Platinum-induced kidney damage: Unraveling the DNA damage response (DDR) of renal tubular epithelial and glomerular endothelial cells following platinum injury

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ABSTRACT

Background: Platinum compounds are potent anticancer drugs but also evoke considerable normal tissue damage. Here, we elucidate the molecular mechanisms contributing to the nephrotoxic effects of cisplatin.
Methods: We comparatively investigated the stress responses of rat kidney tubular (NRK-52E) and glomerular cells (RGE) following treatment with cisplatin (CisPt), oxaliplatin (OxaliPt) and carboplatin (CarboPt). To this end, cell viability, apoptosis, cell cycle progression, DNA damage response (DDR) and repair of DNA adducts were investigated.
Results: CisPt reduced the viability of tubular NRK-52E and glomerular RGE cells most efficiently. Cytotoxicity evoked by CarboPt occurred with a delay, which might be related to a retarded formation of Pt-(GpG) intrastrand crosslinks. RGE cells were more sensitive towards all platinum compounds than NRK-52E cells. Platinum drugs efficiently induced caspase-mediated apoptosis in tubular cells, while RGE cells favored G2/M arrest when treated with equitoxic platinum doses. Mitotic index of NKR-52E and RGE cells was worst affected by OxaliPt. Activation of the DDR was strikingly agent- and cell type-specific. Most comprehensive and substantial stimulation of DDR mechanisms was provoked by CisPt. Repair of Pt-(GpG) intrastrand crosslinks was best in RGE, which was reflected by high mRNA expression of nucleotide excision repair (NER) factors.
Conclusions: There are substantial differences regarding the cause of sensitivity and mechanisms of DDR between tubular and glomerular cells following platinum injury. CisPt is the most potent stimulator of the DDR. We hypothesize that specific DNA adducts and thereby forcefully activated pro-toxic DDR mechanisms contribute to the exceptionally high acute nephrotoxicity of CisPt.

Abbreviations: ApG, adenine-guanine; ATM, Ataxia telangiectasia mutated; ATR, ATM and Rad3 related kinase; Bax, Bcl2-associated X protein; Bcl2, B-cell CLL/lymphoma 2; EdU, 5-ethynyl-2′-deoxyuridine; CarboPt, carboplatin; CENP-F, centromere protein F; Chk1, checkpoint kinase 1; Chk2, checkpoint kinase 2; CisPt, cisplatin; Ctr1, solute carrier family 31 (copper transporter), member 1 (Slc31a1); Csb, excision repair cross-complementing rodent repair deficiency, complementation group 6 (Ercc6); DAPI, 4′,6-diamidino-2-phenylindol; DDR, DNA damage response; DSBs, DNA double-strand breaks; Ercc1, excision repair cross-complementing rodent repair deficiency; ERK2, extracellular regulated kinase 2; Gapdh, glyceraldehyde-3-phosphate dehydrogenase; GpG, guanine-guanine; γH2AX, histone H2AX phosphorylated on serine 139; p-H3, S10 phosphorylated histone H3; IC$_{50}$, inhibitory concentration required to inhibit viability of 50%; IC$_{80}$, inhibitory concentration required to inhibit viability of 80%; Kap-1, KRAB-associated protein-1; MMR, mismatch repair; Mrp2, ATP-binding cassette, subfamily C (CFTR/MRP), member 2 (Abcc2); MTT, 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide; NER, nucleotide excision repair; Nrf2, NF-E2-related factor 2; NRK-52E cells, rat renal proximal tubular epithelial cells; OxaliPt, oxaliplatin; Pt, platinum; RPA, replication protein A2; RGE cells, rat renal glomerular endothelial cells; TC-NER, transcription-coupled nucleotide excision repair; Xiap, X-linked inhibitor of apoptosis; Xpa, xeroderma pigmentosum, complementation group A; Xpg, excision repair cross-complementing rodent repair deficiency, complementation group 5 (Ercc5).

