MOLECULAR BIOPHYSICS AND PHARMACOLOGY

HEAD
VIKTOR BRABEC

SENIOR SCIENTIST
JANA KAŠPÁRKOVÁ

SCIENTISTS
HANA KOSTRHUNOVÁ, JAROSLAV MALINA, OLGA NOVÁKOVÁ, MARIE VOJTÍŠKOVÁ, OLDŘICH VRÁNA

POSTDOCS
ANNA HALÁMIKOVÁ, PAVLA HERINGOVÁ

PHD. STUDENTS
JAKUB FLORIÁN, BARBORA LIŠKOVÁ, JARMILA MLČOUŠKOVÁ, RADANA OLIVOVÁ, TEREZA SUCHÁNKOVÁ, JANA ŠTĚPÁNKOVÁ, LENKA ZERZÁNKOVÁ

TECHNICAL ASSISTANTS
MILADA KOŘÍNKOVÁ

DNA and glutathione interactions in cell-free media of asymmetric platinum(II) complexes cis- and trans-[$PtCl_2$(isopropylamine)(1-methylimidazole)]: relations to their different antitumor effects

The global modification of mammalian and plasmid DNAs by the novel platinum compounds *cis*-[$PtCl_2$(isopropylamine)(1-methylimidazole)] and *trans*- [$PtCl_2$(isopropylamine)(1-methylimidazole)] and the reactivity of these compounds with reduced glutathione (GSH) were investigated in cell-free media using various biochemical and biophysical methods. Earlier cytotoxicity studies had revealed that the replacement of the NH_3 groups in cisplatin by the azole and isopropylamine ligands lowers the activity of cisplatin in both sensitive and resistant cell lines. The results of the present work show that this replacement does not considerably affect the DNA modifications by this drug, recognition of these modifications by HMGB1 protein, their repair, and reactivity of the platinum complex with GSH. These results were interpreted to mean that the reduced activity of this analog of cisplatin in tumor cell lines is due to factors that do not operate at the level of the target DNA. In contrast, earlier studies had shown that the replacement of the NH_3 groups in the clinically ineffective trans isomer (transplatin) by the azole and isopropylamine ligands results in a radical enhancement of its activity in tumor cell lines. Importantly, this replacement also markedly alters the DNA binding mode of transplatin, which is distinctly different from that of cisplatin, but does not affect reactivity with GSH. Hence, the results of the present work are consistent with the view and support the hypothesis systematically tested by us and others that platinum drugs that bind to DNA in a fundamentally different manner from that of conventional cisplatin may have altered pharmacological properties.

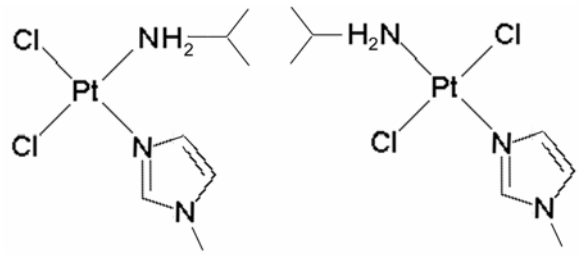

Figure 1: Structures of *cis*-[$PtCl_2$(isopropylamine)(1-methylimidazole)] and *trans*-[$PtCl_2$(isopropylamine)(1-methylimidazole)].

Factors affecting DNA-DNA interstrand cross-links in the antiparallel 3'-3' sense: A comparison with the 5'-5' directional isomer

