Acid-labile interlocked cyclodextrin polymers for therapeutic applications to rare diseases

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Statement of Purpose: Niemann-Pick type C (NPC) disease, one of the lysosomal storage disorders, is an autosomal recessive lysosomal trafficking disorder caused by the mutation of NPC1 protein, which shows the chronic accumulation of cholesterols within lysosomes of cells throughout the body.[1] The lysosomal cholesterol accumulation leads to various clinical symptoms, such as progressive neurodegeneration and hepatosplenomegaly, often resulting in fatality at an early age. Although there is no effective clinical treatment for NPC disease, hydroxypropyl-β-cyclodextrin (HP-β-CD) has received much attention as potential therapeutics for NPC disease. The administration of HP-β-CD reduces the cholesterol content in various organs, leading to prolonging a life-span of NPC disease model mice.[2] However, despite the significant therapeutic effect of HP-β-CD, it requires high dose for NPC disease therapy due to its non-specific inclusion of plasma components and rapid renal clearance. To address these problems, we have designed acid-labile Pluronic/β-CD-based polyrotaxanes (PRXs) bearing acid-cleavable terminal stoppers as new therapeutics for NPC disease.[3,4] The acid-labile polyrotaxanes exert degradation and the release of threaded β-CDs in response to lysosomal acidic environments (Figure 1). Additionally, the acid-labile PRXs are expected to show long blood half-life, because the molecular weight of the PRXs are remarkably high compared to β-CDs. In this study, the acid-labile Pluronic/β-CD-based PRXs were synthesized and their therapeutic effect to NPC disease model mice were investigated.

Methods: The acid-labile PRXs were synthesized by capping Pluronic P123/β-CD pseudopolyrotaxanes with (*N*-triphenylmethyl)glycine, followed by introducing (2-hydroxyethoxy)ethyl (HEE) groups at the threaded β-CD moieties to impart water solubility.[5] The number of threading β-CD on the HEE group-modified PRX (HEE-PRX) and the number of threading HEE groups modified on the PRX were determined to be 11.2 and 62.8. Animal experiments were approved by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University. Homozygous *Npc1* mutant mice (BALB/cNctr-Npc1^{m1N}) were used as NPC disease model mice.[6] The HEE-PRX or HP-β-CD were subcutaneously administered at the dose of 500 mg/kg once a week from three weeks of age, and the change in body weight, motor function, and survival periods were monitored.

Results: The acid-labile HEE groups-modified PRXs (HEE-PRXs) maintained their supramolecular structure at the pH range of 7 to 9 even after 48 h of incubation. On the contrary, the terminal *N*-triphenylmethyl groups were completely cleaved after 24 h incubation at pH 5, and the supramolecular structure of PRXs were degraded into their constituent molecules.

The therapeutic effect of the HEE-PRX to NPC disease model mice was investigated using *Npc1* knockout mice

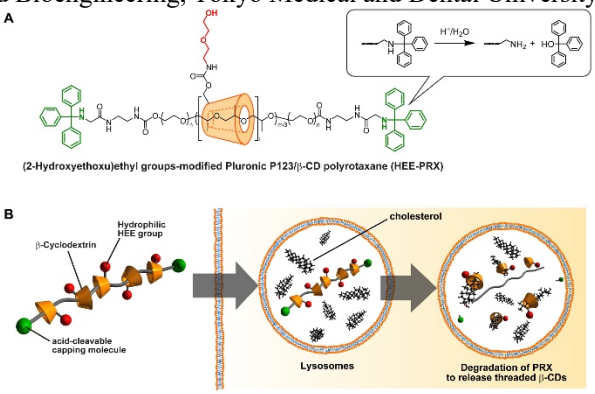

Figure 1. (A) Chemical structure of the HEE-PRX. (B) Schematic illustration for the lysosomal degradation of the HEE-PRXs.

(NPC1 mice). The average life span of untreated NPC1 mice was 71.3 days. The administration of HP-β-CD (500 mg/kg) to NPC1 mice could not significantly prolong the life span (73.8 days), because the concentration of HP-β-CD was insufficient. However, the administration of the HEE-PRX (500 mg/kg) significantly prolonged the life span of NPC1 mice (86.5 days). To confirm the relationship between the life span of NPC1 mice and the tissue cholesterol level, the amount of cholesterols in each tissue was quantified with a GC-MS. The amount of cholesterol in liver for untreated NPC1 mice (8 weeks of age) was determined to be 21.8 ± 4.7 μg/mg of tissue, which was approximately 10-fold higher than that for untreated wild-type mice (1.9 ± 0.2 μg/mg of tissue). The administration of HP-β-CD (500 mg/kg) to NPC1 mice did not significantly change the amount of cholesterol in liver (22.5 ± 1.8 μg/mg of tissue). Therefore, the HP-β-CD administration did not change the life span of NPC1 mice. The administration of the HEE-PRX (500 mg/kg) significantly prohibited the cholesterol accumulation in liver (4.9 ± 1.2 μg/mg of tissue). This is most likely due to the retardation of systemic clearance for HEE-PRXs.

Conclusions: The acid-labile HEE-PRX that can dissociate in response to the lysosomal acidification was synthesized for the treatment of NPC disease. The administration of the HEE-PRX significantly prolonged the life-span of the NPC disease model mice through the inhibition of cholesterol accumulation in tissues. The HEE-PRX would be a promising candidate for the treatment of NPC disease.

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