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1. Introduction

Platinum compounds are highly potent anticancer drugs and widely used in the therapy of malignant diseases. For instance, cisplatin (*cis*-diamminedichloroplatinum(II); CisPt), which is the first platinum compound that has been used for anticancer therapy [1], is preferential for the therapy of various types of cancer, including head and neck, lung, ovarian, cervical, bladder, and testicular cancer [2]. CisPt enters the cell by passive diffusion and by transporters [3–7]. Upon uptake into the cell, its chloride ligands are replaced by water leading to the formation of an electrophilic intermediate that can induce DNA adducts by S$_N$2-like mechanism [2]. Apart from DNA monoadducts and DNA interstrand crosslinks, DNA intrastrand crosslinks (GpG and ApG) are the most abundant adducts formed by CisPt [8,9]. In particular the major GpG intrastrand crosslinks cause a substantial distortion of the DNA double helix [10,11], which results in a block of transcription and replication [12,13], thereby inducing cell death [14]. Carboplatin (*cis*-diammine-1,1-cyclobutane-dicarboxylatoplatinum (II); CarboPt) and oxaliplatin (*trans*-L-1,2-

diaminocyclohexane-oxalatoplatinum(II); OxaliPt) are two other types of platinum compounds. Whereas CarboPt has the same spectrum of antitumor activity as CisPt, OxaliPt demonstrates antitumor efficacy against CisPt resistant tumors, notably colorectal cancer [2]. This might be due to a different mode of action of CisPt, OxaliPt and CarboPt [15,16]. It is also conceivable that (i) mechanisms of transport, (ii) the molecular structure of cytotoxic DNA adducts, (iii) pro-apoptotic DNA damage response (DDR), (iv) the repair of primary or secondary DNA lesions and/or (v) the activation of DNA damage independent cell death pathways or survival mechanisms are of relevance for the differences in response of tumor cells to platinating drugs. Correspondingly, numerous mechanisms have been suggested to be involved in the resistance of tumor cells to platinum compounds [17, 18]. A major mechanism involved in the removal of CisPt-induced intrastrand crosslinks is nucleotide excision repair (NER) [19,20], including transcription-coupled nucleotide excision repair (TC-NER) [21]. The relevance of DNA repair for platinum susceptibility is highlighted by the fact that the expression of ERCC1, which is involved in nucleotide excision repair (NER), predicts the therapeutic efficacy of CisPt in lung and testis tumors [22–27]. Apart from NER, mismatch repair (MMR) has been suggested to be important for the specific detection of CisPt DNA adducts but not of DNA adducts generated by OxaliPt [28].

The clinical use of platinum compounds is limited by numerous adverse effects on normal tissue. The clinically most relevant dose-limiting adverse effects of CisPt are nephrotoxicity [29], while hematotoxicity (i.e. myelosuppression) and neurotoxicity (peripheral neuropathy) are the dose limiting side effects of CarboPt and OxaliPt, respectively [15,30]. The molecular mechanisms underlying the tissue-specific adverse effects of the various platinating drugs are largely unclear. One possible explanation for the preferential cytotoxic effect of CisPt to the kidney are platinum drug-specific transporters. Although CisPt and OxaliPt share some major transporters, they also have compound-specific ones [4,31–33]. However, other mechanisms such as the formation of particular types of DNA lesions, activation of different branches of the DDR and/or pro-apoptotic mechanisms as well as varying capacities to repair CisPt-, OxaliPt- or CarboPt-induced DNA lesions might be of relevance as well. The severity of CisPt-induced peripheral neurotoxicity has been related to insufficient DNA repair [34]. CisPt-induced nephrotoxicity is believed to mainly result from damage to tubular cells [29,35], with the formation of ROS [36], ATR-Chk2 signaling [37] and mechanisms of transport [4,38] being suggested to play pivotal roles. Correspondingly, prevention of ROS formation [39,40] and stimulation of anitoxidative mechanisms by targeting of Nrf2 signaling [41] or inhibition of influx transporters [42] have been considered as nephroprotective strategies. Noteworthy, glomeruli are further particularly sensitive structures of the kidney, which are especially relevant for nephrotoxicity induced by aminoglycosides or cyclosporin [43,44] and, furthermore, play a key role in autoimmune glomerulonephritis [45,46]. Thus, it was speculated that damage to glomeruli provoked by CisPt might also contribute to the renal toxicity of this anticancer drug [47].

In the present study we addressed the question whether various types of renal cells show qualitative and quantitative differences regarding their responsiveness to platinum injury. To this end, we comparatively analyzed the response of normal rat renal tubular epithelial (NRK-52E) and glomerular endothelial (RGE) cells to treatment with equitoxic concentrations of CisPt, OxaliPt and CarboPt, monitoring the activation of cell cycle checkpoints, induction of apoptotic mechanisms, stimulation of mechanisms of the DDR as well as the formation and repair of DNA intrastrand crosslinks and DNA double-strand breaks (DSBs). The results of our extensive studies reveal strikingly complex differences in the stress responses stimulated by the different platinum compounds in tubular epithelial and glomerular endothelial cells.

