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Cisplatin-induced toxicity decreases the mouse vestibulo-ocular reflex

Yasumitsu Takimoto[a,b,c,*], Takao Imai[a], Makoto Kondo[b], Yukiko Hanada[a,b], Atsuhiko Uno[a], Yusuke Ishida[b], Takefumi Kamakura[a], Tadashi Kitahara[d], Hidenori Inohara[a], Shoichi Shimada[b]

[a] Department of Otorhinolaryngology—Head and Neck Surgery, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan
[b] Department of Neuroscience and Cell Biology, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan
[c] Department of Otorhinolaryngology, Suita Municipal Hospital, 2-13-20 Katayama-cho, Suita City, Osaka 564-0082, Japan
[d] Department of Otorhinolaryngology—Head and Neck Surgery, Nara Medical University School of Medicine, 480 Shijocho, Kashihara, Nara 634-8522, Japan

HIGHLIGHTS

- Cisplatin decreased spontaneous locomotor activity.
- Cisplatin decreased vestibulo-ocular reflex for high frequency sinusoidal rotations .
- Cisplatin did not decrease optokinetic response.

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ABSTRACT

Cisplatin is a chemotherapeutic agent commonly used for the treatment of solid tumors, and its side-effects include vestibulotoxicity. Previous studies have reported cisplatin-induced vestibulotoxicity in various animal models, but no study has investigated *in vivo* mouse vestibular dysfunction after cisplatin. The aim of this study was to investigate cisplatin-induced vestibulotoxicity in C57BL/6J mice. Vestibular function was assessed by recording the vestibulo-ocular reflex (VOR). This was done during sinusoidal rotations in the horizontal plane at three frequencies (0.5, 1.0 and 2.5 Hz). A high-resolution, high-frequency digital infra-red camera was used with eye-tracking algorithms. Cisplatin at 16 mg/kg, but not 8 mg/kg, decreased the VOR gain at 2.5 Hz compared with the vehicle control. Following 16 mg/kg cisplatin treatment, the animals showed no change in the optokinetic nystagmus response, suggesting that no major changes in visual or oculomotor functions had occurred. This mouse model may be useful for studying cisplatin-induced vestibulotoxicity and its treatment.

1. Introduction

Cisplatin (*cis*-diamminedichloroplatinum; CDDP) is a commonly used chemotherapeutic agent for the treatment of various solid tumors. However, CDDP-related side effects such as nephrotoxicity, neurotoxicity, ototoxicity and vestibulotoxicity limit its clinical use (Hartmann and Lipp, 2003). CDDP-mediated ototoxicity is thought to involve production of reactive oxygen species and trigger cell-death pathways in hair cells (Rybak and Ramkumar, 2007; Sedo-Cabezon et al., 2014; Waissbluth et al., 2012). Previous studies have reported CDDP-induced vestibulotoxicity (Kobayashi et al., 1987; Schaefer et al., 1981; Wright and Schaefer, 1982). Traditionally, guinea pigs (Nakayama et al., 1996; Sergi et al., 2003; Watanabe et al., 2001) and rats (Banfi et al., 2004; Tian et al., 2013) have been used as animal models for ototoxicity and vestibulotoxicity experiments, with very few studies in mice (Kim et al., 2008). To our knowledge, the only viable animal model study that has shown CDDP-induced vestibular dysfunction is that by Sergi et al. (2003), who reported a decrease in the vestibulo-ocular reflex (VOR) after CDDP administration in guinea pigs. Given the pervasive use of mice in pharmaceutical development and toxicological testing, a mouse model of vestibular dysfunction would be beneficial. Second, a mouse model would facilitate testing of CDDP-induced vestibular dysfunction treatments.

* Corresponding author at: Department of Otorhinolaryngology—Head and Neck Surgery, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan.
E-mail address: yas.say.yas1.24@gmail.com (Y. Takimoto).

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The VOR permits fixation of images by producing eye movements in the opposite direction of the head movement. The vestibular nuclei and cranial motor nuclei innervating extraocular muscles coordinate the VOR. The vestibular nuclei receive afferent signals from peripheral vestibular organs consisting of semicircular canals and otoliths, and send efferent signals to the trochlear and abducens nuclei for eye movements. Generally, the VOR test has been a useful model for assessing vestibular function in various species. The evaluation of eye movements in small animals such as mice is not very common, likely due to the technical difficulty of the procedures (Beraneck et al., 2008; Iwashita et al., 2001; Katoh et al., 1998; Koekkoek et al., 1997; Migliaccio et al., 2011; van Alphen et al., 2001). A recent study indicated that mouse ototoxicity models rarely show functional abnormalities and pathology because of their lower sensitivity to ototoxic drugs than guinea pigs (Poirrier et al., 2010). Previous studies of mouse vestibulotoxicity have used vestibular function analysis that included observation of spontaneous and reflex motor behaviors (Saldana-Ruiz et al., 2013), the swim test and other behavioral observations (Tian et al., 2013; Wu et al., 2001). The aim of this study was to specifically assess CDDP-induced vestibulotoxicity in mice using the VOR test.

