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Subcellular targets of cisplatin cytotoxicity: An integrated view ☆

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ABSTRACT

Cisplatin is a chemotherapeutic drug widely used against a variety of cancers. Its clinical utility is severely limited by its toxicity, which mainly affects, but is not limited to, the inner ear and renal tubules. Cisplatin toxicity is determined by target tissue and cell accumulation, subcellular handling and trafficking through diverse subcellular structures, and interaction with macromolecules. Cisplatin accumulates and stresses different organelles from which delay signaling is activated, including mitochondria, lysosomes, the endoplasmic reticulum, the nucleus, the cell membrane and cytoskeleton, and can also be found in the cytosol. This article critically summarizes the available information in order to establish the connection among its known subcellular effects in a hierarchical and integrative framework. Cisplatin causes different types of cell death in a concentration-dependent manner. Knowledge of the events and signaling leading to the different phenotypes is also intertwined within the model, within the scope of the potential utility of this information in the improvement of the pharmacotoxicological profile of this drug. Perspectives for the key aspects that need to be addressed by future investigation are also outlined.

Contents

Abbreviations: AIF, Apoptosis inducing factor; ATP7A and ATP7B, Copper P-type adenosine triphosphate transporters 7A and 7B; C, Caspase; CAT, Catalase; CL, Cardiolipin; c-Abl, Abelson murine leukemia viral oncogene homologue; CHOP, CCAAT-binding homologous protein; Cyt, Cytosolic; Cyto *c*, Cytochrome *c*; DISC, Death inducing signaling complex; DNA-PK, DNA-dependent protein kinase; CTR, Copper transporter; ER, Endoplasmic reticulum; ERK, Extracellular signal-regulated kinase; FLIP, FLICE-like inhibitor protein; Grp, Glucose-regulated protein; GADD153, Growth arrest- and DNA damage-inducible gene 153; GR, Glutathione reductase; GSH, Glutathione; HMG, High-mobility group; IAP, Inhibitor of apoptosis protein; IRE1, Inositol-requiring enzyme 1; JNK, Jun kinase; LAMP, Lysosome associated membrane protein; LMP, Lysosomal membrane permeabilization; LRP, Lung resistance related protein; MAPK, Mitogen activated protein kinase; MAPKKK, MAPK kinase kinase; MKK, MAPK kinase; MPT, Mitochondrial permeability transition; NFκB, Nuclear factor kappa B; OCT, Organic cation transporter; PERK, Protein kinase RNA-like ER kinase; POX, Peroxidase; ROS, Reactive oxygen species; SOD, Superoxide dismutase; SPHMLase, Sphingomyelinase; TNF-α, Tumor necrosis factor alpha; TNFR, TNF-α receptor; UPR, Unfolded protein response.

☆ Conflict of interest statement: the authors declare that there are no conflicts of interest.

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1. Introduction

Cisplatin (*cis*-diamminedichloroplatinum or CDDP) is a chemotherapeutic drug widely used against a wide variety of solid tumors (Cepeda et al., 2007). It was first described in 1845 by Michel Peyrone (thereafter Peyrone's salt); and its structure was elucidated in 1893 by Alfred Werner. Its antitumor potential was discovered in the 1960s after the observations from Barnett Rosenberg's group on its capacity to inhibit bacterial fission (Rosenberg et al., 1965) and the growth of sarcomas transplanted in mice. Initial clinical studies carried out by Hill's group (Hill et al., 1975) demonstrated its efficacy against several human malignancies, and it was first approved for clinical use in the USA in 1978 (Hill and Speer, 1982). Cisplatin is highly toxic for proliferating cells, because it forms adducts with DNA and impedes DNA replication and mitosis (Saris et al., 1996; Sorenson and Eastman, 1988). However, its therapeutic use and efficacy are limited by its side-effects, mostly nephrotoxicity (mainly tubular necrosis), ototoxicity (cochlear damage), neurotoxicity (mainly peripheral sensory neuropathy) and others. Side effects occur especially at high dosage by acting on several non-proliferating cell types (Barabas et al., 2008; Jaggi and Singh, 2012; Rybak et al., 2009; Sanchez-Gonzalez et al., 2011a). Intrinsic and acquired resistance is another limitation to the therapeutic effect of cisplatin on tumor cells (Cepeda et al., 2007). As such, cisplatin's cytotoxicity is at cross-roads of its therapeutic and side effects. Further knowledge of its cytotoxic mechanisms in tumor and normal cells might help improve the pharmaco-toxicological profile of this drug by exploiting potential differences in its handling or response.

The kidneys accumulate cisplatin. Also other organs such as the liver, prostate, spleen, bladder, muscle, testicle, pancreas, bowel, adrenal, heart, lung, cerebrum and cerebellum also accumulate the drug to a higher or lesser extent (Huo et al., 2005; Junior et al., 2007, McIntosh et al., 1997; Riviere et al., 1990; Wang et al., 2007). The pattern of tissue accumulation does not always coincide with the pattern of tissue toxicity (Staffhorst et al., 2008; Stewart et al., 1982). It might be possible that a given tissue accumulation of cisplatin results in a differential intracellular accumulation from tissue to tissue, or from cell type to cell type, with inverse accumulation within the tissue's extracellular compartment. This might explain why a higher accumulation in a determined organ would result in lower damage when compared to another organ with lower accumulation and higher damage. For example, Junior et al. (2007) have shown higher accumulation of cisplatin in the liver and spleen than in the kidneys. Also human tissue platinum concentrations were highest in liver and prostate (Stewart et al., 1982). However its main toxic effect is nephrotoxicity. Tumors also accumulate cisplatin. Yet, tumor accumulation, i.e. the tumor tissue-to-plasma partition coefficient, is lower than in many organs, even in many in which cisplatin has no significant or much milder effect (Junior et al., 2007; Staffhorst et al., 2008). As we have demonstrated (Sancho-Martinez et al., 2011), lower concentrations of cisplatin are needed to cause cell cycle arrest than to induce cell death. Because most somatic cells are not undergoing division (i.e. they rest in the G0 state) under normal circumstances, this might explain why subtoxic or low toxic doses of cisplatin exert an antitumor effect.

