Tuning the metabolism of the anticancer drug cisplatin with chemoprotective agents to improve its safety and efficacy

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Numerous in vivo studies have shown that the severe toxic side-effects of intravenously administered cisplatin can be significantly reduced by the co-administration of sulfur-containing ‘chemoprotective agents’. Using a metallomics approach, a likely biochemical basis for these potentially useful observations was only recently uncovered and appears to involve the reaction of chemoprotective agents with cisplatin-derived Pt-species in human plasma to form novel platinum–sulfur complexes (PSC’s). We here reveal aspects of the structure of two PSC’s and establish the identification of an optimal chemoprotective agent to ameliorate the toxic side-effects of cisplatin, while leaving its antineoplastic activity largely intact, as a feasible research strategy to transform cisplatin into a safer and more effective anticancer drug.

Introduction

The serendipitous discovery of the antiproliferative effects of cis-diaminedichloroplatinum(ii) or cisplatin [CP] on E. coli cells in the 1960s combined with its approval by the FDA in 1978 heralded the era of platinum-based chemotherapy. Despite the subsequent FDA approval of second- and third-generation platinum-based anticancer drugs, such as carboplatin in 1989 and oxaliplatin in 2002, CP – which is intravenously administered either alone or in combination with other anticancer drugs – remains one of the most effective anticancer drugs that is used worldwide owing to its broad spectrum of activity towards a variety of cancers, including testicular, ovarian, head and neck, colorectal, bladder, cervical and lung cancer as well as melanoma and lymphomas. Extensive studies into the metabolism of CP have revealed that it constitutes a prodrug and it is commonly believed that the binding of the CP-derived monoqua hydrolysis product [(NH₃)₂PtCl(H₂O)]⁺ to DNA represents its likely mode of action. Given the complexity of the intracellular biochemistry of CP, however, further studies are needed to rule out whether other biomolecular mechanisms may also contribute to its overall activity.

Severe toxic side-effects of CP

In contrast to so-called ‘molecularly targeted’ anticancer drugs which target a single pathway to kill cancer cells, CP represents a ‘shotgun’ cytotoxin which offers two major advantages: it is active against many different cell types in a tumour and its utilization is conceptually less susceptible to the development of resistance (although it does occur). Shotgun cytotoxins, however, also exhibit a dark side. With regard to CP, its therapeutic use and efficacy is inherently limited by the severe toxic side-effects that this metal-based drug exhibits on several non-proliferating cell types, which often results in life-long impacts on the quality of life of patients. For example, 30 to 60% of patients suffer from nephrotoxicity, more than 60% of pediatric patients develop bilateral and permanent hearing loss and up to 90% of patients develop neurotoxicity. Although nephrotoxicity in patients can be somewhat ameliorated by increased hydration or the administration of mannitol, no approved procedures exist to completely eliminate ototoxicity, the latter therefore constitutes a primary dose limiting factor. Likewise, there are currently no established clinical procedures to reduce or ameliorate the neurotoxicity of this otherwise very effective anticancer drug.

Strategies to ameliorate the severe toxic side-effects of Pt-based anticancer drugs

Owing to the efficiency of CP and its inherent limitations, considerable research efforts are directed to improve the tumor
selectivity of Pt-based anticancer drugs. This fundamental challenge can be addressed either by synthesizing novel Pt-compounds\cite{31,12} or by improving the delivery of established or newly synthesized Pt-based drugs to the tumor by drug-delivery vehicles.\cite{13} The third principle approach – the one that is of focal interest in the context of this mini-review – aims to reduce the CP-induced severe toxic side-effects by the co-administration of small-molecular-weight ‘chemoprotective agents’, while leaving the anticancer effect of CP largely intact.\cite{14} Compared to the first two approaches, the latter approach is potentially more cost effective since it aims to selectively reduce the severe toxic side-effects of an already FDA-approved Pt-drug with a chemoprotective agent that may – ideally – already be approved by the FDA (i.e. costly drug approval processes are avoided altogether).