2. Methods

2.1. Animals

Adult male C57BL/6J mice, 9 or 10 weeks of age and weighing 20–26 g, were purchased from Japan SLC Inc. (Hamamatsu, Japan). All animal experiments were conducted in accordance with the institutional guidelines set by the Osaka University Graduate School of Medicine Animal Care and Use Committee. Every effort was made to minimize animal suffering and reduce the number of animals used.

2.2. Experimental design

Animals were divided into three groups (n = 10 per group): saline (vehicle) injected control, and 8 and 16 mg/kg CDDP. CDDP (Wako Pure Chemical Industries, Osaka, Japan) was dissolved in saline at a concentration of 0.5 mg/ml. Based on previous studies (Hill et al., 2008; Kim et al., 2008), mice were given 2 or 4 mg/kg CDDP by an intraperitoneal (i.p.) injection once per day for 4 days to obtain a cumulative dose of 8 or 16 mg/kg, respectively. The day after the final injection (day 5), the mice were weighed, and open field, VOR and optokinetic nystagmus (OKN) tests were performed. To evaluate the time course of VOR responses, two additional groups of mice received 16 mg/kg CDDP or saline for 4 days (n = 8 per group) and the VOR test was performed on days 5 and 12. No mortality from CDDP toxicity was noted on either day, and data were obtained from all animals.

2.3. Surgical procedures

To ensure accurate measurements the head required stabilization. Mice received general (3% isoflurane) and local (1% lidocaine) anesthesia. A small incision was made in the skin over the center of the skull and a small metal plate with a screw hole was fixed with dental cement (Sun Medical, Shiga, Japan). Mice were isolated during recovery and closely monitored for 48 h. The animals underwent surgery 2 days before CDDP administration.

2.4. VOR test

The VOR methods were similar to those previously described (Imai et al., 2016). Mice were placed on a metal bar in the center of a turntable, which was surrounded by a plastic cylinder (60 cm in diameter) creating an experimental field. Experiments were performed in darkness. To stabilize the mouse head during rotations, the cemented head plate was screwed to the turntable bar. The head was fixed at 30° with the nose pointing down to align the lateral semicircular canals on the horizontal plane (Calabrese and Hullar, 2006). The turntable (head) was rotated sinusoidally by hand (±5° amplitude) at 0.5, 1.0 and 2.5 Hz using a metronome. The horizontal eye and turntable movements were recorded using a high-resolution infra-red camera recording system (240 Hz sampling rate; Sentech, Kanagawa, Japan). Movements of the turntable and eye were recorded by two cameras and the images were synchronized. The pupil was contracted with an ophthalmic solution (2% pilocarpine hydrochloride, Nippon Tenganyaku Kenkyusho, Nagoya, Japan) to assist with digital image capture. The VOR gain and phase shifts were analyzed according to information from digitally recorded eye and table images. The custom-built metal plate, cylinder, turntable, camera holder and all other custom equipment were made by Bio-Medica (Osaka, Japan).

2.5. OKN test

The OKN test was performed in an illuminated room. Mice were fixed on a table as described above, and this time, surrounded by a rotating plastic cylinder (60 cm diameter) with random dot patterns on the internal surface. The dots consisted of black circles on a white background. The cylinder was rotated by a motor-controlled system (Oriental Motor, Tokyo, Japan), clockwise at 8°/s stimulation. Induced eye movements were recorded using the infrared 240 Hz camera recording system (Sentech, Kamagawa, Japan). The rotating cylindrical screen and all other custom equipment were made by Bio-Medica.

2.6. Open field test

The open field test was performed as previously described (Nakatani et al., 2009). Animals were placed in the center of the open field apparatus (50 × 50 × 40 cm; Muromachi Kikai, Tokyo, Japan). Total distance traveled (m) and mean velocity (m/s) during the open field test were recorded by the ANY-maze tracking system (Stoelting CO, USA) (Kondo et al., 2015, 2014). Data were recorded for 10 min. The mean velocity was calculated across all periods of movement in this study.

2.7. Tissue preparation

Male C57BL/6J mice were deeply anesthetized by intraperitoneal injection of sodium pentobarbital (Nembutal; 200 mg/kg), and decapitated after cessation of spontaneous respiration. The temporal bones (including the organ of Corti, semicircular canals, otoliths, SG, VG and nerve trunk), were dissected immediately with small scissors and fine forceps using an upright microscope (Leica, Wetzlar, Germany), in chilled phosphate buffered saline (PBS, pH 7.4). The temporal bones were stored overnight in 4% paraformaldehyde before decalcification in EDTA solution (10% in PBS, pH 7.2) for a total of 7 days, then cryoprotected in 30% sucrose for 24 h before being frozen for cryostat sectioning into 10 μm thin sections. Sections were stained with a solution of haematoxylin and eosin (H-E) and mounted for morphological evaluation.

2.8. Data analysis

2.8.1. Eye movement analysis

The accuracy of the eye movement analysis has been described previously (Imai et al., 2016). Images of eye movements were stored at 645 × 485-pixel resolution. Using our own custom