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Polyrotaxane-based systemic delivery of β-cyclodextrins for potentiating therapeutic efficacy in a mouse model of Niemann-Pick type C disease

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ABSTRACT

Niemann-Pick type C (NPC) disease is a fatal metabolic disorder characterized by the lysosomal accumulation of cholesterol. Although 2-hydroxypropyl β-cyclodextrin (HP-β-CD) promotes the excretion of cholesterol and prolongs the life span in animal models of NPC disease, it requires extremely high dose. We developed acid-labile β-CD-based polyrotaxanes (PRXs) comprising multiple β-CDs threaded along a polymer chain capped with acid-cleavable stopper molecules for potentiating therapeutic efficacy of β-CD in NPC disease. The acid-labile PRXs dissociate under the acidic lysosomes and release threaded β-CDs in lysosomes, which promotes cholesterol excretion in NPC disease model cells at lower concentration than HP-β-CD. In this study, the therapeutic effect of the PRXs in a mouse model of NPC disease was investigated. Weekly administration of the PRXs significantly prolonged the life span and suppressed neurodegeneration in mice, even at a dose of 500 mg/kg, a markedly lower dose than previously reported for HP-β-CD. Detailed analysis of tissue cholesterol revealed that PRX treatment markedly suppressed the tissue accumulation of cholesterol in the NPC mouse model, but did not alter cholesterol content in wild-type mice. Acid-labile PRX is therefore a promising candidate for potentiating the efficacy of β-CD in the treatment of NPC disease.

1. Introduction

β-Cyclodextrins (β-CDs), a cyclic oligosaccharide comprising seven α-D-glucopyranosides linked by α-1,4-linkages, have attracted considerable attention, because β-CD derivatives are revealed to show therapeutic effect in many diseases, such as Alzheimer's disease [1], Niemann-Pick type C (NPC) disease [2], age-related macular degeneration [3], and atherosclerosis [4]. The β-CD derivatives interact with cholesterols through the formation of an inclusion complex and remove them from the plasma membrane [5,6]. This cholesterol removal effect modulates the localization and function of transmembrane proteins, particularly those localized at the lipid raft domain, and the effect is considered to be related to the therapeutic effect of β-CDs.

Among various diseases, the treatment of NPC disease by 2-hydroxypropyl β-cyclodextrin (HP-β-CD), a highly water-soluble derivative of β-CD, is being extensively studied. This disease is an autosomal recessive disorder caused by mutation in either of the NPC1 or NPC2 proteins [7–9]. Because both proteins play a pivotal role in the transportation of lysosomal cholesterols to the endoplasmic reticulum (ER), dysfunction in these proteins results in the chronic accumulation of low density lipoprotein (LDL)-derived unesterified cholesterols and several glycosphingolipids in lysosomes [7,10]. Patients with NPC disease show fatal clinical symptoms, such as progressive neurodegeneration, as a result of lysosomal cholesterol accumulation [7]. Because HP-β-CD derivatives form an inclusion complex with cholesterols, HP-β-CD mobilizes lysosomal cholesterol to cytoplasm in NPC1-deficient cells, causing cholesterol to be excreted from cells or processed into cholesteryl esters [11]. Moreover, the administration of HP-β-CD in murine and feline models of NPC disease suppressed neurodegeneration and prolonged the life span [2,12–15]. Currently, the therapeutic effect and pharmacological evaluations of HP-β-CD are being widely studied and clinical trials are ongoing [16–19]. However, HP-β-CD is typically administered at high concentrations, ranging from 4000 to 8000 mg/kg in the treatment of NPC and other diseases [1–4,12–14], presumably because of its short half-life in systemic circulation and non-specific interactions with lipoproteins and erythrocytes in the blood stream. Despite its significant therapeutic potential, HP-β-CD exhibits adverse effects such as acute toxicity, pulmonary injury, and ototoxicity when administered at high concentrations [20–23]. These effects might relate to the ability of HP-β-CD to extract cholesterol from the plasma membrane and disrupt lipid rafts [24]. Therefore, improvement in the therapeutic effect of HP-β-CD would be beneficial for reducing the dose

