacturer's instructions for the Applied Biosystems 1700 RT Labeling Kit. Hybridization of cDNA and microarray analysis (Applied Biosystems 1700) was carried out following the manufacturer's chemiluminescence detection kit protocol. Data for each cell line (n = 2) were filtered, normalized, and log2-transformed, before further processing was carried out using Microsoft Excel software/SAM (false discovery rate of 10%; Statistical Analysis of Microarray, Stanford University, Stanford, CA). ABI 1700 gene identities can be accessed via the Panther classification system (www.pantherdb.org). ID mapping, pathway assignment, and over-representation analysis of cellular pathways were performed using the Reactome version 35 database (www.reactome.org).

Results

NCI-H526 SCLC cells were treated with either 4.1 µM cisplatin or 1.35 µM DATCP(IV) for 3 days, which resulted in cell cycle arrest but a cell viability of over 93% (data not shown). Under these conditions, decreases in mitochondrial activity were detectable on day 4 in chemosensitivity assays in the previous study.[16] Cells were harvested and counted and lysates prepared for genome-wide expression analysis. The 40 genes found to be either overexpressed or downregulated to the largest extent in treated NCI-H526 cell in response to cisplatin or DATCP(IV), respectively, in comparison with untreated medium controls, are listed in Table 1. These data demonstrate that the majority of genes are clearly differentially affected by the two compounds. The folate receptor 1 (FOLR1) is the gene that is upregulated by both platinum drugs. Similarly, analysis of the 40 most downregulated genes revealed no concordance.

Because these gene expression differences in NCI-H526 cells may be quantitative rather than qualitative, all genes that were downregulated or upregulated more than four-fold, respectively, in response to one of the two platinum complexes were checked for over-representation in pathway analysis employing the Reactome database. In the case of cisplatin, over-represented pathways involving down-regulated genes included glutathione conjugation, pyruvate metabolism, citric acid cycle, and cellular signal transduction, as well as metabolism of a range of carbohydrates and amino acids (Table 2). Corresponding upregulated pathways comprised metabolic regulation, energy metabolism, and distinct signaling cascades, including mediators like glucagon, phospholipase C (PLC), calmodulin (CaM), adenylate cyclase, and cAMP-responsive element binding protein (CREB). For DATCP(IV), downregulation of genes participating in protein synthesis and turnover, replication, transcription, respiration,

Table 1 Alterations of gene expression in platinum drug-treated NCI-H526 cells. The 40 genes exhibiting highest down- or upregulated expression in treated NCI-H526 small cell lung cancer cells in response to cisplatin or DATCP(IV), respectively, compared with untreated cells (fold Δ: n-fold change in gene expression treated/untreated cells)

