**Development of a Novel Hydrogen Sulfide-Donating Liposome Using Methemoglobin for the Prevention of Cisplatin-Induced Nephrotoxicity**

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**Background and aims.** Hydrogen sulfide (H2S) exhibits diverse biological activities at low concentrations and is being explored for therapeutic applications. However, due to its gaseous nature and toxicity at high concentrations, a delivery system capable of sustained H2S release is essential. Methemoglobin (metHb), a biological molecule that reversibly binds H2S, is a potential candidate for controlled H2S delivery. In this study, we developed H2S-bound metHb-encapsulated liposomes (H2S-metHbV) as a novel H2S donor and evaluated their potential for medical application.

**Methods.** H2S-metHbV was prepared by adding sodium hydrosulfide to metHb-encapsulated liposomes. H2S release was quantified using UV-Vis. spectroscopy. Cytotoxicity was assessed in LLCPK-1 renal epithelial cells. The renoprotective effect was evaluated in a cisplatin (CDDP)-induced nephrotoxicity mouse model. Additionally, B16F10 tumor-bearing mice were used to assess the impact of H2S-metHbV on CDDP’s anti-tumor activity.

**Results.** H2S-metHbV, approximately 200 nm in size, showed sustained H2S release, with 75% released over 12 hours at pH 7.4. It exhibited no cytotoxicity toward LLCPK-1 cells. H2S-metHbV dose-dependently alleviated CDDP-induced nephrotoxicity *in vivo*. Additionally, it did not impair the anti-tumor efficacy of CDDP in the B16F10 tumor bearing mice.

**Conclusion/Discussion.** H2S-metHbV functioned as a safe and sustained H2S donor without showing toxicity *in vitro* or *in vivo*. It effectively protected against CDDP-induced nephrotoxicity without affecting anti-tumor efficacy. These findings suggest that H2S-metHbV has a potential for medical application such as a prophylactic agent for chemotherapy-induced kidney injury.