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Apolipoprotein-mimetic nanodiscs reduce lipid accumulation and improve liver function in acid sphingomyelinase deficiency

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Abstract

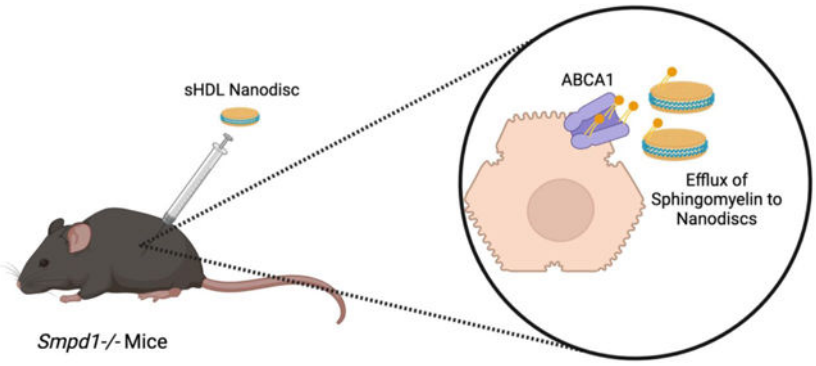
Acid sphingomyelinase deficiency (ASMD) is a severe lipid storage disorder caused by the diminished activity of the acid sphingomyelinase enzyme. ASMD is characterized by the accumulation of sphingomyelin in late endosomes and lysosomes leading to progressive neurological dysfunction and hepatosplenomegaly. Our objective was to investigate the utility of synthetic apolipoprotein A-I (ApoA-I) mimetics designed to act as lipid scavengers for the treatment of ASMD. We determined the lead peptide, 22A, could reduce sphingomyelin accumulation in ASMD patient skin fibroblasts in a dose dependent manner. Intraperitoneal administration of 22A formulated as a synthetic high-density lipoprotein (sHDL) nanodisc mobilized sphingomyelin from peripheral tissues into circulation and improved liver function in a mouse model of ASMD. Together, our data demonstrates that apolipoprotein mimetics could serve as a novel therapeutic strategy for modulating the pathology observed in ASMD.

Graphical Abstract

[†]Denotes equal contribution

Conflicts of Interest:

Dr. Schwendeman declares financial interests for board membership, as a paid consultant, for research funding, and/or as equity holder in EVOQ Therapeutics. The University of Michigan has a financial interest in EVOQ Therapeutics, Inc.



Synthetic high-density lipoprotein nanodiscs accept excess sphingomyelin from peripheral tissues in a mouse model of ASMD.

Keywords

Apolipoprotein mimetics; Acid Sphingomyelinase Deficiency

Introduction

Acid sphingomyelinase deficiency (ASMD), is an autosomal recessive disorder resulting from loss of function mutations in *SMPD1*, leading to lysosomal accumulation of sphingomyelin and other lipids.[1-3] Disease progression can lead to hepatosplenomegaly and reduced pulmonary function, and, in the most severe cases, neurological deterioration, leading death typically at 2-3 years of age.[4]

Prior studies have shown ASMD patients have reduced levels of high-density lipoproteins (HDL) in circulation that are enriched in sphingomyelin content.[5,6] When apolipoprotein A-1 (ApoA-1) is secreted by the liver, it interacts with the ATP binding cassette transporter A1 (ABCA1) on cell membranes to form nascent HDL particles from phospholipids.[7,8] Recent work has taken advantage of ApoA-1 function for the development of synthetic HDL as therapeutics for cardiovascular disease.[9-12] Because of the difficulty producing significant quantities of recombinant ApoA-1, several short ApoA-1 mimetic peptides have been developed.[13-15] These peptides efflux cellular lipids via the ABCA1 transporter and increase circulating HDL levels. Based on this existing data, we decided to investigate if redistribution of accumulated sphingomyelin by apolipoprotein mimetics could serve as a new treatment strategy for ASMD using a genetic mouse model of the disease.[16]

Materials and Methods

Detailed materials and methods can be found in the supplementary information.

Results and Discussion

We compared 3 different apolipoprotein-mimetic peptides in their ability to solubilize sphingomyelin lipid vesicles. The 18A peptide contains 18 amino acids designed to mimic

the amphipathic a-helix found in ApoA-1 with no sequence homology to the full-length protein.[15] The 5A peptide contains two helices based on 18A, with 5 alanine substitutions in the second helix.[14] The 22A peptide is based on the most prevalent amino acid present at each position in the a-helices of ApoA-1, with modifications to improve lipid binding.[13] Full length ApoA-1 was used as a reference, and it induced moderate solubilization of sphingomyelin (~25% reduction in turbidity) but was surpassed by 22A and 18A (Figure 1A). We next investigated the ability of the peptides to remove sphingomyelin from ASMD patient fibroblasts (Figure 1B), as accumulation of sphingomyelin is a hallmark feature of ASMD. At 10μM, 22A, 18A, and 5A all showed similar efflux capabilities (~15%). Treatment at 100μM resulted in enhanced efflux, however, this came at the cost of cytotoxicity for 18A and 5A (Figure 1C). This cytotoxicity is likely due in part to disruption of the cell membrane, as the peptide with the strongest lipid binding, 18A, showed the most severe drop in cell viability. From these experiments, 22A stood out as the most promising peptide due to its dose-dependent increase in sphingomyelin efflux and superior safety profile compared to the other treatments.