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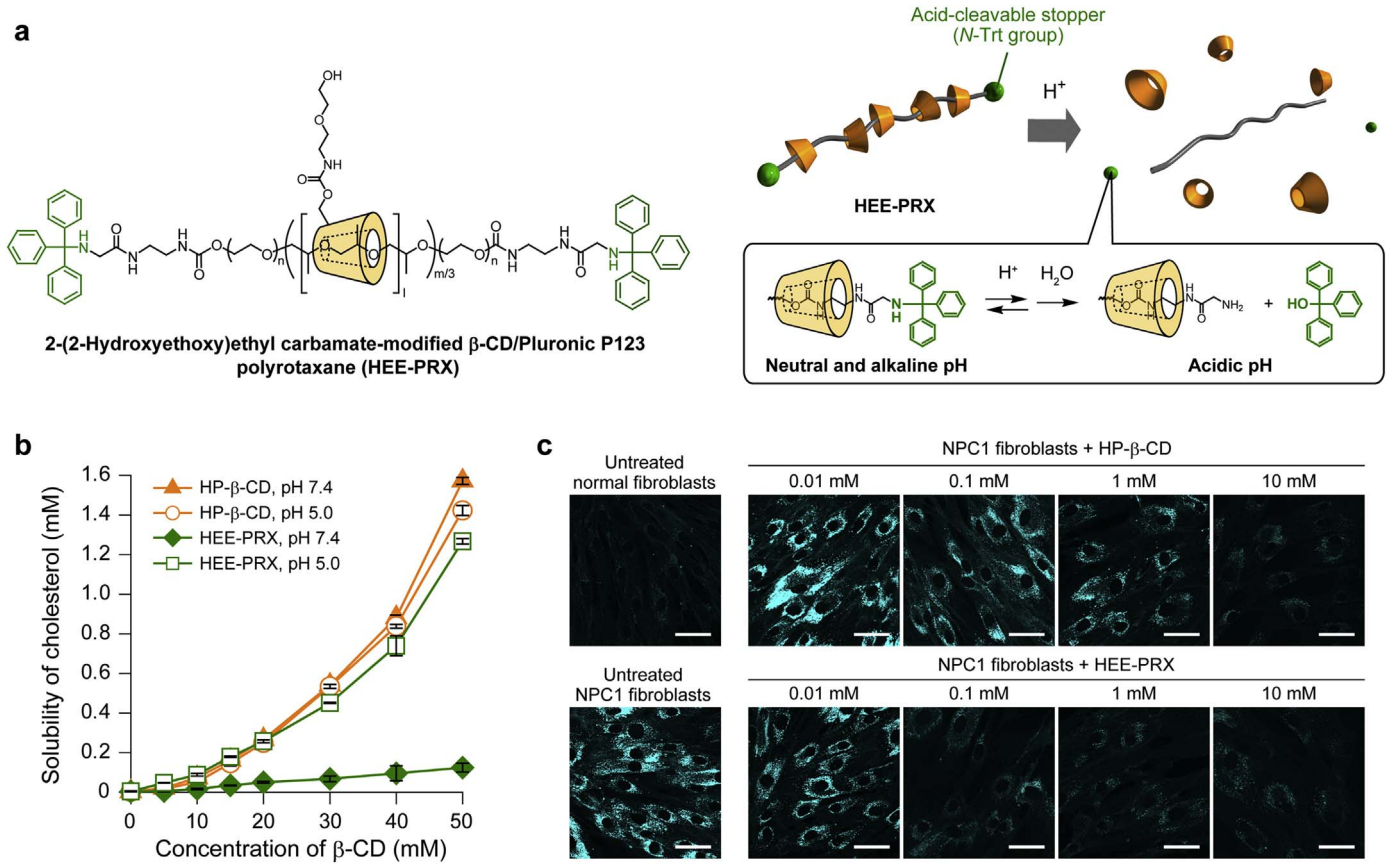

