Available online at www.sciencedirect.com
ScienceDirect
Toxicology and Applied Pharmacology 215 (2006) 228–236
Toxicology and Applied Pharmacology
www.elsevier.com/locate/ytaap

D-Methionine attenuated cisplatin-induced vestibulotoxicity through altering ATPase activities and oxidative stress in guinea pigs

Po-Wen Cheng [a,c], Shing-Hwa Liu [a], Yi-Ho Young [d], Shoei-Yn Lin-Shiau [b,*]

[a] Institute of Toxicology, College of Medicine, National Taiwan University, Taipei, Taiwan
[b] Institute of Pharmacology, College of Medicine, National Taiwan University, No. 1, Section 1, Jen-Ai Road, Taipei, Taiwan
[c] Department of Otolaryngology, Far Eastern Memorial Hospital, Taipei, Taiwan
[d] Department of Otolaryngology, College of Medicine, National Taiwan University, Taipei, Taiwan

Received 29 November 2005; revised 27 February 2006; accepted 28 February 2006
Available online 19 April 2006

Abstract

Cisplatin has been used as a chemotherapeutic agent to treat many kinds of malignancies. Its damage to the vestibulo-ocular reflex (VOR) system has been reported. However, the underlying biochemical change in the inner ear or central vestibular nervous system is not fully understood. In this study, we attempted to examine whether cisplatin-induced vestibulotoxicity and D-methionine protection were correlated with the changes of ATPase activities and oxidative stress of ampullary tissue of vestibules as well as cerebellar cortex (the inhibitory center of VOR system) of guinea pigs. By means of a caloric test coupled with electronystagmographic recordings, we found that cisplatin exposure caused a dose-dependent (1, 3, or 5 mg/kg) vestibular dysfunction as revealed by a decrease of slow phase velocity (SPV). In addition, cisplatin significantly inhibited the Na^+, K^+-ATPase and Ca^{2+}-ATPase activities in the ampullary tissue with a good dose–response relationship but not those of cerebellar cortex. Regression analysis indicated that a decrease of SPV was well correlated with the reduction of Na^+, K^+-ATPase and Ca^{2+}-ATPase activities of the ampullary tissue. D-Methionine (300 mg/kg) reduced both abnormalities of SPV and ATPase activities in a correlated manner. Moreover, cisplatin exposure led to a significant dose-dependent increase of lipid peroxidation and nitric oxide concentrations of the vestibules, which could be significantly suppressed by D-methionine. However, cisplatin did not alter the levels of lipid peroxidation and nitric oxide of the cerebellum. In conclusion, cisplatin inhibited ATPase activities and increased oxidative stress in guinea pig vestibular labyrinths. D-Methionine attenuated cisplatin-induced vestibulotoxicity associated with ionic disturbance through its antioxidative property.

Keywords: Cisplatin; Vestibulotoxicity; Vestibulo-ocular reflex; Caloric test; Na^+, K^+-ATPase; Ca^{2+}-ATPase; Lipid peroxidation; Nitric oxide; D-Methionine

Introduction

Cisplatin (*cis*-diamminedichloroplatinum II) is a widely used antineoplastic agent, effective against a variety of tumors. Its site of action is believed to be DNA to which it binds irreversibly, forming intra-strand crosslinks between adjacent guanine residues (Eastman, 1986). It is thought that its chemotherapeutic effects are mediated through this action. The major toxic effects of cisplatin include ototoxicity, nephrotoxicity, neurotoxicity, myelosuppression, and gastrointestinal toxicity (Ozols and Young, 1985; Stewart et al., 1987; Stoter et al., 1989). Although nephrotoxicity can be the dose-limiting side effect of cisplatin cancer therapy, currently the most frequent dose-limiting factor is ototoxicity (Blumenreich et al., 1985; Forastiere et al., 1987; Berry et al., 1990). Most studies of cisplatin ototoxicity are limited to the cochlea. Reports on cisplatin vestibulotoxicity are relatively rare. Furthermore, some controversial studies in humans exist. Cancer patients might have a risk of vestibulotoxicity after treatment by cisplatin, which could be documented by electronystagmography, rotational chair testing, vestibular autorotation test or caloric testing (Schaefer et al., 1981; Black et al., 1982; Hartwig et al., 1983; Kobayashi et al., 1987; Kitsigianis et al., 1988a, 1988b). On the contrary, Myers et al. (1993) concluded that no clear evidence of vestibular toxicity could be found in patients receiving cisplatin chemotherapy. The variability of vestibulotoxic effects might be due to the

* Corresponding author. Fax: +886 2 2391 5297.
E-mail address: syl@ha.mc.ntu.edu.tw (S.-Y. Lin-Shiau).

0041-008X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.taap.2006.02.014

neurootological methods used for monitoring the deficit of vestibular function, and inter-individual susceptibility as documented in cisplatin-treated humans. In animal studies, cisplatin did cause the impairment of vestibular function (Sergi et al., 2003, 2004). Although past histopathological studies demonstrated a preservation of the vestibular neuroepithelium after high doses of cisplatin (Wright and Schaefer, 1982; Schweitzer et al., 1986), more recent studies have shown that vestibular hair cells were damaged in guinea pigs even after lower doses of cisplatin (Nakayama, 1992, 1996; Sergi et al., 2003). Based on these findings, we consider that the degree of vestibular functional impairment induced by cisplatin may be correlated with the extent of morphological damage, and that cisplatin-induced biochemical changes of the vestibules such as enzyme activities and oxidative stress need further studies. Although the neuroprotective agents (such as D-methionine, sodium thiosulfate, *N*-acetylcysteine) against cisplatin-induced cochleotoxicity have been reported (Campbell et al., 1996; Muldoon et al., 2000; Thomas Dickey et al., 2004), none of them were studied on their protection against cisplatin-induced vestibulotoxicity.