A study of the unusual 3'–3' 1,4-GG interstrand cross-link (IXL) formation in duplex DNA by a series of polynuclear platinum anticancer complexes has been performed. To examine the effect of possible preassociation through charge and hydrogen-bonding effects the closely related compounds [{*trans*-$PtCl(NH_3)_2\}_2(\mu$-*trans*-$Pt(NH_3)_2$-$\{NH_2\ (CH_2)_6NH_2\}_2)]^{4+}$ (BBR3464, **1**), [{trans-$PtCl\ (NH_3)_2\}_2(\mu$-$NH_2\ (CH_2)_6$-$NH_2)]^{2+}$ (BBR3005, **2**), [{*trans*-$PtCl(NH_3)_2\}_2(\mu$-$H_2N\ (CH_2)_3NH_2\ (CH_2)_4)]^{3+}$ (BBR3571, **3**) and [{*trans*-$PtCl\ (NH_3)_2\}_2$-$\{\mu$-$H_2N\ (CH_2)_3N\ (COCF_3)\ (CH_2)_4\}]^{2+}$ (BBR3571-$COCF_3$, **4**) were studied. Two different molecular biology approaches were used to investigate the effect of DNA template upon IXL formation in synthetic 20-base-pair duplexes. In the "hybridisation directed" method the monofunctionally adducted top strands were hybridised with their complementary 5'-end labeled strands; after 24 h the efficiency of interstrand cross-linking in the 5'–5' direction was slightly higher than in the 3'–3' direction. The second method involved "postsynthetic modification" of the intact duplex; significantly less crosslinking was observed, but again a slight preference for the 5'–5' duplex was present. 2D [^{1}H,^{15}N] HSQC NMR spectroscopy studies of the reaction of [^{15}N]-**1** with the sequence 5'-d{TATACATGTATA}$_2$ allowed direct comparison of the stepwise formation of the 3'–3' IXL with the previously studied 5'–5' XL on the analogous sequence 5'- d$(ATATGTACATAT)_2$. Whereas the preassociation and aquation steps were similar, differences were evident at the monofunctional binding step. The reaction did not yield a single distinct 3'–3' 1,4-GG IXL, but numerous crosslinked adducts formed. Similar results were found for the reaction with the dinuclear [15N]-**2**. Molecular dynamics simulations for the 3'–3' IXLs formed by both **1** and **2** showed a highly distorted structure with evident fraying of the end base pairs and considerable widening of the minor groove.

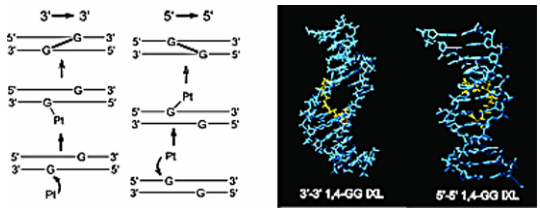

Figure 2: Left panel: A schematic showing the directionality of the two possible DNA–DNA interstrand cross-links (3'–3' and 5'–5') that multinuclear platinum complexes can form. Right panel: Snapshots of the molecular dynamics simulations run on the 3'–3' 1,4-GG and 5'–5' 1,4-GG interstrand cross-links formed by trinuclear platinum complex BBR3464. The 3'–3' 1,4-GG interstrand crosslink is highly distorted with obvious widening of the minor groove and fraying of the base pairs. The 5'–5' 1,4-GG interstrand crosslink is much less distorting with the duplex maintaining its integrity.

Energetics, conformation, and recognition of DNA duplexes modified by methylated analogues of $[PtCl(dien)]^+$

In early studies of empiric structure–activity relationships, monodentate Pt^{II} complexes were considered to be biologically inactive. Examples of such inactive monodentate Pt^{II} compounds are $[PtCl(dien)]^+$ (dien=diethylentriamine) and $[PtCl(NH_3)_3]^+$. DNA is considered the major biological target of platinum compounds. Thus, monodentate DNA binding of Pt^{II} compounds was previously expected to display insignificant biological effects because it was assumed to affect DNA conformation and downstream cellular processes markedly less than the cross-links of bifunctional Pt^{II} complexes. More recently it was shown that some monodentate Pt^{II} complexes do exhibit biological effects; the active monodentate Pt^{II} complexes commonly feature bulkier amine ligands than the hitherto used dien or NH_3 groups. We were therefore interested in determining whether a simple but marked enhancement of the bulkiness of the dien ligand in monodentate $[Pt(NO_3)(dien)]^+$ by multiple methylation of this ligand affects the early phases in which platinum compounds exert their biological activity. More specifically, the goals of this study, performed in cell-free media, were to determine how the modification of DNA duplexes by methylated analogues of $[Pt(NO_3)(dien)]^+$ affects their energetics and how the alterations of this biophysical parameter are reflected by the recognition of these duplexes by DNA polymerases and the DNA repair system. We have found that the impact of the methylation of $[Pt(NO_3)(dien)]^+$ on the biophysical properties of DNA (thermodynamic, thermal, and conformational properties) and its biochemical processes (DNA polymerization and the repair of DNA adducts) is remarkable. Hence, we conclude that monodentate DNA binding of Pt^{II} compounds may considerably affect the biophysical properties of DNA and consequently downstream cellular processes as a result of a large increase in the bulkiness of the nonleaving ligands in this class of metal complex.