Fig. 1. (a) Chemical structure of 2-(2-hydroxyethoxy)ethyl (HEE) carbamate-modified β-CD/Pluronic-based polyrotaxane bearing *N*-Trt end groups (HEE-PRX) and schematic illustration for the acid-induced cleavage of the *N*-Trt end groups in HEE-PRX and the subsequent release of threaded β-CDs. (b) Amount of solubilized cholesterol in the presence of HP-β-CD and HEE-PRX at various concentrations at pH 7.4 and 5.0 ($n = 3$). (c) Filipin-stained normal and NPC1-deficient human fibroblasts after 24 h treatment with HP-β-CD and HEE-PRX at various concentrations. For HEE-PRX, the concentration was regarded as the concentration of threaded β-CDs (scale bars: 50 μm).

and avoiding intrinsic adverse effects.

To address these issues, we have proposed the use of polyrotaxanes (PRXs), supramolecular compounds comprising multiple CDs threaded along a linear polymer chain [25–29], as an intracellular delivery vehicle for β-CD (Fig. 1a) [30–32]. Because the hydrophobic cavity of β-CDs is occupied with a polymer chain, the threaded β-CDs in the PRXs do not extract cholesterol from the plasma membrane, thus avoiding toxicity [30–32]. Furthermore, we have developed acid-labile PRXs possessing *N*-triphenylmethyl (*N*-Trt) groups as acid-cleavable stoppers to allow the dissociation of PRXs at acidic pH, such as in lysosomes, and the intracellular release of threaded β-CDs (Fig. 1a) [32]. The release of threaded β-CDs in acidic lysosomes led to improved cholesterol accumulation in NPC disease-derived cells at concentrations approximately 100-fold lower than for HP-β-CD [30–32]. Therefore, acid-labile PRXs are potential noninvasive and effective therapeutic candidates in NPC disease. However, their therapeutic effects in animal models of NPC disease have not yet been clarified.

In this study, we described the therapeutic effect of β-CD/Pluronic-based acid-labile PRXs in a mouse model of NPC disease (*Npc1*$^{-/-}$ mice). The effect of weekly administration of PRXs on the main pathologies of *Npc1*$^{-/-}$ mice, such as short survival, neurodegeneration, and tissue cholesterol accumulation, was investigated in comparison with HP-β-CD. Furthermore, the pharmacokinetics of subcutaneously administered PRXs were investigated in comparison with HP-β-CD to verify their in vivo characteristics. Taken together, the findings of this study clearly show that PRXs have significant potential for potentiating therapeutic efficacy of β-CD derivatives in vivo.

2. Materials and methods

2.1. Materials

2-(2-Hydroxyethoxy)ethyl carbamate-modified polyrotaxane (HEE-PRX) composed of Pluronic P123 as an axle polymer, β-CDs modified with HEE groups as cyclic molecules, and *N*-Trt end groups as acid-cleavable bulky stoppers were synthesized according to our previous report [32]. The number of threading β-CD and HEE groups modified on the PRX were determined as 11.2 and 62.5, respectively (i.e., the number of HEE groups was 5.6 per β-CD). The number-averaged molecular weight of the HEE-PRX was 29,000. HP-β-CD (averaged molecular weight of 1480, the number of HP groups was 5.9 per β-CD) was obtained from Sigma-Aldrich (Milwaukee, WI, USA). HiLyte Fluor 680 (HF680) (Anaspec, Fremont, CA, USA)-modified HEE-PRX and HP-β-CD were synthesized according to our previous report [32].

2.2. Solubility of cholesterol by inclusion complexation with β-CDs

The solubility of cholesterol was measured on an HPLC according to our previous report [32]. The detailed procedure was described in Supplementary data.

2.3. Cholesterol reducing effect in culture model

Human skin fibroblasts derived from a patient with Niemann-Pick type C disease (NPC1) (GM03123) and normal human skin fibroblasts (GM05659) were obtained from the Coriell Institute for Medical Research (Camden, NJ, USA). These cells were cultured in Dulbecco's