Cisplatin upregulated	Fold Δ	DATCP(IV) upregulated	Fold Δ	Cisplatin downregulated	Fold Δ	DATCP(IV) downregulated	Fold Δ
ASCL1	200.5	MPP4	946.7	GSTP1	0.002	TUBA	0.007
MAGEA4	72.9	MMP26	56.2	AVIL	0.005	RPLP2	0.008
MAGEC2	38.9	DKK2	46.7	LOC51161	0.007	TMSB10	0.012
PAGE-5	35.3	FBXL13	42.9	BASP1	0.008	STMN1	0.013
ISL1	24.7	CKMT2	38.6	PRSS3	0.011	RPL41	0.015
DCX	22.9	EYA1	32.1	TMPRSS3	0.012	RPL17	0.015
XAGE1D	17.5	BCL6	32.0	IL13RA1	0.015	XRCC5	0.016
RBP1	15.5	NPY	27.7	CD9	0.015	RPS21	0.020
GS3955	14.9	OPB2B	22.7	DDX1	0.015	UBE4A	0.020
DNER	14.8	DGKQ	22.5	RNPC1	0.015	TUBA4	0.023
SERPINB8	13.9	FOLR1	22.4	SPOCK1	0.015	RPS19	0.027
COL1A2	13.3	HNMT	22.1	NKX6-1	0.017	CALM2	0.028
STMN2	13.2	SCNN1D	20.6	PTPN18	0.018	DGKZ	0.030
FLJ32942	12.9	EDIL3	19.8	SLC1A5	0.018	RASGEF	0.031
TUBA3E	11.1	EMCN	19.7	THY1	0.018	UBB	0.031
KLHL1	11.0	KIF9	18.9	FABP5	0.020	RPL13A	0.033
HT021	10.7	CYP2C18	18.0	CSNK1G1	0.021	RPSS12	0.036
SYT1	9.8	WNT16	17.7	COL27A1	0.021	HIST1H4C	0.036
SLC43A3	8.9	BFSP1	17.1	RGS13	0.024	HNRPA2B1	0.037
FOXG1B	8.8	SLC3A1	14.5	NMU	0.026	AHCY	0.037
PNMA5	8.7	HAVCR1	14.3	AZGP1	0.029	RPL26	0.039
PAGE3	7.9	HUS1B	14.2	VIL1	0.029	SLC25A5	0.040
DDC	7.3	PTGFR	13.4	ELF3	0.030	ATP1A1	0.041
ELAVL4	7.1	KCNK1	12.9	SCGB2A1	0.031	COX7C	0.042
SIX3	6.9	IFNA14	12.4	ID1	0.032	RPS16	0.044
CaMKIINalpha	6.7	ADAM33	12.3	TNFSF8	0.033	TPT1	0.045
NCALD	6.5	SLC4A4	12.1	NMT1	0.033	MORF4L	0.046
KCNMB2	6.4	CDH13	11.9	MLL	0.033	RPL41	0.048
MS4A8B	6.2	PPEF2	11.6	FASN	0.034	TMSB4X	0.048
CROT	6.2	GDF8	11.3	MAZ|KIF22	0.035	RPL14	0.049
APOBEC3B	6.1	DMP1	10.7	LY6E	0.036	HNRPA1	0.049
NKX2-1	5.9	LATS2	10.5	MYO10	0.037	HDGF	0.051
FOLR1	5.7	ARCH	10.3	MFNG	0.038	SNRPF	0.052
XAGE3	5.6	SLC35B4	10.2	JAK1	0.038	ACTB	0.055
PKIB	5.5	TGM5	10.2	EN2	0.039	UCHL1	0.057
MAGEC1	5.4	HTR3E	9.9	RASD2	0.039	NGFRAPF1	0.058
LPL	5.4	SPACA4	9.5	SYT7	0.040	SMT3H2	0.059
GRP	5.3	PRX	9.1	HNRPA0	0.040	BTF3	0.060
GBA3	5.2	DNAJB5	9.0	VAMP2	0.041	RPL31	0.060
NKX2-2	4.8	RFPL1	8.9	RGL	0.041	PPIA	0.060

cell cycle regulation, p53-dependent and -independent damage response, glycolysis/gluconeogenesis, and others were found (Table 3). Upregulated transcripts included those involved in metabolism of xenobiotics, metal ion transport, H-RAS activation, and cell junction organization. According to the Reactome database, with the single exception of the "metabolism of carbohydrates" (REACT_474/1383/1520) partially overlapping pathways, none of the processes mostly affected by either cisplatin and DATCP(IV) was the same, which indicates important differences in their mechanisms of cytotoxicity.

Discussion

The development of orally applicable platinum-based anticancer drugs is currently being intensively pursued in order to avoid intravenous administration, allowing for outpatient care.[1,2] Among the first oral platinum coordination complexes established are picoplatin(II) and satraplatin(IV), which have shown promise in preclinical and clinical trials but have so far failed to gain approval.[4] Platinum(IV) compounds are considered prodrugs that are converted to their cytotoxically active platinum(II) forms primarily at the target site.[23] Oxoplatin is converted to platinum(II)

Table 2 Over-representation pathway analysis of genes more than four-fold down- or upregulated in NCI-H526 small cell lung cancer cells treated with cisplatin. Gene expression was assessed using Applied Biosystems Human Genome Survey Microarray V2.0, and data were analyzed using the Reactome database