2. Materials and methods

2.1. Materials

Rat renal proximal tubular epithelial (NRK-52E) and rat glomerular endothelial (RGE) cells originate from the German Collection of Microorgansisms and Cell Culture (DSMZ) (Braunschweig, Germany). Cisplatin, oxaliplatin and carboplatin were obtained from the pharmaceutical department of the University Hospital Düsseldorf and originate from TEVA (Ulm, Germany). The following antibodies were used: antibody detecting Ser139 phosphorylated histone H2AX (γH2AX) (Millipore (Billerica, USA)), ERK2, PARP-1, CENP-F and β-actin antibodies (Santa Cruz Biotechnology (Santa Cruz, USA)), p-p53, p-Chk1, caspase 3 (additionally detects cleaved caspase 3), caspase 7 and cleaved caspase 7 (Cell Signaling (Denvers, Massachusetts, USA)), p-Chk2 and Ki-67 (Abcam (Cambridge, England)), p-RPA32 and p-KAP-1 (Bethyl Laboratories (Montgomery, USA)), GAPDH and p-c-Jun (Epitomics (Burlingame, USA)). The antibody against Pt-(GpG) intrastrand crosslinks was kindly provided by J. Thomale (Essen, Germany). The antibody detecting S10-phosphorylated histone 3 (p-H3) is part of the "HCS Mitotic Index Kit" (Life Technologies, Carlsbad, CA, USA). The fluorescent antibody Alexa Fluor 488 was obtained from Life Technologies (Carlsbad, CA, USA), and the horseradish peroxidase-conjugated antibodies anti-rat IgG, anti-mouse IgG, anti-rat IgG and anti-rabbit IgG were purchased from Rockland (Gilbertsville, PA, USA).

2.2. Cell culture

NRK-52E cells were grown in DMEM (Sigma, Steinheim, Germany), RGE cells in RPMI (Sigma, Steinheim, Germany) containing 10% of fetal calf serum (FCS) (PAA Labratories, Cölbe, Germany; Biochrom, Berlin, Germany) and 1% penicillin/streptomycin (Sigma, Steinheim, Germany) at 37 °C in an atmosphere containing 5% CO_2. If not stated otherwise, treatment of logarithmically growing cells was performed 24 h after seeding. Ionizing radiation was performed with a caesium-137 radiation source.

2.3. Determination of cell viability

Cell viability was determined using the MTT assay [48]. Briefly, the yellow tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (Sigma, Steinheim, Germany) is metabolized to a purple formazan dye by mitochondrial dehydrogenases, which are only active in living cells. Untreated control and platinum-treated cells were incubated with the tetrazolium salt (5 mg/ml in PBS) for 30–40 min at 37 °C. Afterwards the dye was solubilized with DMSO (MERCK, Darmstadt, Germany) and absorption was measured at 560 nm. Relative viability in the untreated controls was set to 100%. In addition, cell viability was also determined by use of the Alamar Blue assay [49]. Here, viable cells metabolize the non-fluorescent dye resazurin (Sigma, Steinheim, Germany) to fluorescent resorufin. The cells were incubated for up to 2.5 h with the resazurin solution (final concentration 40 μM) before fluorescence was measured (excitation: 535 nm, emission: 590 nm, 5 flashes, integration time: 20 μs). Relative viability in the untreated controls was set to 100%. Additionally, alteration in cell viability was recorded in real-time by measuring the electrical impedance via the iCElligence system (ACEA Bioscience, San Diego, USA). 200 μl medium was added to the E-Plate L8 for background measurements. Subsequently, cells were seeded in additional 250 μl medium. Appropriate cell densities were determined by preliminary experiments. Cell attachment was monitored by measuring the impedance for 4 h (240 sweeps; time interval: 1 min). Afterwards impedance was recorded over a time period of 72 h (192 sweeps; time interval 30 min). 24 h after seeding, cells were treated with the platinating drugs by adding the corresponding concentrations in 50 μl medium. Changes in the electrical impedance were expressed as cell index