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Influence of *Npc1* genotype on the toxicity of hydroxypropyl-β-cyclodextrin, a potentially therapeutic agent, in Niemann–Pick Type C disease models ☆

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ABSTRACT

Hydroxypropyl-β-cyclodextrin (HPBCD) is an attractive drug candidate against Niemann–Pick Type C (NPC) disease. However, the safety of HPBCD treatment for NPC patients remains to be elucidated. In this study, we examined the acute toxicity of HPBCD in *Npc1*-deficient mice. When treated with HPBCD (20,000 mg/kg, subcutaneously), over half of the wild-type ($Npc1^{+/+}$) or $Npc1^{+/-}$ mice died by 72 h after the injection. In contrast, all of the $Npc1^{-/-}$ mice survived. Marked pathophysiological changes, such as an elevation in serum transaminase and creatinine levels, hepatocellular necrosis, renal tubular damage, interstitial thickening, and hemorrhages in lungs, were induced by the HPBCD treatment in $Npc1^{+/+}$ or $Npc1^{+/-}$ mice. However, these

Abbreviations: NPC, Niemann–Pick Type C disease; HPBCD, Hydroxypropyl-β-cyclodextrin; ALT, Alanine aminotransferase; CHO, Chinese hamster ovary.

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pathophysiological changes were significantly alleviated in *Npc1*$^{-/-}$ mice. In addition, *in vitro* analysis showed that the *Npc1* gene deficiency and treatment with U18666A, an Npc1 inhibitor, remarkably attenuated the cytotoxicity of HPBCD in Chinese hamster ovary cells. These results suggest that the *NPC1* genotype exacerbates the cytotoxicity of HPBCD and *Npc1*$^{-/-}$ mice have substantial resistance to the lethality and the organ injury induced by HPBCD injection compared with *Npc1*$^{+/+}$ or *Npc1*$^{+/-}$ mice. We suggest that the *Npc1* genotype should be considered in the safety evaluation of HPBCD using experimental animals and cells.

1. Introduction

Niemann–Pick Type C (NPC) disease, an autosomal recessive disorder caused by mutations in either of the *Npc1* or *Npc2* genes, is characterized by progressive neurological deterioration and death during childhood [1]. Marked lysosomal accumulation of unesterified cholesterol and shortage of esterified cholesterol in cellular compartments are observed in NPC disease, and cholesterol sequestration may be a key factor in developing the disease. Recently, some reports have shown that hydroxypropyl-β-cyclodextrin (HPBCD), a cyclic oligosaccharide derivative that has a solubilizing ability on lipophilic compounds, including cholesterol, attenuated cholesterol sequestration in systemic cells and prolonged the lifespan in *Npc*1 null mice [2–4]. In addition, Matsuo et al. [5] reported that treatment with HPBCD improved hepatosplenomegaly and central nervous system dysfunction in two patients with NPC disease.

HPBCD has been used as a pharmaceutical additive with high aqueous solubility and extremely low toxicity and has been used clinically with cardinal remedies in parenteral formulations [6]. Based on these facts, HPBCD is compassionately used to treat patients with NPC disease. In our previous study, patients were administered high-dose HPBCD (2000–2500 mg/kg) infusions twice or more per week without severe adverse events [5]. However, Chien et al. [7] reported that chronic HPBCD infusion induced the pneumonia in healthy pigs and suggested the risk of lung toxicity by HPBCD treatment for NPC disease. In addition, some reports have demonstrated that HPBCD caused organ injury, such as renal and liver dysfunction, in animals [8,9]. Therefore, the safety of HPBCD treatment for NPC patients remains to be elucidated.

Based on these facts, this study was conducted to evaluate the acute toxicity of HPBCD in NPC disease. We examined the toxic effects of HPBCD on mice as determined by survival rate, changes in serum biochemical parameters, and histological analysis in wild-type or homozygous and heterozygous *Npc1* mutant mice. In addition, to evaluate the effects of NPC disease on cellular injury induced by HPBCD, we examined the effects of NPC1 inhibition by gene deletion and pharmacological inhibition using U18666A on the HPBCD-induced cell injury in *in vitro* cultured cells.

2. Material and methods

2.1. Reagents

HPBCD was kindly donated by Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan). Mayer's hematoxylin, 1% eosin alcohol solution, and mounting medium for histological examination (malinol) were from MUTO Pure Chemicals (Tokyo, Japan). Dulbecco's modified Eagle's medium and F-12 medium were obtained from Gibco-Life Technologies (Life Technologies Japan, Tokyo, Japan). HyClone™ fetal bovine serum (FBS) was purchased from Thermo Scientific (Logan, UT, USA). The cell counting kit and Cellstain® Double Staining Kit were obtained from Dojindo Laboratories (Kumamoto, Japan). All other reagents and solvents were of reagent grade. De-ionized and distilled bio-pure grade water was used throughout the study.

2.2. Animal experiments

Age-matched (9–11 weeks) male wild-type (*Npc1*$^{+/+}$) mice and homozygous (*Npc1*$^{-/-}$) and heterozygous (*Npc1*$^{+/-}$) mutant (BALB/cNctr-*Npc1*^{m1N}) mice [10] were used. A total of 75 mice were used in this study, 35