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Short communication

Improving the safety of metal-based drugs by tuning their metabolism with chemoprotective agents

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ABSTRACT

Metal-based drugs remain a tiny minority of all drugs that are on the market. The success story of the quintessential metal-based drug cisplatin (CP), which is intravenously administered to 70% of all cancer patients, however, demonstrates the inherent potential of metal-based drugs. A distinct disadvantage of CP is the dose-limiting severe toxic-side effects that it exerts in patients. To better understand the biomolecular basis for its toxicity, we employed a metallomics method to observe all platinum metabolites that are formed in blood plasma. These investigations revealed that a highly toxic CP-derived hydrolysis product – the highly toxic monoaqua hydrolysis complex (MHC) – is formed within 5 min. More importantly, the application of this research tool has unraveled the mechanisms by which the chemoprotective agents sodium thiosulfate, D-methionine, *N*-acetyl-cysteine and L-glutathione modulate the metabolism of CP in plasma, namely by rapidly reacting with the MHC to form platinum-sulfur complexes. Since CP remained in plasma for a considerable time, the possibility of 'tuning' its metabolism with chemoprotective agents in a desirable way has emerged. These observations are highly relevant because these chemoprotective agents were previously shown to significantly reduce the toxicity of CP in animal models, often without appreciably affecting its anticancer efficiency. Collectively, these results suggest that the toxicity of other metal-based drugs may be overcome if their metabolism in the bloodstream is adequately tuned with a suitable chemoprotective agent. This principle strategy has considerable potential in terms of harnessing the full potential of bringing more metal-based drugs to the market.

1. Introduction

Barnett Rosenberg's discovery of the antiproliferative effects of *cis*-diamminedichloroplatinum(II) or *cis*-platin [CP] on *E. coli* cells in the 1960s followed by the FDA approval of this platinum compound in 1978 heralded the era of platinum-based chemotherapy [1]. Although the FDA approved the second- and third-generation platinum-based anticancer drugs carboplatin in 1989 and oxaliplatin in 2002 [2], CP remains one of the most effective anticancer drugs that offers a 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 [3–5]. Detailed studies into the metabolism of CP have revealed that it is actually a prodrug. After the uptake into cells CP is activated by hydrolysis and the generated monoaqua hydrolysis complex $[(NH_3)_2PtCl(H_2O)]^{+1}$ (MHC) will eventually bind to the DNA which precludes cell replication and will result in cell death [5]. In spite of the inherent complexity of the intracellular biochemistry of CP [3,6–8], however, further studies are needed to establish if other biomolecular events may also contribute to its anticancer activity [9].

2. Severe toxicity of the 'shotgun cytotoxin' CP

There are two types of anticancer drugs. 'Molecularly targeted' anticancer drugs target a single pathway to kill cancer cells and are therefore prone for resistance to develop in the target cells. 'Shotgun cytotoxins', on the other hand, are active against many different cell types in a tumor environment and are therefore less susceptible to the development of resistance [10] (resistance to CP, however, does occur [11]). Shotgun cytotoxins, however, also exhibit an inherent disadvantage: systemic toxicity. The therapeutic use and efficacy of CP, for example, is inherently limited by the severe toxic side-effects that this metal-based drug exhibits on several non-proliferating cell types [8], which often results in life-long impacts on the quality of life of patients [12]. For example, 30 to 60% of patients suffer from nephrotoxicity [13,14], more than 60% of pediatric patients develop bilateral and permanent hearing loss [15] and up to 90% of patients exhibit some symptoms of neurotoxicity [4]. Although nephrotoxicity in patients can be somewhat ameliorated by increased hydration or the administration of mannitol [16], no procedure has been clinically approved to completely eliminate ototoxicity. These side-effects therefore essentially

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constitute a primary dose limiting factor [17]. Likewise, there are currently no established clinical procedures to ameliorate its neurotoxicity [18]. In terms of developing a strategy to mitigate these severe toxic side-effects of CP it is critical to identify which platinum species play an important role. To this end, the MHC is likely to be a key player since a) we have detected this species in human blood plasma within 5 min after the addition of CP [19,20], b) ~15% of total platinum in plasma of patients was present as the MHC after a 1-h infusion of cisplatin [21] and c) the MHC is 8-times as toxic as CP [22,23].

3. Strategies to improve the safety of Pt-based anticancer drugs

Based on the established efficiency CP and the related platinum-based anticancer drugs carboplatin and oxaliplatin as well as their severe toxic-side effects, considerable research efforts are currently underway to enhance the tumor selectivity of Pt-based anticancer drugs to achieve actual benefits for cancer patients. This fundamental challenge is being addressed by synthesizing novel Pt-compounds [5,24,25] and/or by improving the delivery of established or newly synthesized Pt-based drugs to the tumor by selective drug-delivery vehicles [26–29]. The third principle approach – the one that is the focus of our group – 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 [30]. This latter approach is potentially more cost effective than the first two approaches as it aims to selectively reduce the severe toxic side-effects of an already FDA-approved Pt-drug with a chemoprotective agent that is – ideally – already approved by the FDA for other purposes. Thus, the third approach altogether avoids costly drug approval processes which are required for novel drug entities or novel drug delivery vehicles.

