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Serendipitous effects of β-cyclodextrin on murine model of Krabbe disease

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β-cyclodextrins are cyclic oligosaccharides assembled in a ring configuration containing a lipophilic central cavities and hydrophilic outer surfaces. Traditionally, cyclodextrins are used as excipients and

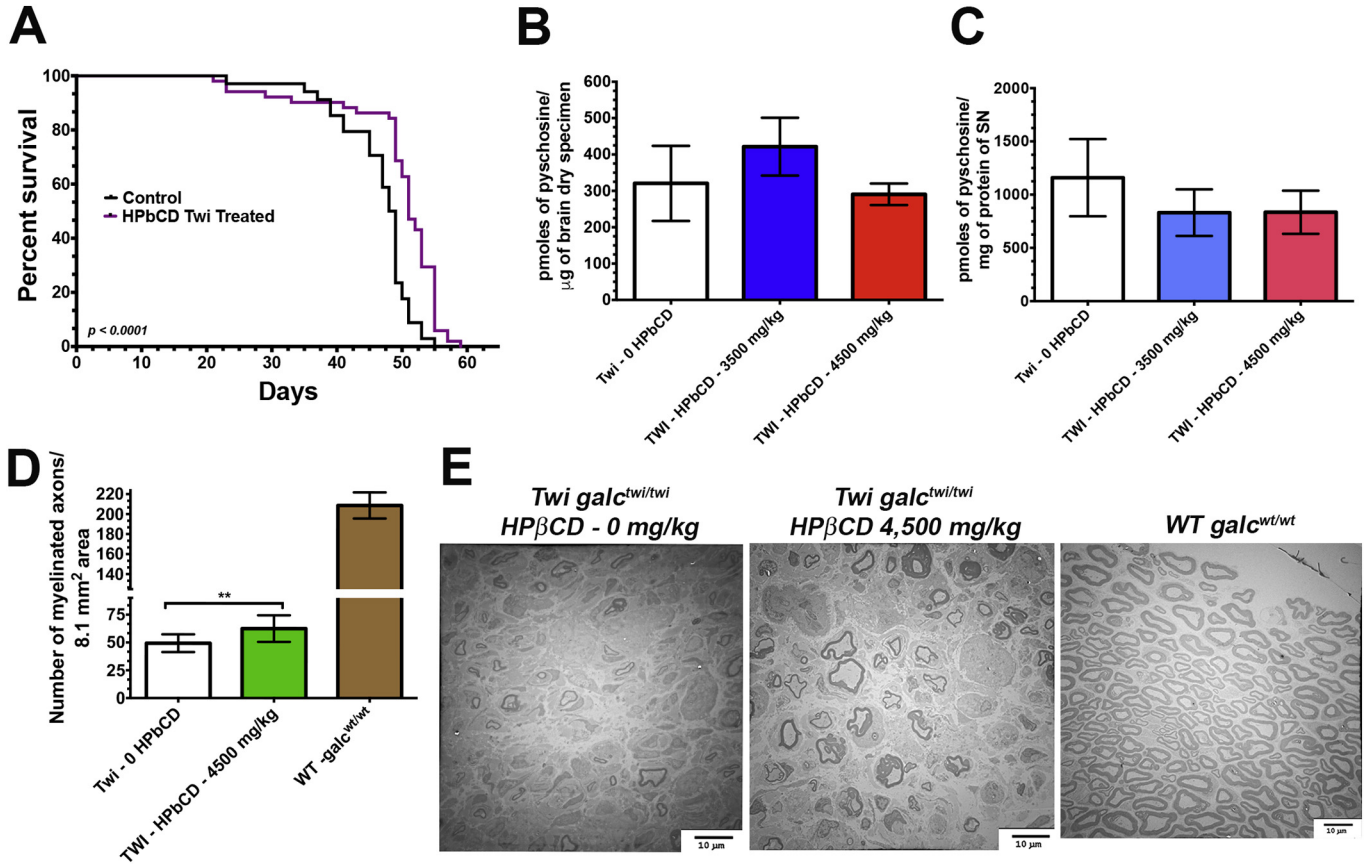

Fig. 1. The effects of 2-hydroxypropyl-β-cyclodextrin (HPβCD) in the *Twitcher* mouse model for Krabbe disease. (A) Survival analysis of *Twitcher* (*Twi galc*$^{twi/twi}$) mice treated with HPβCD (n = 34) and controls (n = 51). Equal number of males and females of *Twi galc*$^{twi/twi}$ were used in both groups. From post-natal day 3 until their death, treated *Twi* mice group received 4500 mg/kg of HPβCD subcutaneously diluted in phosphate saline every 2 days. An initial group of 12 mice receiving 3500 mg/kg subcutaneously also in the same frequency and duration are included in this survival analysis. Psychosine levels measured by LC-MS/MS in the brain (B) and sciatic nerve (C) specimens showed no statistically significant differences between mice receiving HCβCD (n = 6) and those receiving only saline (n = 6). (D) The number of myelinated axonal bulbs were significantly increased in the mice receiving HCβCD (n = 6) in comparison to controls (n = 6) ($p < 0.05$). (E) The transmission electron microscopy images of sciatic nerves dissected post-mortem showed increased preservation of myelinated fibers in the group of *Twi* mice receiving HPβCD.

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absorption enhancers of hydrophobic molecules [1]. Among different cyclodextrin derivatives, 2-hydroxypropyl-β-cyclodextrin (HPβCD) showed to be an efficacious therapeutic agent for Niemann-Pick Disease Type C1 (NPC1), an autosomal-recessive and fatal neurodegenerative disorder [2–6], and it is currently being evaluated in clinical trials [7,8]. During *in vivo* experiments of small molecule candidates identified in a cell-based LC-MS/MS medium-throughput screening for psychosine-reducing molecules [9], we observed beneficial therapeutic effects of the HPβCD in the *Twitcher* mouse (*Twi*), C57BL/6 *galc*$^{twi/twi}$, the murine model of globoid-cell leukodystrophy (GLD) or Krabbe disease, an inborn lysosomal disease caused by the deficiency of the lysosomal β-galactocerebrosidase (GALC) [10,11]. The *Twi* mouse model recapitulates the severe neurological course and demyelinating processes in both central (CNS) and peripheral (PNS) nervous systems due to the elevated cytotoxicity of psychosine in myelin-forming cells in the setting of GALC deficiency [11–14]. To solubilize highly hydrophobic small molecule "hits" for murine experiments, HPβCD was used as a dissolvent as previously described [1]. In early experiments, the *Twi* mice group receiving HPβCD alone at 3500–4500 mg/kg subcutaneously from third day of life and then every two days showed slower progression of neurological symptoms and expanded lifespan (Fig.1A). No statistically significant differences in the levels of the cytotoxic psychosine were noticeable in the both brain and sciatic nerves of HPβCD-treated *Twi* mice (Fig.1B and 1C), indicating a distinct mechanism of action of HPβCD. To further investigate the effects of HPβCD, ultrastructural studies performed blindly in proximal and distal segments of sciatic nerves showed statistically significant preservation of myelinated axons in the cohort receiving HPβCD (Fig.1C). It is unlikely that HPβCD has any effect in the CNS given its inability to cross the blood-brain barrier (BBB) [2,15,16] and the unchanged psychosine levels in the brains of HPβCD-treated *Twi* mice (Fig.1B). Altogether, these serendipitous findings demonstrate the therapeutic potential of HPβCD in the demyelinating disease processes of GLD by yet unknown mechanisms. In addition, these results highlight the importance of careful selection of additives for dissolution of hydrophobic small molecules for animal studies.

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