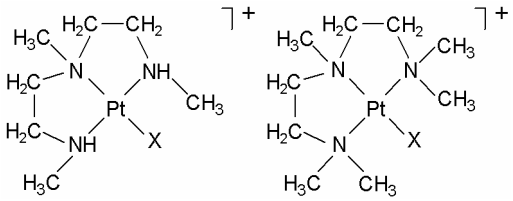

Figure 3: Structures of $[Pt(NO_3)(dien\text{-}Me_3)]^+$ and $[Pt(NO_3)(dien\text{-}Me_5)]^+$.

DNA interactions of dinuclear Ru^{II} arene antitumor complexes in cell-free media

The new dinuclear ruthenium drugs - in which two {(η^6-p-isopropyltoluene)RuCl[3-(oxo-κO)-2-methyl-4-pyridinonato-κO_4]} units were linked by flexible chains of different length $[(CH_2)_n$ (n = 4, 6, 8, 12)] - were found to exert promising cytotoxic effects in human cancer cells. DNA modifications by these new dinuclear Ru^{II} arene compounds, which differed in the length of the linker between the two Ru^{II} centers, were examined by biochemical and biophysical methods. The complexes bind DNA forming intrastrand and interstrand cross-links in one DNA molecule in the absence of proteins. An intriguing aspect of the DNA-binding mode of these dinuclear Ru^{II} compounds is that they can crosslink two DNA duplexes and also proteins to DNA—a feature not observed for other antitumor ruthenium complexes. Thus, the concept for the design of interhelical and DNA–protein cross-linking agents based on dinuclear Ru^{II} arene complexes with sufficiently long linkers between two Ru centers may result in new compounds which exhibit a variety of biological effects and can be also useful in nucleic acids research.

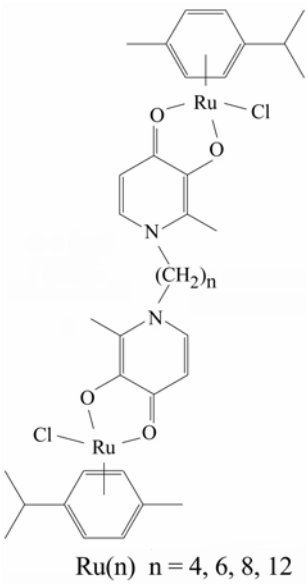

Figure 4: Structures of the dinuclear Ru^{II} arene complexes and their abbreviations.

Mechanistic studies of the modulation of cleavage activity of topoisomerase I by DNA adducts of mono- and bi-functional Pt^{II} complexes

Using electrophoresis and replication mapping, we show that the presence of DNA adducts of bifunctional antitumor cisplatin or monodentate [PtCl(dien)]Cl (dien = diethylenetriamine) in the substrate DNA inhibits eukaryotic topoisomerase 1 (top1) action, the adducts of cisplatin being more effective. The presence of camptothecin in the samples of platinated DNA markedly enhances effects of Pt–DNA adducts on top1 activity. Interestingly, the effects of Pt–DNA adducts on the catalytic activity of top1 in the presence of camptothecin differ depending on the sequence context. A multiple metallation of the short nucleotide sequences on the scissile strand, immediately downstream of the cleavage site impedes the cleavage by top1. On the other hand, DNA cleavage by top1 at some cleavage sites which were not platinated in their close proximity is notably enhanced as a consequence of global platination of DNA. It has been suggested that this enhancement of DNA cleavage by top1 may consist in its inability to bind to other cleavage sites platinated in their close neighborhood; thus, more molecules of top1 may become available for cleavage at the sites where top1 normally cleaves and where platination does not interfere.