P value	Identifier event	Name of this event
Cisplatin downregulated		
0.0006	REACT_18414	Dephosphorylation of NCAM1 bound pFyn
0.0033	REACT_6926	Glutathione conjugation
0.0047	REACT_22296	Upregulation of cytosolic proteins by activated PPARA
0.0057	REACT_1046	Pyruvate metabolism and citric acid (TCA) cycle
0.0086	REACT_25287	The Na^+/K^+-transporting ATPase
0.0109	REACT_6854	Glutathione conjugation of cytosolic substrates
0.0109	REACT_14820	Metabolism of polyamines
0.0135	REACT_34	Ethanol oxidation
0.0163	REACT_12527	EGFR non-clathrin mediated endocytosis
0.0163	REACT_18333	Recruitment of FAK to NCAM1:Fyn in lipid rafts
0.0187	REACT_474	Metabolism of carbohydrates
0.0226	REACT_12387	Sprouty sequesters Cbl away from active EGFR
0.0226	REACT_18259	SOS binds Grb2 bound to pFAK:NCAM1
0.0251	REACT_13	Metabolism of amino acids and derivatives
0.0417	REACT_2071	Pyruvate metabolism
0.0461	REACT_1785	Citric acid cycle (TCA cycle)
0.0461	REACT_12495	Assembly in clathrin-coated vesicles (CCVs)
Cisplatin upregulated		
0.0009	REACT_13723	Neurotransmitter release cycle
0.0011	REACT_1665	Glucagon signaling in metabolic regulation
0.0013	REACT_12079	PLC-gamma1 signalling
0.0057	REACT_9053	CaM pathway
0.0101	REACT_1505	Integration of energy metabolism
0.0141	REACT_15333	Adenylate cyclase inhibitory pathway
0.0215	REACT_18312	NCAM1 interactions
0.0238	REACT_15497	PKA-mediated phosphorylation of CREB

species by intracellular-reducing agents such as ascorbic acid and glutathione. Furthermore, exposure to 0.1 M HCl, representing gastric acidity, resulted in two-fold increased antiproliferative activity.[16] Reduction/activation of oxoplatin at a low pH is an advantage for targeted release in the acidic microenvironment of solid tumors.

Although 40 years have passed since the discovery of the anticancer activity of cisplatin, the mechanism of action of platinum complexes is still unclear.[24] The question of whether platinum(IV) compounds have intrinsic activity or whether they serve as prodrugs that are reduced to platinum(II) molecules before reaching their DNA target remains to be resolved. Platinum(IV)–ammine complexes containing the chelating ligand 1,2-diaminocyclohexane combined with a variety of coordinating anions were found to react with 9-methylxanthine, 9-methylhypoxanthine, and guanosine-5′-monophosphate, providing evidence that not all platinum(IV) compounds represent prodrugs.[25] Oxoplatin is capable of forming DNA adducts in a rather slow process.[15] Oxoplatin was furthermore found to accumulate in tumor tissue, and metabolization resulted in the formation of several species, amongst them cisplatin, pointing to the role of oxoplatin as a prodrug of cisplatin; however, this hypothesis has not been validated so far. Oxoplatin reacted with 0.1 M HCl as well as DATCP(IV) yielded identical infrared spectra and cytotoxic effects.[16] The reduction potential of the platinum-based drugs depends mainly on the axial ligands, with chloride substituents reduced most easily, hydroxide groups most stable, and carboxylate ligands lying between the two extremes.[23,26]

According to the ATCC, the NCI-H526 cell line, originating from a bone metastasis of an SCLC patient prior to therapy, expresses neuron-specific enolase, brain enzyme of creatine kinase, and p53 mRNA. Comparison of the gene expression patterns of control and treated NCI-H526 cells revealed significant differences in the expression pattern of target genes for cisplatin and DATCP(IV). Cisplatin-downregulated transcripts are involved in glutathione conjugation, pyruvate metabolism and citric acid cycle, cell signal transduction and metabolism of a range of carbohydrates and amino acids, and upregulation of pathways employed in metabolic regulation, energy metabolism, and cell signaling in NCI-H526 cells, which point to a restricted and selective cellular response.

