Trappsol® Cyclo(TM) hydroxypropyl beta cyclodextrin administered intravenously in patients with Niemann-Pick type C disease reduces cholesterol in liver tissue

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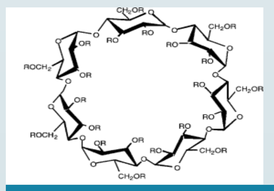
Abstract

Niemann-Pick disease type C (NPC) is a rare and often fatal genetic disorder characterized by cholesterol accumulation in the lysosomes of cells comprising the liver, lung, spleen, brain and other organs. Hydroxypropyl beta-cyclodextrins (HPβCDs) have been shown in pre-clinical studies to release cholesterol from cells, normalize cholesterol homeostasis, delay symptom onset, and increase lifespan. Here we present data from the first 8 NPC patients participating in "A Phase I study to evaluate the single and multiple-dose pharmacokinetics of intravenous Trappsol®Cyclo™ HPβCD in patients with Niemann-Pick disease type C (NPC-1) and the effects of dosing upon biomarkers of NPC disease" (NCT02939547). Trappsol®Cyclo™ is the proprietary formulation of HPβCD of Cyclo Therapeutics, Inc. The trial is randomized, double-blinded, with no control group. Subjects received 7 doses at either 1500 mg/kg body weight or 2500 mg/kg over 8 to 9 hours twice monthly. Biopsy tissue from skin and liver were obtained at baseline and (after the 7th dose). Eight micron sections were stained with filipin, a fluorescent marker which binds to unesterified cholesterol, with H&E, or control buffer. Quantification of filipin fluorescence was analyzed using a custom algorithm and standards for no change, or mild, moderate or marked reduction in staining of tissue. Results showed that filipin staining in liver tissue in 5 of 8 subjects had marked reductions in filipin staining, 2 with moderate reductions and 1 with mild reduction. Filipin staining in skin tissue fibroblasts showed essentially no change. These are the first results showing that Trappsol®Cyclo™ HPβCD reduces unesterified cholesterol accumulation in liver tissue of NPC patients. These results are consistent with previously presented biochemical data showing that Trappsol®Cyclo™ HPβCD decreases cholesterol synthesis and increases cholesterol metabolism (WORLD Poster 2019). A more complete interpretation of the present data will be possible at study unblinding.

Introduction

Niemann-Pick disease type C (NPC) is a rare and often fatal genetic disorder characterized by cholesterol accumulation in the lysosomes of cells comprising the liver, lung, spleen, brain and other organs. Hydroxypropyl beta cyclodextrins (HPβCDs) have been shown in pre-clinical studies to release cholesterol from cells, normalize cholesterol homeostasis, delay symptom onset, and increase lifespan. Here we present data from the first 8 NPC patients participating in "A Phase I study to evaluate the single and multiple-dose pharmacokinetics of intravenous Trappsol®Cyclo™ HPβCD in patients with Niemann-Pick disease type C1(NPC-1) and the effects of dosing upon biomarkers of NPC disease" (NCT02939547). Trappsol®Cyclo™ is the proprietary formulation of HPβCD of Cyclo Therapeutics, Inc.



β-Cyclodextrin, R=H
HPβCD, R=$OCH_2CH(CH_3)OH$ or H

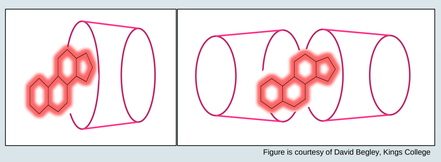


Figure is courtesy of David Begley, Kings College

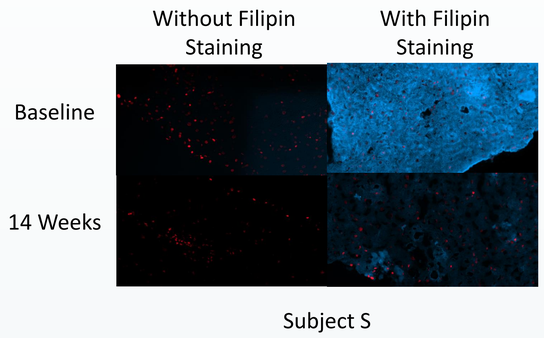
This schematic represents interaction of cylinder shaped cyclodextrins and cholesterol in a 1:1 or 1:2 ratio