### Reducing CP-induced toxic side-effects by ‘chemoprotection’

Numerous studies with animal models or patients have demonstrated that chemoprotective agents, such as sodium thiosulfate (STS),\cite{15} \(N\)-acetyl-L-cysteine (NAC),\cite{15,16} amifostine,\cite{15,17} sodium diethylthiocarbamate,\cite{9,19} D-methionine,\cite{9,19} L-methionine,\cite{19,20} L-glutathione (GSH),\cite{21} cimetidine,\cite{22} sodium salicylate,\cite{23} or newly synthesized Pt-based drugs to the tumor by drug-delivery vehicles.\cite{13} The third principle approach – the one that

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Graham George was educated at King's College London (BSc, 1979) and the University of Sussex (DPhil, 1983). After postdoctoral fellowships at the University of Sussex and at Exxon Research & Engineering Co. in New Jersey, USA, he continued at Exxon as a Principal Investigator until moving to the Stanford Synchrotron Radiation Laboratory in 1992. In 2003 took up the position of full professor and Tier 1 Canada Research Chair of X-ray Absorption Spectroscopy at the University of Saskatchewan. His research directions include a career-long interest in X-ray spectroscopy and the development of new methods for understanding the roles and mechanisms of metals in biology. He is married to fellow professor and Canada Research Chair Ingrid Pickering, and they have three children.

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Ingrid Pickering received her BA in Natural Sciences from the University of Cambridge (UK) in 1986 and following this used Physical Chemistry to study heterogeneous catalysis at the Royal Institution and received a PhD from Imperial College London (UK) in 1990. After two years as a postdoctoral fellow with Exxon Research and Engineering Co. (NJ, USA), she moved to the Stanford Synchrotron Radiation Laboratory (CA, USA) in 1992.

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Aru Narendran is a full professor in the departments of pediatrics and oncology, Faculty of Medicine, University of Calgary. His primary research and clinical interests focus on the development of new agents and novel therapeutics for refractory pediatric malignancies. He is the recipient of a number of awards including the Odile Schweiguth International Prize for pediatric oncology research and the young investigator award from the Children’s oncology group (COG). Currently, he directs the POETIC pre-clinical and drug discovery laboratory with the mandate to identify agents and regimens for early phase clinical trials for currently difficult to treat pediatric cancer patients.
Conclusions

The bloodstream represents the first biological compartment where chemical reactions between intravenously administered CP and a chemoprotective agent may ensue. Although relevant chemistry between CP (and its metabolites) and a chemoprotective agent may also occur in internal organs, it is very difficult to tune the metabolism of CP therein and it will therefore not be further discussed. Conceptually, the intravenous administration to a patient with an appropriate chemoprotective agent should allow one to modulate the metabolism of CP in the bloodstream, which will ultimately determine which Pt-species are left in the bloodstream to subsequently interact with healthy (unintended) and tumor tissue cells (intended) (Fig. 1). In order to evaluate the potential of this ‘chemoprotection approach’, one needs to first understand the metabolism of CP in the bloodstream itself before one can probe the effect that chemoprotective agents may exert on its metabolism (Fig. 1) as well as other blood constituents, such as erythrocytes as well as plasma proteins and metalloproteins.

It is commonly believed that intravenously administered CP does not hydrolyze in the bloodstream, owing to the comparatively high concentration of $\text{Cl}^-$ (~100 mM) in human blood plasma. After the addition of CP to human plasma in vitro, however, highly toxic CP-derived hydrolys products (CPHP) and plasma protein bound Pt-species (Pt-PP) were present within as little as 5 min, while the majority of Pt was still present as the parent drug. All chemoprotective agents that we have investigated – STS, NAC, $\alpha$-methionine and GSH – affected the metabolism of CP in plasma by producing additional Pt-peaks that did not match the retention time of the Pt-peaks that were detected when only CP was added to plasma (in this case Pt-peaks corresponding to CP,

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Fig. 1 Conceptual depiction of the chemical reactions that occur between cisplatin (CP) and chemoprotective agents in human plasma in vitro as well as the repercussions that these Pt-species will exert in the whole organism in vivo. CPHP refers to all CP-derived hydrolys products and PP refers to plasma proteins.
CPHP's and Pt-PP's were detected. The additional Pt-peaks were therefore assigned to novel Pt-containing sulfur complexes or PSC's in blood plasma. The mechanism of formation of these PSC's likely involves the reaction of the highly reactive monoaqua hydrolysis product of CP – [(NH₃)₂PtCl(H₂O)]⁺ – with each chemoprotective agent. Based on this demonstrated ‘tunability’ of the metabolism of CP with chemoprotective agents in blood plasma, it is now possible to discuss potential advantages and disadvantages of this principle approach to possibly improve Pt-based anticancer drugs that are currently in use.

The advantages of a ‘chemoprotection approach’ are that the identification of an inexpensive and safe chemoprotective agent that can neutralize highly toxic CPHP's should allow one to reduce the severe toxic side effects of CP while minimally affecting the efficacy of the parent drug CP (Fig. 1). There are three potential disadvantages that have to be considered in the context of this principle approach, (a) the chemoprotective agent itself may exert adverse toxic effects by affecting the integrity of endogenous plasma metalloproteins, such as transferrin, ferritin and Zn bound to human serum albumin (which would result in potential toxicity), (b) the chemoprotective agent may decrease the plasma lifetime of the parent CP (which would decrease the efficacy of the latter) and/or (c) the PSC may not be effectively excreted, which in turn could result in possible organ toxicity (e.g. if the PSC traverses the blood–brain barrier).