Most apolipoprotein mimetics in development are dosed as synthetic high-density lipoprotein nanodiscs (sHDL) instead of free peptide. Prior studies from our lab have demonstrated sHDL formulations of 22A have higher areas under the curve than free peptide.[17] With this in mind, we determined the differences between sHDL and free 22A *in vitro* to further inform the best formulation for *in vivo* experiments. We prepared the sHDL by combining the 22A peptide with the phospholipid DMPC at a 2:1 ratio. The resulting particles were 6-8 nm in size and possessed disc-like morphology as determined by DLS and TEM respectively (Figure 2A-B). The sHDL nanodiscs showed slightly reduced sphingomyelin efflux capabilities compared to the free peptide when used at 10μM peptide concentration (15% vs 11%) (Figure 2C). This difference is likely attributable to effects of the peptide complex with DMPC, which reduces the amount of exposed peptide able to directly bind sphingomyelin. Despite the small change in efflux, the sHDL formulation of 22A possessed a superior safety profile in ASMD fibroblasts compared to free peptide, showing no decrease in survival at concentrations up to 500μM. From this, we decided to investigate the effects of the sHDL formulation of 22A in a mouse model of ASMD.

Given the increased sphingomyelin content as a result of ASMD, we sought to determine if sHDL would serve as an acceptor of excess sphingomyelin from tissues and mobilize it into the bloodstream. Despite the absence of ASM activity in this mouse model, the presence of neutral sphingomyelinases localized to the Golgi, ER, and plasma membrane represented an opportunity for remobilized sphingomyelin to be metabolized.[18,19] WT and *Smpd1*−/− mice were administered a single injection of 100 mg/kg sHDL nanodiscs intraperitoneally (I.P.), and serum was collected for sphingomyelin quantification. Both WT and *Smpd1*−/− mice had similar amounts of sphingomyelin present in the serum prior to treatment. Following sHDL injection, *Smpd1*−/− mice showed a ~20% increase in circulating sphingomyelin that persisted through the 24-hour time point (Figure 3A). A similar increase was observed in the WT mice 24-hours after treatment. In addition to serum sphingomyelin levels, we compared the lipoprotein profiles of WT and *Smpd1*−/− mice before and after sHDL injection. Prior to injection, *Smpd1*−/− mice had similar levels of very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and HDL compared to WT (Figure 3B). This deviates from

what is observed in humans, as the enriched sphingomyelin content of immature HDL particles has been shown to inhibit the enzymes required for HDL maturation.5 Six hours after sHDL injection in WT and *Smpd1*−/− mice, we observed a small decrease in HDL and a substantial increase in VLDL (Figure 3C). This change in VLDL levels is the result of rapid exchange of cholesterol and phospholipids from endogenous lipoproteins to sHDL and is consistent with previous reports on lipoprotein profiles following sHDL administration.[17] Twenty-four hours post-injection, we observed a drop in VLDL and slight increase in HDL levels relative to baseline for both WT and *Smpd1*−/− mice (Figure 3D). Post-column analysis of lipoprotein fractions revealed the VLDL species appearing after sHDL nanodisc injection to be enriched in sphingomyelin and cholesterol content, with 30-40% of the total amount of sphingomyelin and cholesterol contained in the VLDL (not shown).

We next performed a 4-week study in which *Smpd1*−/− mice received daily I.P. injections of 100 mg/kg sHDL nanodiscs starting at 6-weeks of age. *Smpd1*−/− mice demonstrated deficits in motor performance and increased body weight compared to WT mice, which were not affected by sHDL treatment. (Figure 4A-B). This is likely due to limited quantities of peripherally administered sHDL nanodiscs able to cross the BBB (data not shown). Serum was collected at the end of treatment and analyzed for markers of liver function. Treatment of *Smpd1*−/− mice with sHDL nanodiscs resulted in significant reductions in serum alkaline phosphatase (ALKP) and aspartate aminotransferase (AST), two common markers of healthy liver function, rescuing them to levels observed in WT mice (Figure 4C-E).