The vestibulo-ocular reflex (VOR) system is an important modulator to stable vision during natural activity. It is a simple reflex arc, transmitting the velocity command from the vestibule to the ocular motor neurons to ensure that velocity of eye movement matches head velocity (Smith and Crawford, 1998). The function of VOR system and cerebellar circuitry can be examined by the caloric test coupled with electronystagmographic (ENG) recordings in guinea pigs (Young et al., 1991). The slow phase velocity (SPV) instead of the duration of caloric nystagmus is monitored for the assessment of the altered sensitivity of the sensory epithelium on the vestibular apparatus (Henriksson, 1956; Kavanagh and Babin, 1986). Through the pattern of caloric nystagmus, lesions in the vestibular labyrinth, vestibular nuclei and/or their connection to the cerebellum can be assessed (Vibert et al., 1993). Lesions of the vestibular labyrinth or nuclei typically decrease the gain (maximum slow phase velocity/maximum head velocity) of VOR. In contrast, lesions of the cerebellum might increase the VOR gain, mediated by a loss of inhibitory effect of the archi-cerebellum on the vestibular nuclei (Baloh et al., 1979; Sharpe et al., 1981; Ito, 1993).

Reports concerning the mechanism of cisplatin vestibulotoxicity were relatively rare. There are in vitro and in vivo reports supporting that cisplatin could inhibit Na^+, K^+-ATPase and/or Ca^{2+}-ATPase activities of renal tissues (Nechay and Neldon, 1984; Tay et al., 1988; Brady et al., 1990) and those of cochleae (Cheng et al., 2005); therefore, it is suspected that both ATPase activities might have a role in the pathogenesis of cisplatin-induced vestibular dysfunction. In addition, nitric oxide synthase and apoptosis were induced in the vestibule of guinea pigs after the application of cisplatin (Watanabe et al., 2000, 2001a, 2001b). The tumor suppressor protein, p53, was suggested to play a critical role in initiating apoptosis in utricular organotypic cultures of rats treated with cisplatin (Zhang et al., 2003). Furthermore, antioxidants have been proposed to be useful in preventing cisplatin vestibulotoxicity (Sergi et al., 2004). D-Methionine, an antioxidant amino acid and a sulfur-containing chemoprotectant, has been proved to provide excellent protection from cisplatin-induced hearing loss by decreasing both of loss of cochlear hair cells (Campbell et al., 1996; Korver et al., 2002) and damage to the stria vascularis (Campbell et al., 1999; Cheng et al., 2005) in animal studies. Reser et al. (1999) have shown that L- and D-methionine exhibit equivalent protection against cisplatin-induced elevation of hearing thresholds and loss of outer hair cells in rats. At least some of the protection occurs because methionine reduces the cochlear oxidative stress after cisplatin administration (Campbell et al., 2003).

Reactive oxygen species (ROS) act as possible mediators of cellular injury through nonspecific modification and disruption of proteins, phospholipids and nucleic acids. Critical sites of ROS attack are the cell membrane and membranes of intracellular organelles. The disruptive action of ROS may cause alterations of the membrane structure and function, including fluidity, permeability, activity of enzymes, channels, and receptors. It has been shown that ROS could inhibit Na^+, K^+-ATPase and Ca^{2+}-ATPase of in vitro membrane ion transport systems, and that antioxidants might prevent the decrease in both enzyme activities (Soszynski et al., 1996; Lehotsky et al., 1999). Moreover, Na^+, K^+-ATPase and Ca^{2+}-ATPase play an important role in maintaining cellular ionic homeostasis and physiologic function of the inner ear as well as the nervous system. Based on these facts, we hypothesized that the dysfunction of VOR system might be due to the changes of ATPase-specific activities of the vestibule, the brainstem or the cerebellum of guinea pigs after receiving cisplatin for 7 consecutive days, and that the change of oxidative stress detected by the increased levels of lipid peroxidation and nitric oxide may play, at least in part, a role in the altered enzyme activities. Our previous study has disclosed that cisplatin caused no reduction of ATPase activities in the brainstem of guinea pigs (Cheng et al., 2005). Therefore, in the present study, we focused our effort on the measurement of ATPase activities of the vestibule and the cerebellum. Additionally, the VOR function was examined to see whether it was correlated with the quantitative changes of Na^+, K^+-ATPase and Ca^{2+}-ATPase activities of the cisplatin-damaged vestibules. These experimental data are required to clarify the possible role of Na^+, K^+-ATPase and Ca^{2+}-ATPase in the pathogenesis of vestibular damage after cisplatin treatment. The further experiment that D-methionine inhibited cisplatin-induced vestibulotoxicity probably through preventing the decrease of ATPase activities and attenuating oxidative stress would support our working hypothesis.

Materials and methods

Animal preparations. Hartley strain albino guinea pigs from an in-house breeding colony were housed at ambient temperature of 23 ± 2 °C, humidity 50–60% and given a solid guinea pig diet and tap water ad libitum. The study was conducted in accordance with the Guiding Principles in the use of Animals in Toxicology. Guinea pigs with strong positive Preyer reflexes (a characteristic ear twitch responding to an auditory stimulus to verify the normal hearing), normal activity and appearance were enrolled into this study. The VOR of experimental animals on the roll and yaw planes (Dieterich and Brandt, 1995) were visually confirmed with passive head rotation in the dim light. Complete data set was