**Tuning the metabolism of CP with an optimal chemoprotective agent**

In the context of translating this chemoprotection approach into practical benefits for patients (e.g. reduction of ototoxicity, neurotoxicity and/or nephrotoxicity), it is important to clearly distinguish between information that can be obtained from *in vitro* studies (e.g. using human plasma) and information that can only be derived from *in vivo* studies (e.g. using an appropriate animal model). *In vitro* studies are crucial to establish whether the mode of action of a chemoprotective agent involves the formation of a complex with CPHP's in plasma. If this is the case, the structure of the formed PSC can either be elucidated by X-ray absorption spectroscopy (Fig. 2) and/or mass spectrometry (Fig. 3). In *in vitro* studies can also establish an effective molar ratio between the chemoprotective agent and CP which will preclude the formation of free CPHP's in plasma and minimize a chemoprotective agent-induced perturbation of endogenous plasma metalloproteins. Furthermore, the antitumour activity of the formed PSC's and their acute (IC₅₀) as well as their potential long-term toxicity can be established by assessing toxicogenomic endpoints in cell culture experiments using appropriate cell lines. After the completion of these *in vitro* studies, *in vivo* studies with an appropriate animal model are absolutely necessary to further optimize the molar ratio between the chemoprotective agent and CP, their order of injection as well as the time lag between the administration of the two drugs. In addition, *in vivo* studies are required to detect a potentially adverse organ accumulation of a formed PSC (e.g. in the brain) and to assess the clearance of formed PSC complexes from the bloodstream to the kidneys and ultimately the urine.

**Future outlook**

The elucidation of the mechanisms by which chemoprotective agents affect the metabolism of CP in the bloodstream is absolutely critical in the context of developing a clinical treatment protocol that can be employed to significantly reduce the toxic side-effects of CP in patients while maintaining its antitumor efficacy. This task is now within reach since state-of-the-art metallomics tools can be applied in conjunction with animal studies. In principle, the approach of selectively inactivating the toxic hydrolysis products of CP in the bloodstream by an optimal chemoprotective agent, while maximizing the lifetime of the active anticancer prodrug CP in the blood circulation would effectively transform this anticancer drug into a much safer one.

Fig. 2 X-ray absorption spectroscopy-derived structure of the [Pt(S₂O₃)₄]⁶⁻ that was isolated from PBS-buffer (STS : CP = 400 : 1). (A) Shows the Pt L₃ EXAFS oscillations and (B) the Pt–S phase corrected Fourier transforms, for data (red lines), together with the best fit (blue lines). Best fits indicated four equivalent Pt–S at distance R of 2.31 Å, with σ², the mean square deviation in R, of 0.0031 Å². The inset shows the energy minimized geometry optimized density functional theory structure for [Pt(S₂O₃)₄]⁶⁻ constrained to S₄ point group symmetry to assist with convergence. The computed Pt–S bond lengths were 2.33 Å, in excellent agreement with the EXAFS results, and both are in agreement with the Pt–S distance of the sole reported crystal structure of a terminal thiosulfate bound to a Pt(II) ion.
This strategy may also allow one to improve the therapeutic potential of CP (as well as other toxic metal-based anticancer drugs) by escalating the dose that is administered to a patient. This would be particularly useful as this would allow one to treat even those patients in which the tumor has developed drug resistance and where the CP dose can often not be further increased because of the inherent severe toxic side-effects of CP.

**Acknowledgements**

MS is a Fellow in the Canadian Institutes of Health Research Training grant in Health Research Using Synchrotron Techniques (CIHR-THRUST). AN is supported by a research grant from the Alberta’s Children’s Hospital Foundation. GNG and IJP are supported by Canada Research Chairs, with research at the University of Saskatchewan further supported by NSERC, CIHR, SHRF and the Government of Saskatchewan. Use of the Stanford Synchrotron Radiation Lightsource (SSRL), SLAC National Accelerator Laboratory is supported by the U.S. DOE, Office of Science, OBES under Contract No. DE-AC02-76SF00515. The SSRL Structural Molecular Biology Program is supported by the DOE Office of Biological and Environmental Research and by the National Institutes of Health (NIH), National Institute of General Medical Sciences (NIGMS) including P41GM103393. The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of NIGMS or NIH.

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