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Immunohistochemical detection of platinated DNA in the cochlea of cisplatin-treated guinea pigs ☆

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Abstract

Cisplatin-induced ototoxicity is correlated with functional and morphological changes in the organ of Corti, the stria vascularis and the spiral ganglion. However, the cochlear sites of cisplatin uptake and accumulation have not been properly identified. Therefore, we have developed an immunohistochemical method to, indirectly, detect cisplatin in semithin cryosections of the guinea pig cochlea (basal turn) using an antiserum containing antibodies against cisplatin-DNA adducts. Platinated DNA was present in the nuclei of most cells in the organ of Corti and the lateral wall after cisplatin administration. Nuclear immunostaining was most pronounced in the outer hair cells, the marginal cells and the spiral ligament fibrocytes. This study is the first to demonstrate the presence of cisplatin in histological sections of the cochlea.

Keywords: Cisplatin ototoxicity; Cisplatin-DNA adducts; Cochlea; Guinea pig; Organ of Corti; Stria vascularis

1. Introduction

Cisplatin-induced ototoxicity is characterized by an initial high-frequency hearing deficit, which is associated with a loss of the hair cells in the organ of Corti (for reviews, see De Oliveira, 1989; Schweitzer, 1993). In addition, functional and morphological changes have been observed in the stria vascularis, whereas there is also evidence that the spiral ganglion is affected after cisplatin administration (Schweitzer, 1993; Cardinaal et al., 2000; Hamers et al., 2003; Van Ruijven et al., 2004). However, despite several attempts, the cochlear sites of cisplatin uptake and accumulation have not been properly identified.

Numerous methods are available to demonstrate cisplatin in biological fluids or homogenized tissue samples (cf., Johnsson et al., 1995; Verschraagen et al., 2002). Using a radiographic method, Schweitzer (1993) detected [^{195m}Pt]-labelled cisplatin in homogenated samples of the organ of Corti and the stria vascularis. However, these techniques cannot be used to detect cisplatin in histological sections. Since the cisplatin molecule contains a central platinum (Pt) atom (Fig. 1), X-ray microanalytic techniques are the obvious choice for demonstrating the presence of cisplatin in ultrathin sections of tissues. Using X-ray microanalysis, Pt has been detected in cisplatin-treated kidney tissue (Berry et al., 1982; Makita et al., 1986; Saito and Aran, 1994a) and tumor cell lines (Khan and Sadler, 1978).

Abbreviations: ABC, avidin–biotin–peroxidase complex; DAB, 3,3′-diaminobenzidine tetrahydrochloride; NGS, normal goat serum; PBS, phosphate-buffered saline; PEG 6000, polyethyleneglycol (MW 6000); Pt, platinum; PVP, poly(vinylpyrrolidone)

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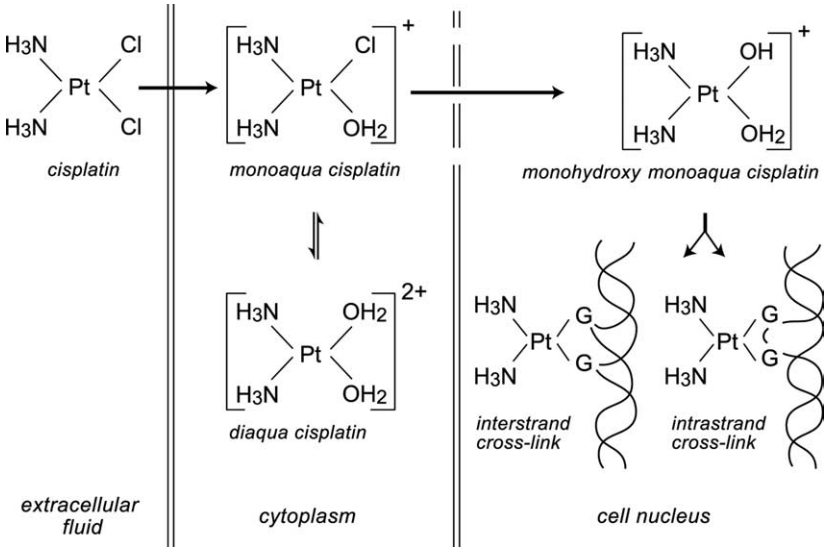

Fig. 1. The cisplatin molecule crosses the plasma membrane and is transformed within the cytoplasm into several charged species. Especially the monoaqua and monohydroxy monoaqua species are reactive and interact with nuclear DNA, resulting in the formation of interstrand and intrastrand cross-links (Adapted from Kartalou and Essigmann, 2001).

In contrast, studies investigating the cochlear distribution of cisplatin using ultrastructural X-ray microanalysis have failed to detect Pt in the organ of Corti (Maruyama et al., 1993; Saito and Aran, 1994a; Welb, 1995), whereas data on the stria vascularis are conflicting. Although Maruyama et al. (1993) detected Pt in ultrathin sections of the stria vascularis after cisplatin administration by means of X-ray microanalysis, Welb (1995) could not corroborate this finding with sensitive techniques such as electron energy-loss spectrometry and laser microprobe mass spectrometry. The failure to detect cisplatin in ultrathin sections of the cochlea by means of X-ray microanalysis in the former studies may be explained by the fact that the intracellular concentration of Pt was below the detection limit (Saito and Aran, 1994a). On the other hand, cisplatin could be extracted from the cochlear tissues during chemical fixation and subsequent histological processing (Saito and Aran, 1994a).

Alternatively, (intra)cellular distribution of cisplatin can be assessed immunohistochemically. However, because of the aforementioned considerations as well as the fact that the production of antibodies against small molecules, such as cisplatin, is rather difficult and time-consuming, we decided to take another, indirect approach. It has been demonstrated that, in tumor cells, the uncharged cisplatin molecule is transformed into several charged species that interact with nuclear DNA to form interstrand and intrastrand cross-links (Fig. 1; Reed et al., 1996; Fuertes et al., 2003). Antibodies against these cisplatin-DNA adducts react with cell nuclei in histological sections of tumor tissue (Bergström et al., 1997; Meijer et al., 2001). In addition, nuclear immunoreactivity for these adducts has been observed in, for instance, sections of cisplatin-treated kidney tissue (Terheggen et al., 1987; Bergström et al., 1997; Meijer et al., 1999) and dorsal root ganglia (Terheggen et al., 1989; Meijer et al., 1999). These findings imply that cisplatin-DNA adducts are not only formed in tumor cells, but also in normal tissue. We have surmised that a similar molecular mechanism underlies the drug's ototoxic effect and that platination of DNA also may take place in cochlear tissues after cisplatin administration. Therefore, we have developed an immunohistochemical method to, indirectly, detect cisplatin in semithin cryosections of the guinea pig cochlea using an antiserum containing antibodies against cisplatin-DNA adducts.

2. Materials and methods

2.1. Animals

Albino, female Dunkin–Hartley guinea pigs (body weight 250–350 g) were purchased from Harlan Laboratories (Horst, The Netherlands) and housed in the Animal Care Facility of the Central Laboratory Animal Institute of Utrecht University. Animals had free access to both food and water and were kept under standard laboratory conditions. The experimental procedures were approved by the University's Committee on Animal Research (DEC-UMC #89055). Animal care was under the supervision of the Central Laboratory Animal Institute of Utrecht University.

2.2. Drug administration

Platinol® injection fluid, containing 1 mg cisplatin per ml, was obtained from Bristol–Myers Squibb (Woerden, The Netherlands). This stock solution was diluted in