Table 3 Over-representation pathway analysis of genes more than four-fold down- or upregulated in NCI-H526 small cell lung cancer cells treated with DATCP(IV). Gene expression was assessed using Applied Biosystems Human Genome Survey Microarray V2.0, and data were analyzed using the Reactome database

P value	Identifier event	Name of this event
DATCP(IV) downregulated		
<0.001	REACT_1477	Eukaryotic translation elongation
<0.001	REACT_1014	Translation
<0.001	REACT_17015	Metabolism of proteins
<0.001	REACT_71	Gene expression
<0.001	REACT_6305	Respiratory electron transport, ATP synthesis
<0.001	REACT_22393	Respiratory electron transport
<0.001	REACT_6828	APC/C-mediated degradation of cell cycle proteins
<0.001	REACT_21279	Regulation of mitotic cell cycle
0.0001	REACT_24994	Regulation of mRNA stability
0.0001	REACT_6954	APC/C:Cdc20 degradation of mitotic proteins
0.0002	REACT_25325	Destabilization of mRNA by AUF1 (hnRNP D0)
0.0003	REACT_9029	Cyclin A:Cdk2-associated events at S phase
0.0008	REACT_383	DNA replication
0.0008	REACT_829	Regulation of DNA replication
0.0010	REACT_20605	Metabolism of mRNA
0.0011	REACT_2014	Synthesis of DNA
0.0013	REACT_1625	p53-dependent G1 DNA damage response
0.0056	REACT_2160	p53-independent DNA damage response
0.0121	REACT_1383	Glycolysis
0.0281	REACT_19195	Adherens junctions interactions
0.0306	REACT_1520	Gluconeogenesis
0.0321	REACT_578	Apoptosis
0.0426	REACT_6759	Formation of ATP by chemiosmotic coupling
0.0436	REACT_474	Metabolism of carbohydrates
DATCP(IV) upregulated		
0.0001	REACT_18425	Prostanoid ligand receptors
0.0021	REACT_13705	Phase 1 – functionalization of compounds
0.0036	REACT_20582	Zinc efflux and compartmentalization by the SLC30 family
0.0045	REACT_20547	Metal ion SLC transporters
0.0048	REACT_7963	Packaging of telomere ends
0.0048	REACT_13433	Biological oxidations
0.0091	REACT_19305	Transport of glucose, bile salts, metal ions, and amine compounds
0.0189	REACT_19118	SLC-mediated transmembrane transport
0.0243	REACT_23928	SOS1 activates H-Ras
0.0324	REACT_20676	Cell junction organization
0.0385	REACT_24024	Gab2 binds the p85 subunit of class 1A PI3 kinases

In contrast, DATCP(IV) suppresses expression of a host of genes participating in many aspects of cellular processes like protein synthesis and turnover, replication, transcription, respiration, cell cycle regulation, p53-dependent and -independent damage response, and glycolysis/gluconeogenesis. Upregulated transcription of genes involved in the metabolism of xenobiotics and metal ion transport seems to be important in drug resistance. The finding of only one single overlapping pathway, namely "metabolism of carbohydrates and glycolysis/gluconeogenesis", corroborates the fundamental differences in the mechanisms of cytotoxicity induced by the two platinum compounds and contradicts the exclusive role of DATCP(IV) as a prodrug of cisplatin.

All platinum(IV) complexes that have reached clinical trials thus far have yielded a platinum(II) central atom, reductively formed in vivo by endogenous molecules such as glutathione and ascorbate.[5] Unexpectedly, the extracellular reduction of ormaplatin was primarily accomplished by protein sulfhydryl groups but not by glutathione, predominantly leading to the expected *cis*-dichlorido-(D,L-*trans*)-1, 2-diamineplatinum(II) among other products, due to substitution of chloride ligands.[27] In clinical trials of ormaplatin, approximately 60% of the platinum in blood was bound to proteins (50% irreversibly) at the end of infusion, and the drug exhibited severe and unpredictable neurotoxicity.[17,28–31] Similarly, the reaction of

iproplatin with glutathione yielded *cis*-di(isopropylamine) chlorido-glutathionato-platinum(II) and not the expected *cis*-dichlorido-species. Therefore, binding of one of the available coordination sites of this platinum(II) product to glutathione precludes the formation of bifunctional adducts with DNA.[29,32] Reaction with cysteine-rich cellular proteins and zinc-finger transcription factors as well as disruption of protein-DNA complexes may represent alternative targets for this iproplatin metabolite.[33,34] The intracellular fate of DATCP(IV) has not been clarified so far, but analogically to the platinum complexes ormaplatin and iproplatin as well as in accordance with the present study, DATCP(IV) seems to bind to a host of cellular proteins, which results in shutdown of their transcription, rather than conversion to free platinum(II) compounds and DNA damage preceding impairment of transcription.