4. Chemoprotective agents reduce the severe toxic side-effects of CP

To date a large number of studies with animal models and/or patients have demonstrated that the chemoprotective agents sodium thiosulfate (STS) [31–35], *N*-acetyl-L-cysteine (NAC) [31,36–38], amifostine [33,39,40], sodium diethyldithiocarbamate [17,33,41–43], D-methionine (DM) [16,44–48], L-methionine [44,49], L-glutathione (GSH) [50–52], cimetidine [53], sodium salicylate [54,55], L-carnosine [56], 2,3-dimercapto-1-propanesulfonic acid [57], and procaineamide hydrochloride [58–61] can significantly reduce the severe toxic side-effects of CP. At present amifostine is the only chemoprotective agent that has been clinically approved by the FDA specifically for CP therapy [62], but it did not significantly reduce the CP-induced ototoxicity in patients compared to the CP only treated group [23]. Of the aforementioned chemoprotective agents STS and NAC have been approved by the FDA for the treatment of cyanide poisoning and for acetaminophen overdose, while DM is in phase 3 clinical trials to reduce noise-induced hearing loss [12]. Last but not least, GSH represents an endogenous compound that would not require FDA approval for its administration.

5. Mechanism by which chemoprotective agents modulate the metabolism of CP *in vitro*

In view of the rather extensive body of literature which demonstrates that ameliorating agents can effectively reduce the toxicity of CP *in vivo*, it is perhaps surprising that the underlying mode of action as to where and how the relevant bioinorganic chemistry unfolds in the organism at the molecular level has remained elusive as long as it has. This undesirable situation must be attributed on the on hand to the complexity of biological systems (*e.g.* blood plasma contains thousands of proteins [63]) and a lack of appropriate analytical tools to gain insight into the metabolism of CP on the other. To simultaneously address both of these aspects, we have developed a metallomics tool [19,64] which is based on the hyphenation of size-exclusion chromatography (SEC) coupled on-line to an inductively coupled plasma atomic emission spectrometer (ICP-AES). This SEC-ICP-AES system was first employed to directly observe the metabolism of CP in plasma. After spiking plasma with CP and incubating the obtained mixture at 37 °C, the analysis of plasma aliquots over a 24 h period revealed CP and the time dependent formation of CP-derived hydrolysis products as well as platinated plasma proteins [19]. This metallomics tool was subsequently employed to establish the comparative metabolism of cisplatin and carboplatin using the same human plasma stock and the obtained results were in accord with what one would expect from the literature [19]. Once the validity of the results that can be obtained with the developed metallomics tool had been established, we employed it to investigate the effect of chemoprotective agents on the metabolism of CP. Our results revealed that all of the aforementioned chemoprotective agents chemically react with CP-derived hydrolysis products in human and/or rabbit plasma to form platinum-sulfur complexes (PSC's) which corresponded to the detection of additional Pt-peaks that did not match the retention time of the Pt-peaks that were detected when only CP was added to plasma [65–68]. The mechanism of formation of these PSC's likely involves the reaction of the highly reactive monoaqua hydrolysis product of CP – $[(NH_3)_2PtCl(H_2O)]^{+1}$ – with each chemoprotective agent.

6. Understanding the *in vitro* results in the context of the organism

At this point it is instructive to ponder in what way the *in vitro* formation of CP-derived platinum metabolites after the addition of CP to plasma and the formation of platinum-sulfur complexes after the addition of CP and a chemoprotective agent may be exploited. In principle, we need to distinguish between two distinct ways by which all Pt-species that are either present (CP) or are formed in the bloodstream (CP-derived hydrolysis products, platinated plasma proteins, platinum-sulfur complexes) will effect cancer cells and healthy tissues cells: their *uptake into cells* [69,70] and the associated interactions with biomolecular targets therein (*e.g.* DNA binding) as well as their *interaction with the surface* of all cells that are in direct contact with the bloodstream (*e.g.* induction of apoptosis [3]).

With regard to the metabolism of CP in plasma and the subsequent cell uptake of the Pt-species from the bloodstream (Fig. 1), each Pt-species will have a certain propensity to be uptaken into cancer cells (intended anticancer effect, red lightning bolt in Fig. 1) as well as healthy tissue cells (unintended toxic-side effect, red lightning bolt in Fig. 1). Owing to the different 'flux' of each of the Pt-species into cancer cells and healthy tissue cells, the net uptake of all Pt-species by cancer cells and healthy tissue cells may be rather similar since CP represents a shotgun cytotoxin. With regard to the toxicity that individual Pt-species will exert by interacting with the cell surface, however, we have − to a first approximation – only to consider two kinds of Pt-species, namely CP itself and the MHC, which is 8-times more toxic than CP. Therefore, the MHC, which is rapidly formed in blood plasma is likely to play a key role in mediating the severe toxic side-effects of CP (Fig. 1, yellow lightning bolts). Thus, the rapid formation of the MHC in the blood stream and its inherent toxicity play a crucial role in the context of translating the chemoprotection approach to benefits to patients.

With regard to the chemoprotective agent-driven modulation of the metabolism of CP in plasma and the subsequent cell uptake of the Pt-species from the bloodstream (Fig. 2), the situation is now different as it is currently unknown to what extent the formed platinum-sulfur complexes are uptaken into cells. Assuming that neither the platinum-sulfur complexes nor the platinated plasma proteins are appreciably uptaken by cells, only CP will remain the blood circulation for it to be uptaken by cancer cells and healthy tissue cells. While it is unknown whether there may be a slight selectivity of CP to enter cancer cells over healthy tissue cells (Fig. 2, red lightning bolts), the fact that no highly toxic MHC remains in the blood circulation would imply that the severe toxic