Overall, our findings represent the first investigations of apolipoprotein mimetics in ASMD. Their ability to remove sphingomyelin from ASMD patient fibroblasts and mobilize sphingomyelin *in vivo* are both positive effects, however these changes were not able to halt disease progression in the complete absence of acid sphingomyelinase activity. Future experiments in models possessing residual enzyme activity, such as the murine model of non-neurological ASMD that features low levels of lysosomal sphingomyelinase activity developed by Marathe et al. [20], could reveal a more prominent role for apolipoprotein mimetics in managing ASMD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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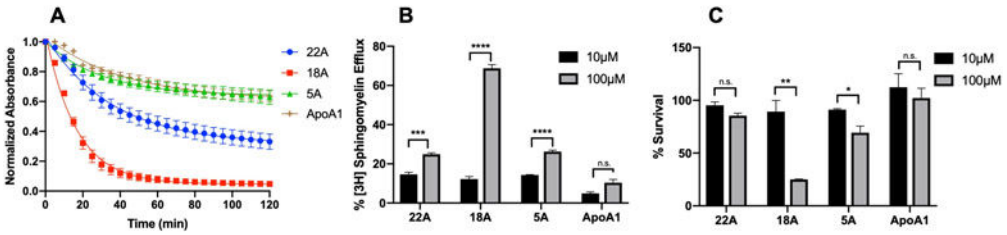

Figure 1:

Evaluation of apolipoprotein mimetic peptides *in vitro*. Solubilization of sphingomyelin vesicles during 2-hour incubation with 5mg/mL apolipoprotein mimetic peptides measured by reduction in turbidity at 600nm (**A**). Sphingomyelin efflux from ASMD fibroblasts after 24-hour treatment with 10μM or 100μM peptide (**B**). Viability of ASMD patient cells following 24-hour treatment with peptides. (**C**). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, n.s. not significant. N=3

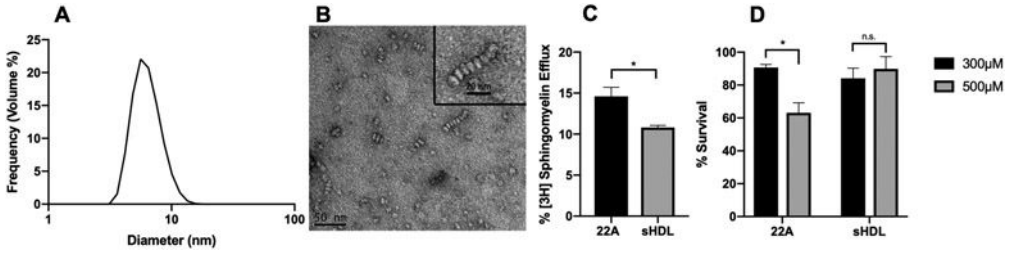

Figure 2:

Comparing sHDL to free peptide *in vitro*. Size distribution of sHDL nanodiscs measured by dynamic light scattering (DLS) (**A**). Morphology of sHDL nanodiscs observed using negative-stain TEM (**B**). Sphingomyelin efflux comparison in ASMD skin fibroblasts following 24-hour treatment with free 22A peptide or 22A-DMPC (2:1) sHDL at 10μM peptide concentration (**C**). Cell viability following 24-hour treatment with free 22A peptide or sHDL at 300μM and 500μM (**D**). *$p<0.05$, n.s. not significant. N=3

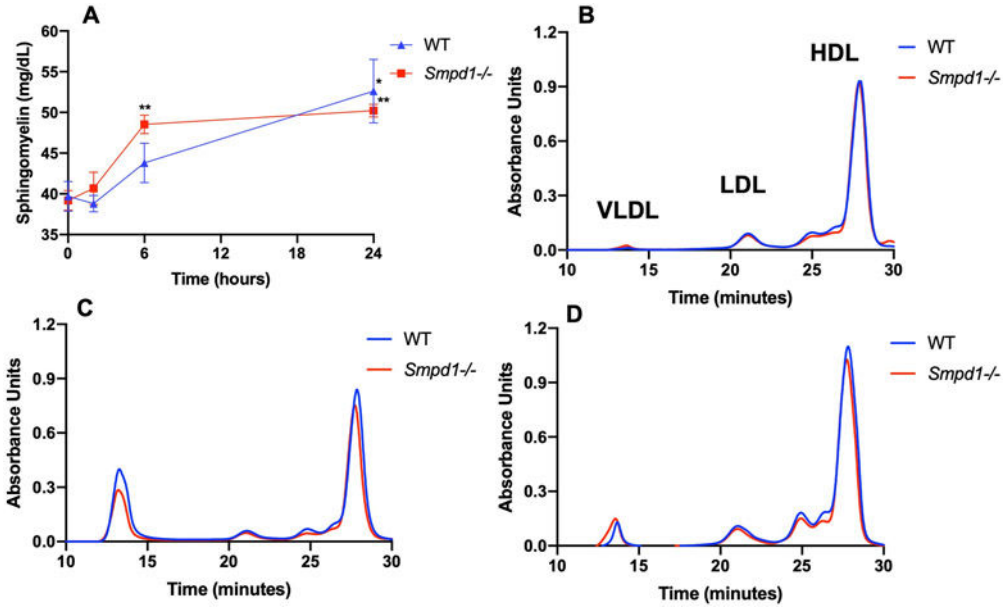

Figure 3:

