Original Article

Modulation of copper transporters in protection against cisplatin-induced cochlear hair cell damage

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Abstract Cisplatin belongs to platinum-based drugs and is widely used in cancer chemotherapy. Ototoxicity is one of the major dose limiting side-effects of cisplatin. For toxicity to occur cisplatin must first be transported from the bloodstream into cochlear cells. Three copper transporters are considered pathways for regulating the uptake and translocation of cisplatin into cells: Ctr1, ATP7A and ATP7B. Our recent study with cochlear organotypic cultures shows that cochlear hair cells can be destroyed by cisplatin at low concentrations from 10μm to 100μn. However, high doses of cisplatin cannot damage hair cells, maybe due to intrinsic feedback reactions that increase export of platinum by ATP7B when the platinum concentration is high in extracellular space. Cimitidine is a specific copper transporter inhibitor that can block the entrance of copper and platinum, and may prevent cisplatin-induced cochlear hair cell injury. To evaluate this hypothesis, we treated cochlear organotypic cultures with cisplatin (10 μm or 50 μm) alone, or cisplatin combined with cimitidine at concentrations ranging from 10-2000 μm for 48 hours. cisplatin at 10 μm damaged about 20% hair cells. In contrast, when cimitidine (10 μm, 100 μm and 2000 μm) was added to the culture, near 100% cochlear hair cell survived. At higher concentration (50 μm), cisplatin destroyed about 80% of cochlear hair cells. However, 100 μmcimitidine rescued about 50% hair cells from cisplatin damage, and 2000μm cimitidine protected about 80% hair cells. The data of western blot showed that CTR1 and ATP7B expressions were increased in cisplatin treated cochlear tissue, but cimitidine significantly reduced CTR1 and ATP7B. In addition, ATP7A expression was depressed a little after cisplatin treatment. Considering that Ctr1 is involved in copper and platinum influx, but the ATP7A and ATP7B are copper export transporters, the results suggest that cimitidine can effectively block the entrance by copper transporters and stop the influx of cisplatin.

Keywords cisplatin, copper transporter, ototoxicity, cochlear hair cell

Introduction

Cisplatin is one of the most widely used chemotherapeutic agents for the treatment of various malignant tumors. The antitumor effects of cisplatin largely arise from intrastrand and interstrand cross linking of DNA leading to G2 cell cycle arrest thereby blocking tumor proliferation .[1,2] Cisplatin also induces a complete blockage at the S phase of the cell cycle inhibiting total mRNA transcription .[3]

In addition to its potent anti-tumor actions, cisplatin is highly toxic to the kidney, liver, nervous system, bone marrow, inner ear, etc. [4-10] The ototoxic effects of cisplatin produce significant hearing loss and permanent destruction of cochlear hair cells and spiral ganglion

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neurons (SGNs). [11-14] Cisplatin-induced damage to cochlear hair cells (HCs) is initiated in outer hair cells (OHC) followed by inner hair cells (IHC). The HC damage begins at the basal turn of the cochlea and gradually spreads to the apex in vivo. [12, 14, 15] However, cisplatin destroys both OHCs and IHCs evenly through the entire length of the cochlea in vitro. [13] Besides the damage to HCs, cisplatin also damages SGNs in the modiolus, and the marginal epithelium on the stria vascularis .[12] Therefore, HCs, SGNs and stria vascularis are three major targets of cisplatin toxicity in the cochlea. [12, 14]

The ototoxic mechanisms of cisplatin involve multiple factors, such as generation of highly toxic free radicals and reactive oxygen species (ROS) with reduction of antioxidant enzymes, [12, 16] destruction of DNA ,[17]damage of mitochondria, [8] activation of caspase cascade, [11, 12, 14, 18] and activation of p53. [18, 19] Although a complicated network of apoptotic signals is involved in cisplatin ototoxicity, the major apoptotic pathway induced by cisplatin in the cochlea is initiated from cell death receptors on the cell membrane, and then converged to p53 signaling pathway to complete the programmed self-destruction finally .[11, 12, 14, 18, 19]

For toxicity to occur cisplatin must first be transported from the bloodstream into cells. Cisplatin can be activated once it enters the cytoplasm when the chloride atoms on cisplatin are displaced by water molecules. The aquatic cisplatin becomes potent electrophile and then react with nucleic acid to target DNA. In addition, previous studies have demonstrated that when cisplatin is transported into the cell, it can bind with glutathione, and then becomes a cisplatin-glutathione complex to exert its toxic effects [20]. Therefore, the entry of cisplatin is the first important step responsible for its intracellular toxic effects.

The pathways that regulate the uptake and translocation of cisplatin into HCs, SGNs, and cochlear supporting cells are poorly understood. However, research evidence suggests that cisplatin uptake is mediated by copper transporters that mediate copper and platinum homeostasis in the cell. [12, 14, 21-23] Cells tightly regulate copper homeostasis through three copper transporters, Ctr1, ATP7A and ATP7B. Ctr1 imports copper and platinum-basedcompoundssuchascisplatin, carboplatin, and oxaliplatin into the cell. [21] ATP7A and ATP7B are copper exporters which eject the copper and platinum-based compounds from cytoplasm. [22, 23, 24] Cells over expressing ATP7B show increased efflux of cisplatin and carboplatin from the cytoplasm and reduced accumulation of these compounds .[24, 25, 26] These results suggest that ATP7B is important in drug efflux and cisplatin resistance. In contrast, cells that over express ATP7A accumulate large amounts of cisplatin in vesicles. These cells are also resistant to toxicity presumably by preventing escape of platinum to potentially lethal targets such DNA.[27]Therefore, Ctr1, ATP7A, and ATP7B regulate the uptake and extrusion of copper and platinum-based compounds.

The efficiency of copper and/or platinum input or output depends on the requirement of the cell and the level of intracellular copper/platinum. Cells may detect intracellular and extracellular copper/platinum levels for the appropriate regulation. If the level of copper in the cells is high, cell can enhance its export; otherwise it may gain its import. For example, high extracellular copper leads to the endocytosis of Ctr1 from the membrane and subsequent protein degradation. This reduces the uptake of copper/platinum from the extracellular environment preventing excess copper uptake into the cytoplasm. [24-28] These important findings suggest that high level of extracellular copper/platinum is detectable by the cell, which causes Ctr1 to rapidly withdraw from the membrane into the cytoplasm where Ctr1 is quickly degraded; and that this negative feedback mechanism reduces the uptake of copper/platinum and reduces its toxicity.

In order to test if HCs can also regulate input and output of cisplatin to achieve a self-defense, post-natal cochlear organotypic cultures were treated with various doses of cisplatin for 24 or 48 hours. The missing of HCs and the changes in expression of copper transporters by cisplatin treatment was evaluated in present study. In addition, the protective effects of copper transporter inhibitor, cimetidine was also evaluated to illuminate the possibility of regulating copper transporters.

Materials and Methods

Cochlear organotypic cultures and cisplatin treatment

Rat pups (Sprague Dawley) at postnatal day 3 were

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