Assessment of Ocular and Cervical Vestibular-evoked Myogenic Potentials in Cisplatin-induced Otolith Toxicity in Guinea Pigs

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Abstract
The purposes of this study were to determine if cervical vestibular-evoked myogenic potentials (cVEMPs) and ocular VEMPs tests are good electrophysiological examinations to represent cisplatin-induced otolith toxicity and to examine if D-methionine (D-met) pre-injection protects the otolith organs against cisplatin-induced changes of enzyme activities and oxidative status. Guinea pigs were intraperitoneally injected with sterile 0.9% saline alone, cisplatin (5 mg/kg) alone, D-met (300 mg/kg) alone, or D-met (300 mg/kg) 30 min before cisplatin (5 mg/kg) in combination, once daily for 7 consecutive days. Each animal undergoes the oVEMP and cVEMP tests before and one week after treatment. The saline group receives equivalent volume injections as the treatment groups. Each animal's body weight was measured before and after 7 days after the treatment. Immediately after the final oVEMP and cVEMP tests, the animals are immediately put under deep anesthesia with an intraperitoneal injection of sodium pentobarbital (60 mg/kg) and then decapitated. Using microdissection techniques, the utricular and saccular membranous tissue from two animals per group are collected, pooled, and preserved in 0.3 mL Tris buffer (1 M, pH 7.0) in a 2 mL microcentrifuge tube and stored at -10 °C until assayed, resulting in a size of six in each group. Immediately before measurements of enzyme-specific activities of Na+, K+-ATPase, Ca2+-ATPase, LPO, and NO, the pooled samples of the otolith organ specimens are homogenized in the 2 mL microcentrifuge tube by an ultrasonic tissue disruptor (Branson, Digital Sonifier S-450D, Danbury, CT, USA).

Methods and Materials
Forty-eight guinea pigs are randomly divided into four groups, comprising one saline control and three treatment groups. Animals are intraperitoneally injected with sterile 0.9% saline alone, cisplatin (5 mg/kg) alone, D-met (300 mg/kg) alone, or D-met (300 mg/kg) 30 min before cisplatin (5 mg/kg) in combination, once daily for 7 consecutive days. Each animal undergoes the oVEMP and cVEMP tests before and one week after treatment. The saline group receives equivalent volume injections as the treatment groups. Each animal's body weight was measured before and after 7 days after the treatment. Immediately after the final oVEMP and cVEMP tests, the animals are immediately put under deep anesthesia with an intraperitoneal injection of sodium pentobarbital (60 mg/kg) and then decapitated. Using microdissection techniques, the utricular and saccular membranous tissue from two animals per group are collected, pooled, and preserved in 0.3 mL Tris buffer (1 M, pH 7.0) in a 2 mL microcentrifuge tube and stored at -10 °C until assayed, resulting in a size of six in each group. Immediately before measurements of enzyme-specific activities of Na+, K+-ATPase, Ca2+-ATPase, LPO, and NO, the pooled samples of the otolith organ specimens are homogenized in the 2 mL microcentrifuge tube by an ultrasonic tissue disruptor (Branson, Digital Sonifier S-450D, Danbury, CT, USA).

Results
The body weight change (mean±SEM) in each group was 29.8±9 g, -35.1±12 g, 5.9±17 g, and -11.1±10 g for the groups of 0.9% saline, cisplatin (5 mg/kg), D-met (300 mg/kg), and cisplatin (5 mg/kg) combined with D-met (300 mg/kg), respectively.

The response rate, mean latencies, interwave latency, and amplitudes of cVEMP testing in guinea pigs for four groups were shown in Table 1 and Figure 2.

The response rate, mean latencies, interwave latency, and amplitudes of the oVEMP test in guinea pigs before and after the treatment of cisplatin (5 mg/kg) or/and D-met (300 mg/kg) for four groups were shown in Table 2 and Figure 3.

The means of LPO and NO levels reflecting oxidative stress in guinea-pig saccular and utricular membranous tissue were displayed in Table 3.

Conclusion
The cVEMP and oVEMP tests were feasible for the evaluation of cisplatin-related otolith dysfunction. D-met significantly attenuated cisplatin-induced damage to otolith organs in guinea pigs. The D-met-mediated improvement in otolith function correlated with a significant attenuation of increased oxidative stress and reduced ATPase activities.

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References