Conclusion

Here we demonstrate that the effects of DATCP(IV) on global gene expression of an SCLC cell line differ fundamentally from those of cisplatin. It is concluded that the metabolite itself, or intracellular reaction products thereof, impair a host of important proteins, resulting in the shutdown of a whole panel of genes involved in pathways effecting metabolism of cellular constituents and energy production. Thus, our data suggest that this compound may act as an anticancer drug originally and not by serving only as a prodrug of cisplatin, as was previously deduced from its chemical properties, such as the reduction potentials of tetrachlorido-platinum(IV) complexes.[25]

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Disclosure

The authors report no conflict of interest associated with this work.

References

1. Kostova I. Platinum complexes as anticancer agents. *Recent Patents Anti-Cancer Drug Discov*. 2006;l(1):1–22.
2. Kelland L. The resurgence of platinum-based cancer chemotherapy. *Nature Rev Cancer*. 2007;7(8):573–584.
3. Wang D, Lippard SJ. Cellular processing of platinum anticancer drugs. *Nature Rev Drug Discov*. 2005;4(4):307–320.
4. Olszewski U, Hamilton G. A better platinum-based anticancer drug yet to come? *Anticancer Agents Med Chem*. 2010;10(4):293–301.
5. Hall MD, Mellor HR, Callaghan R, Hambley TW. Basis for design and development of platinum(IV) anticancer complexes. *J Med Chemistry*. 2007;50(15):3403–3411.
6. Hall MD, Alderden RA, Zhang M, et al. The fate of platinum(II) and platinum(IV) anti-cancer agents in cancer cells and tumours. *J Struct Biol*. 2006;155:38–44.
7. Bhargava A, Vaishampayan UN. Satraplatin: leading the new generation of oral platinum agents. *Expert Opin Investig Drugs*. 2009;18(11):1787–1797.
8. Hambley TW, Battle AR, Deacon GB, et al. Modifying the properties of platinum(IV) complexes in order to increase biological effectiveness. *J Inorg Biochem*. 1999;77(1–2):3–12.
9. Foltinová V, Švihálková Šindlerová L, Horváth V, et al. Mechanisms of effects of platinum (II) and (IV) complexes. Comparison of cisplatin and oxaliplatin with satraplatin and LA-12, new PT(IV)-based drugs. *Scripta Medica Facultatis Medicae Universitatis Brunensis Masarykianae*. 2008;81:105–116.
10. Nakai T, Ando M, Okamoto Y, et al. Modulation of oxidative DNA damage and DNA-crosslink formation induced by *cis*-diammine-tetrachloro-platinum(IV) in the presence of endogenous reductants. *J Inorg Biochem*. 2011;105(1):1–5.
11. Cubo L, Hambley TW, Sanz Miguel PJ, et al. The preparation and characterization of trans-platinum(IV) complexes with unusually high cytotoxicity. *Dalton Trans*. 2011;40:344–347.
12. Carr JL, Tingle MD, McKeage MJ. Satraplatin activation by haemoglobin, cytochrome C and liver microsomes in vitro. *Cancer Chemother Pharmacol*. 2006;57:483–490.
13. Konovalova AL, Presnov MA, Zheligovskaia NN, Treshchalina EM. Antitumor effect of complex compounds of tetravalent platinum. *Doklady Akademii Nauk SSSR*. 1977;234(1):223–226.
14. Presnov MA, Konovalova AL, Kozlov AM, et al. The antitumor activity of oxoplatinum. *Neoplasma*. 1985;32(1):73–83.
