TRAIL-induced cell death and caspase-8 activation are inhibited by cisplatin but not carboplatin

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Objective: Platinum (Pt) based drugs including cisplatin and carboplatin are widely used as anticancer drugs in various human cancers. Many studies have shown that chemotherapeutic agents synergistically enhance cell death induced by death ligands. However it has been recently reported that cisplatin may inhibit tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced cell death through inactivation of caspases. Thus, we investigated whether carboplatin also inhibits TRAIL-induced cell death.

Methods: HeLa cells were treated with TRAIL in the presence of cisplatin or carboplatin, and cell death was analyzed using the crystal violet staining method. Caspase activation was checked through detection of Bid cleavage by Western blotting using anti-Bid antibody.

Results: Cisplatin inhibits TRAIL-induced cell death in HeLa cells; however, carboplatin enhanced TRAIL-induced cell death. Whereas cisplatin inhibited caspase-8-mediated Bid cleavage, carboplatin had no effect on caspase-8 activity.

Conclusion: Although cisplatin and carboplatin are platinum-containing cancer therapeutic agents, they have the opposite effects on TRAIL-induced cell death.

Key Words: Carboplatin, Caspase-8, Cisplatin, TRAIL
In this study, we confirmed that cisplatin inhibited TRAIL-induced cell death, but carboplatin showed no inhibitory effect on TRAIL-induced cell death.

MATERIALS AND METHODS

1. Reagents and antibody
Cisplatin (Choongwae Pharma Corporation, Seoul, Korea) and Carboplatin (Korea United Pharm, Inc, Seoul, Korea) were purchased. Recombinant active caspase-8 and pan-caspase inhibitor z-VAD-fmk were purchased from Calbiochem. Polyclonal anti-Bid antiserum was generated in rabbit using recombinant Bid protein as antigen.

2. Cell culture and cell viability measurement
HeLa cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. The cells were plated on 48-well plates for the cell death assay. Cell viability was determined by crystal violet staining as described.10 In brief, cells were stained with 0.4% crystal violet in methanol for 10 minutes at room temperature and then washed with tap water. Stained cells were extracted with 4% methanol, and dye extracts were measured at 540 nm wavelength using a microplate reader.

3. Analysis of Bid cleavage
Recombinant active caspase-8 (100 ng) was pretreated with cisplatin (50 μg), z-VAD-FMK (0.1 mM), or carboplatin (50 μg), and incubated with recombinant Bid (100 ng) protein in caspase assay buffer (10 mM PIPES, 100 mM NaCl, 0.1 mM EDTA, 10 mM DTT, 10% sucrose, 0.1% CHAPS, pH 7.4) for 1 hour at 37°C. Cleavage of Bid was analyzed by Western blot with anti-Bid antibody.

4. Western blotting
Caspase-8 reaction mixture was separated by 15% SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membrane. The membranes were blotted with the anti-Bid antibody.

RESULTS

1. Cisplatin inhibits TRAIL-induced cell death in HeLa cells, but not carboplatin
A number studies have demonstrated that chemotherapeutic agents including cisplatin synergistically enhanced cell death by death ligands such as TRAIL, FasL, or TNF-alpha. However in a previous study, we showed that cisplatin inhibited death ligand-induced cell death.9 Thus, we first investigated whether or not carboplatin, a second generation analogue of cisplatin, also inhibits TRAIL-induced cell death. HeLa cells were treated with TRAIL for 24 hours in the presence of cisplatin or carboplatin, and the cell viability was analyzed by crystal violet staining. Cisplatin inhibited cell death by TRAIL, while carboplatin did not inhibit TRAIL-induced cell death in HeLa cells. Moreover, carboplatin treated HeLa cells were more sensitive to TRAIL than ordinary HeLa cells treated with TRAIL alone (Fig. 1A). The crystal violet-stained cells were extracted with 4% methanol, and dye extracts were measured at 540 nm wavelength using a microplate reader. Similar to the results in Fig. 1A, cisplatin had an inhibitory effect in a dose dependent manner, while carboplatin enhanced cell death in a dose dependent manner (Fig. 1B). These results suggest that carboplatin has no inhibitory effect on TRAIL-induced cell death inconsistent with cisplatin; however, carboplatin does sensitize the cells to TRAIL-induced cell death.

2. Cisplatin but not carboplatin inhibits TRAIL-induced cell death via inactivation of caspase-8
TRAIL induces the trimerization of the death receptors DR4/DR5 upon ligand binding. It subsequently recruits adapter proteins such as FADD and caspase-8 leading to the formation of DISC. The activated caspase-8 cleaves Bid to generate the truncated Bid (tBid) that translocates to mitochondria.
TRAIL-induced cell death and caspase-8 activation are inhibited by cisplatin but not carboplatin.

**Fig. 2.** Cisplatin directly inhibits activity of caspase-8, but not carboplatin. (A) Pretreated recombinant human caspase-8 (100 ng) with cisplatin (250 μg) was incubated with recombinant Bid (100 ng) in 50 μl of caspase assay buffer for 1 hour at 37°C. (B) Pretreated recombinant human caspase-8 (100 ng) with carboplatin (250 μg) in the presence or absence of pan-caspase inhibitor zVAD-fmk was incubated with recombinant Bid (100 ng) in 50 μl of caspase assay buffer for 1 hour at 37°C. Cleavage of Bid was analyzed by Western blot with anti-Bid antibody.

**Fig. 3.** Structure of cisplatin and carboplatin. Cisplatin contains a platinum atom complexed with two ammonia groups and two chloride residue, and carboplatin contains a platinum atom complexed with two ammonia groups and 1,1-cyclobutane-dicarboxyl residue.

...dria and causes mitochondrial dysfunction. Thus caspase-8-dependent Bid cleavage is a key mechanism in TRAIL-induced cell death. Our previous study showed that cisplatin inactivates caspases. To test whether carboplatin inhibits activation of caspase-8 or not, cleavage of recombinant Bid protein by caspase-8 was monitored by Western blotting. Consistent with previous results, cisplatin inactivated caspase-8 (Fig. 2A); however, carboplatin has no inhibitory effect on caspase-8 activation (Fig. 2B). These results suggest that carboplatin has no effect on caspase-8 activity unlike cisplatin.

**DISCUSSION**

Cisplatin and carboplatin are widely used to treat various human cancers. These platinum-based chemotherapeutic drugs cause DNA adducts leading to cell death. They also bind to many extracellular and intracellular proteins, although they have different binding abilities. The formation of protein adducts with platinum-based drugs may be a key event for the tumor killing activities as well as side effects. The chemical structure of cisplatin is quite different from that of carboplatin, although both drugs contain platinum. Cisplatin contains a platinum atom complexed with two ammonia groups and two chloride residues, and the chloride residue in cisplatin interacts with the sulfur group of the cysteine residue forming a cisplatin-protein complex. Cisplatin interacts with caspases by direct binding of the chloride residue in cisplatin with the sulfur group of the cysteine residue in caspases and lead to the inactivation of the caspasas due to formation of a complex of cisplatin and caspase. However, the two chlorides in cisplatin have been substituted with 1,1-cyclobutane-dicarboxyl residue in carboplatin (Fig. 3), possibly removing the functional moiety for binding to caspases. Based on the fact that carboplatin has no inhibitory effect on TRAIL-induced cell death, we speculate that carboplatin does not directly interact with caspase-8. These results provide evidence that carboplatin functions differently from cisplatin.

**REFERENCES**