Studies of the mechanism of action of platinum(II) complexes with potent cytotoxicity in human cancer cells

The biological activity of 12 platinum(II)-based DNA intercalators of the type $[Pt(I_L)(A_L)]^{2+}$, where I_L is an intercalating ligand (1,10-phenanthroline or a methylated derivative) and A_L is an ancillary ligand (diamino-cyclohexane, diphenylethylenediamine or 1,2-bis(4-fluorophenyl)-1,2-ethylenediamine) was examined. The chiral compounds and the racemic compounds were tested against a panel of human cancer cell lines, with a number of complexes displaying activity significantly greater than that of cisplatin (up to 100-fold increase in activity in the A-427 cell line). The activity of the complexes containing diphenylethylenediamine and 1,2-bis(4-fluorophenyl)-1,2 ethylenediamine was significantly lower compared to the complexes containing diaminocyclohexane. Further in vitro testing, such as DNA unwinding, competition assays, and DNase I footprinting, was conducted on the most active compound [(5,6-dimethyl-1,10-phenanthro-line)(1*S*,2*S*-diaminocyclohexane)platinum(II)]$^{2+}$ and its enantiomer to provide information about the mechanism of action. These complexes display activity in cisplatin resistant cell lines, have higher cellular uptake than cisplatin, and do not activate caspase-3 as cisplatin does, indicating that these complexes exhibit a different mechanism of action.

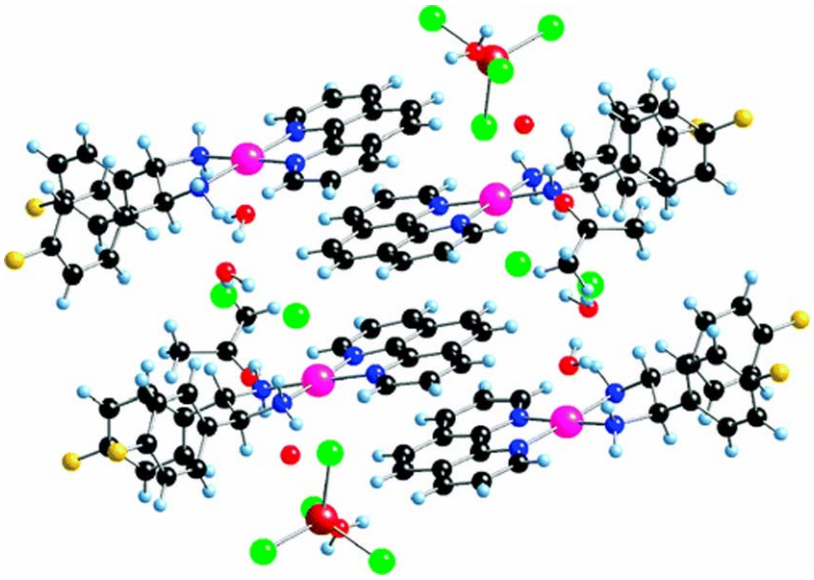

Figure 5: Structures of the platinum(II)-based DNA intercalating complexes.

Oligopyridine–ruthenium(II)–amino acid conjugates: synthesis, characterization, DNA binding properties and interactions with the oligonucleotide duplex d(5-CGCGCG-3)$_2$

The binding properties of diastereomeric oligopyridine–ruthenium(II)–amino acid conjugated complexes of the general formulas Λ- and Δ-[Ru(bpy)$_2$(4,4′(CO$_2$Y)$_2$-bpy)]$^{2+}$, where Y = L-AlaCONH$_2$, L-LysCONH$_2$, L-HisCONH$_2$, L-TyrCONH$_2$ with calf-thymus DNA and the oligonucleotide duplex d(5-CGCGCG-3′)$_2$, were investigated by means of circular dichroism (CD), NMR spectroscopy and DNA thermal denaturation (T_m) curves were studied. CD and T_m data indicate that all diastereomeric complexes bind to the DNA major groove, Δ-diastereomers in a similar manner, while Λ-diastereomers in dependence of the nature of the amino acid. NMR studies of d(5′CGCGCG-3′)$_2$, and the complexes Δ- **1**, Δ- **2**, Λ- **1** and Λ- **2** indicate that Δ- **1** and Δ- **2** were bound having the ancillary bpy ligands towards the DNA groove, while the corresponding Λ- **1** and Λ- **2** were orientated in a similar way, facing the ligand 4,4′(CO$_2$Y)$_2$bpy towards the DNA major groove. Photoinduced DNA cleavage was observed in all cases studied, which take place through singlet oxygen production. Δ- **4** and Λ- **4** show the lower photoinduced cleavage yield, probably because the singlet oxygen (^{1}O$_2$) oxidizes not only the DNA phosporodiesteric bonds but the tyrosine's phenolic OH bond as well.