15. Nováková O, Vrána O, Kiseleva VI, Brabec V. DNA interactions of antitumor platinum(IV) complexes. *Eur J Biochem*. 1995;228: 616–624.
16. Olszewski U, Ach F, Ulsperger E, et al. In vitro evaluation of oxoplatin: an oral platinum(IV) anticancer agent. *Met Based Drugs*. 2009:348916.
17. Schilder RJ, LaCreta FP, Perez RP, et al. Phase I and pharmacokinetic study of ormaplatin (tetraplatin, NSC 363812) administered on a day 1 and day 8 schedule. *Cancer Res*. 1994;54(3):709–717.
18. Trask C, Silverstone A, Ash CM, et al. A randomized trial of carboplatin versus iproplatin in untreated advanced ovarian cancer. *J Clin Oncol*. 1991;9(7):1131–1137.
19. Choi S, Filotto C, Bisanzo M, et al. Reduction and anticancer activity of platinum(IV) complexes. *Inorg Chem*. 1998;37:2500–2504.
20. Platts JA, Ermondi G, Caron G, et al. Molecular and statistical modeling of reduction peak potential and lipophilicity of platinum(IV) complexes. *J Biol Inorg Chem*. 2011;16(3):361–372.
21. Montaña AM, Batalla C. The rational design of anticancer platinum complexes: the importance of the structure-activity relationship. *Curr Med Chem*. 2009;16(18):2235–2260.
22. Galanski M. Recent developments in the field of anticancer platinum complexes. *Recent Pat Anticancer Drug Discov*. 2006;1(2):285–295.
23. Harper BW, Krause-Heuer AM, Grant MP, et al. Advances in platinum chemotherapeutics. *Chemistry*. 2010;16:7064–7077.
24. Gibson D. The mechanism of action of platinum anticancer agents: what do we really know about it? *Dalton Trans*. 2009;48:10681–10689.
25. Talman EG, Kidani Y, Mohrmann L, Reedijk J. Can Pt(IV)-amine complexes act as 'prodrugs'? *Inorg Chim Acta*. 1998;283:251–255.
26. Reithofer MR, Bytzek AK, Valiahdi SM, et al. Tuning of lipophilicity and cytotoxic potency by structural variation of anticancer platinum(IV) complexes. *J Inorg Biochem*. 2011;105:46–51.
27. Chaney SG, Gibbons GR, Wyrick SD, Podhasky P. An unexpected biotransformation pathway for tetrachloro-(d,l-trans)-1,2-diaminocyclohexaneplatinum(IV) (tetraplatin) in the L1210 cell line. *Cancer Res*. 1991;51:969–973.
28. Pendyala L, Walsh JR, Huq MM, et al. Uptake and metabolism of iproplatin in murine L1210 cells. *Cancer Chemother Pharmacol*. 1989;25:15–18.

29. Pendyala L, Arakali AV, Sansone P, et al. DNA binding of iproplatin and its divalent metabolite cis-dichloro-bis-isopropylamine platinum (II). *Cancer Chemother Pharmacol*. 1990;27:248–250.
30. McKeage MJ, Boxall FE, Jones M, Harrap KR. Lack of neurotoxicity of oral bisacetatoamminedichlorocyclohexylamine-platinum(IV) in comparison to cisplatin and tetraplatin in the rat. *Cancer Res*. 1994;54:629–631.
31. O'Rourke TJ, Weiss GR, New P, et al. Phase I clinical trial of ormaplatin (tetraplatin, NSC 363812). *Anticancer Drugs*. 1994;5(5):520–526.
32. Volckova E, Weaver E, Bose RN. Insight into the reactive form of the anticancer agent iproplatin. *Eur J Med Chem*. 2008;43:1081–1084.
33. Eastman A. Glutathione-mediated activation of anticancer platinum(IV) complexes. *Biochem Pharmacol*. 1987;36(23):4177–4178.
34. Kido Y, Khokhar AR, Siddik ZH. Glutathione-mediated modulation of tetraplatin activity against sensitive and resistant tumor cells. *Biochemical Pharmacol*. 1994;47:1635–1642.