Intracellular determinants such as the red-ox status also condition cisplatin toxicity. This is because the molecule of cisplatin has much lower toxicity and reactivity than its aquated metabolites, which have much higher avidity for nucleophilic sites in macromolecules (Kartalou and Essigmann, 2001). Inside the cells, a low chloride environment, its chloride ions are substituted by water molecules (Andrews and Howell, 1990; Ekborn et al., 2003; Johnson et al., 1980). This process is modulated by the level of available molecules with free thiol groups, which capture cisplatin species and prevent them from binding other targets (Dabrowiak et al., 2002; Sadowitz et al., 2002).

Cisplatin causes cell death both by apoptosis and non-apoptotic, necrotic-like processes (Cepeda et al., 2007; Price et al., 2004; Ramirez-Camacho et al., 2008; Sato et al., 2001). The mode of cell death has been linked to cisplatin concentration. In tumor (Guchelaar et al., 1998; Sancho-Martinez et al., 2011) and non-tumor cells (Lieberthal et al.; 1996; Sancho-Martinez et al., 2011), low concentrations of cisplatin induce apoptosis, whereas higher ones cause necrosis. Both apoptosis and necrosis have been also found in vivo, after treatment with this drug, in tumors and renal cells (Meyer and Madias, 1994; Sato et al.; 2001). In the case of renal toxicity, necrosis is mostly found in the proximal tubule (along with apoptosis), whereas in the distal tubule only apoptosis is observed (Kroning et al., 2000; Megyesi et al., 1998; Price et al., 2004). This has been explained by a lower concentration of cisplatin reaching the distal tubule, as the bulk of filtered drug is reabsorbed in the proximal tubule (Kroning et al., 1999; Sancho-Martinez et al., 2011). Necrosis is a form of cell death that induces an inflammatory and innate immune response (Festjens et al., 2006; Nunez et al., 2010; Zong and Thompson, 2006). Necrotic cells contribute to activate the inflammatory response known to participate in the pathophysiological mechanisms of cisplatin's nephrotoxicity (Sanchez-Gonzalez et al., 2011a, 2011b). The consequences of tumoral necrosis in the context of the antitumoral effect of cisplatin are not well determined. All this means that, at least for a better control of cisplatin's side effects, both the injury sites and pathways leading to activation of apoptosis and those leading to necrosis need to be identified for appropriate and individual pharmacological targeting. The next sections of this review critically compile the information known on the effects of cisplatin at the subcellular level, which may lead to one form or another of cell death. They also intend to integrate this information in order to clarify the key effects of cisplatin compromising cell viability, and the intertwining and hierarchical organization of the responses; in order to put in perspective the main aspects that need further exploration for a better knowledge and improvement of its pharmaco-toxicological profile.

2. Transmembrane handling, intracellular trafficking and subcellular distribution

Classically, passive diffusion was considered the main mechanism of cellular uptake of cisplatin. This was based on the observation that, in general (i) accumulation is proportional to extracellular drug concentration, (ii) accumulation is not saturable, and (iii) structural analogs of cisplatin do not inhibit its accumulation (Gately and Howell, 1993). However, more recent observations challenge this concept. It was observed that, in different cell types, the whole uptake of cisplatin, or at least a part of it, seems to be dependent on energy consumption and could be modulated by pharmacological agents such as inhibitors of Na+/K+-ATPase (i.e. ouabain), inhibitors of membrane transporters and channels, such as amphotericin B, and digitonin (Hall et al., 2008; Kroning et al., 2000). It was also observed that a fraction of cisplatin uptake might be modulated by intracellular mediators such as H-ras, protein kinase C, protein kinase A or the calcium–calmodulin pathway (reviewed in Gately and Howell, 1993).

The present body of evidence (extensively reviewed in Hall et al., 2008) indicates that the specific intracellular accumulation of cisplatin in each cell type is the result of the combined action of specific mechanisms of internalization, sequestration and externalization, which differ substantially from one cell type to another. Passive diffusion may also contribute differently to the overall cellular handling of cisplatin depending on its extracellular concentration, with the higher contribution corresponding to the higher concentration. These differences underlie the intrinsic and acquired resistance or sensitivity of tumor cells to the drug. Along with tissue and physical barriers and intracellular red-ox status, they may also be behind the different toxic effects of cisplatin through the organism. Cisplatin's transportome is formed by membrane transporters and channels. It plays a very important role in tumor cell chemosensitivity and chemoresistance (Huang et al., 2004). A recent review by Burger et al. (2011) showed that there are a limited number of influx and efflux transporters implicated in the cellular accumulation of cisplatin. Yet, the mechanisms of cellular uptake and