Single dose of sHDL nanodiscs increases circulating levels of sphingomyelin and alters lipoprotein profile in WT and *Smpd1*−/− mice. Mice were injected I.P. with 100mg/kg of sHDL and serum was analyzed at 2, 6, and 24 hours post-injection for sphingomyelin levels (**A**). Distribution of lipoproteins in serum for WT and *Smpd1*−/− mice prior to treatment with sHDL (**B**), 6 hours after sHDL injection (**C**), and 24 hours after injection (**D**) was evaluated using SEC with detection at 265nm. *p<0.05. **p<0.01 compared to initial SM levels. N=3

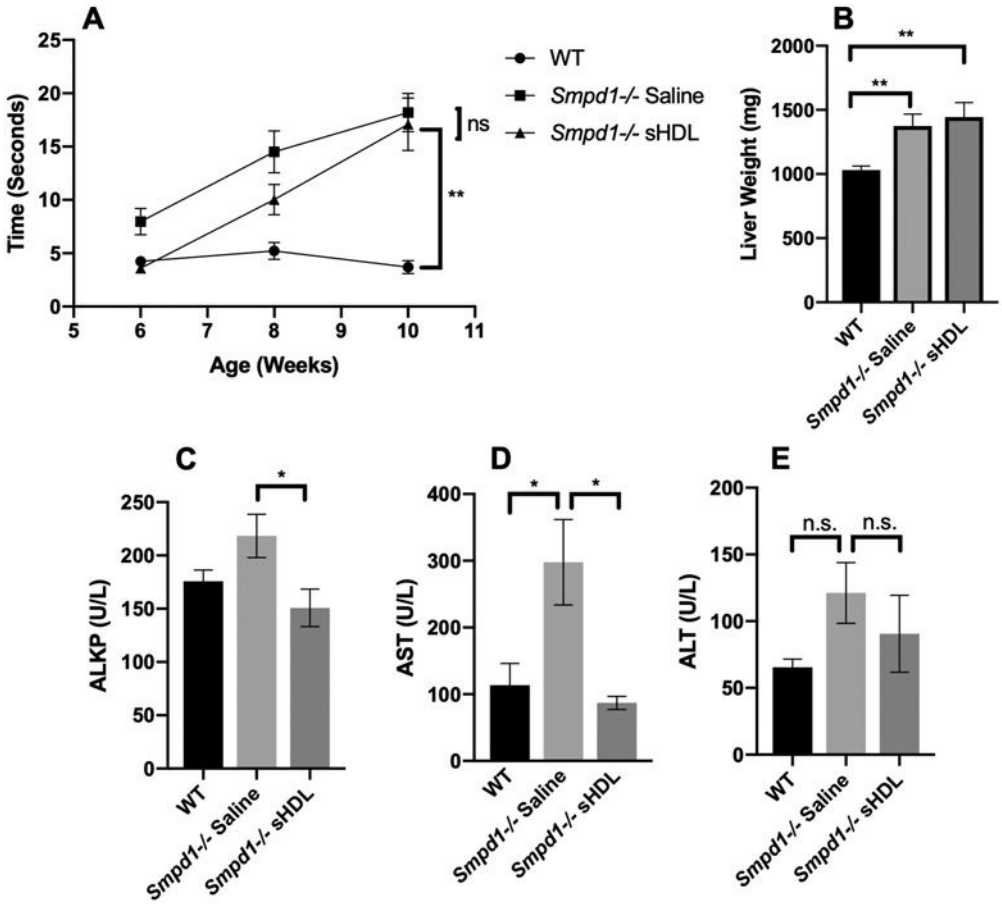

Figure 4:

Effects of repeated dosing of sHDL in *Smpd1−/− mice*. *Smpd1*−/− or WT mice were given daily injections of saline or 100mg/kg sHDL I.P. for 4 weeks starting at 6 weeks of age. The motor deficits of mice were evaluated via time to traverse a balance beam (A). Livers were weighed at the end of treatment (B). Serum was collected at the end of treatment and analyzed for ALKP (C), AST (D), and ALT (E). N=6-11 mice per group. Error bars are SEM. *p<0.05, **p<0.01