Methodology

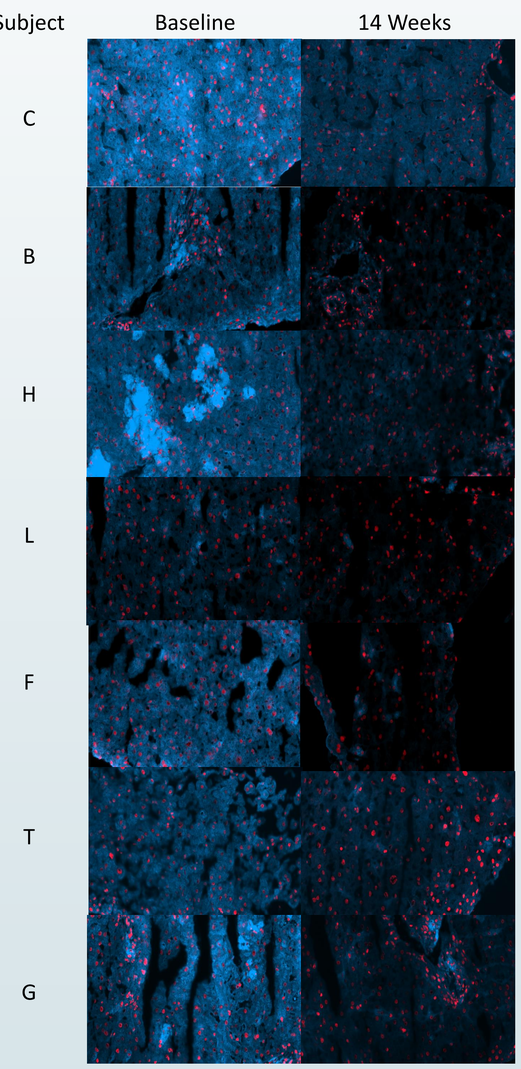
The trial was a randomized, double-blinded study with no control group. Subjects received 7 doses of study drug intravenously at either 1500 mg/kg body weight or 2500 mg/kg body weight over 8 to 9 hours twice monthly. Biopsy tissue from skin and liver were obtained at baseline and after the 7th dose.

Biopsy tissue was flash frozen, stored and shipped at -80C to HistologiX Ltd., Nottingham, UK for sample preparation and analysis. Eight-micron sections were stained with Filipin III (Sigma), a marker which fluoresces on binding to unesterified cholesterol, with H&E, or buffer. To assist in quantification of signal, a red nuclear binding fluorescent stain was added. Samples were digitally scanned at x20 using Zeiss AxioScan whole fluorescent slide scanner. Quantification of fluorescence was analyzed using Indica Labs HALO® image analysis and a custom algorithm including standards marked, moderate, mild or no change in signal.

Results



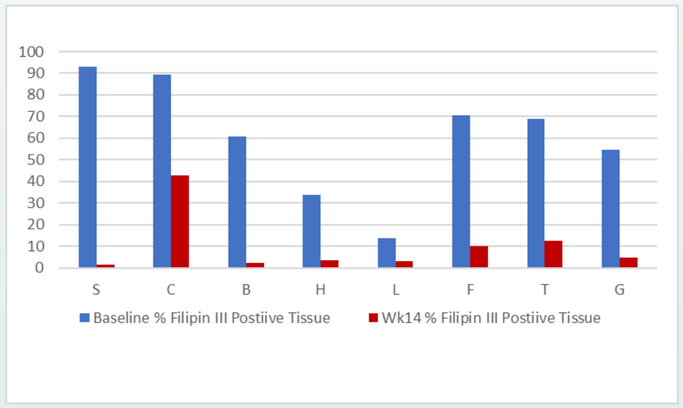
Subject S



Changes in Filipin III Staining of Liver Tissue

Patient No	Treatment Phase	Area of Tissue with Filipin III Staining (%)	Percentage Decrease in Filipin Staining	Area of Tissue with No Filipin III Staining (%)	Increase in Tissue Area Staining Negative for Filipin III
S	Pre-Treatment	93.0		7.0	
	Post-Treatment	1.4	98%	98.6	91.6
C	Pre-Treatment	89.3		10.7	
	Post-Treatment	42.9	52%	57.1	46.4
B	Pre-Treatment	60.7		39.3	
	Post-Treatment	2.3	96%	97.7	58.5
H	Pre-Treatment	33.7		66.3	
	Post-Treatment	3.6	89%	96.4	30.1
L	Pre-Treatment	13.7		86.3	
	Post-Treatment	3.1	78%	96.9	10.6
F	Pre-Treatment	70.7		29.3	
	Post-Treatment	10.1	86%	89.9	60.6
T	Pre-Treatment	68.8		31.2	
	Post-Treatment	12.5	82%	87.5	56.2
G	Pre-Treatment	54.8		45.2	
	Post-Treatment	4.9	91%	95.1	49.9

Tissue Area Percentage staining for Filipin III in Liver Core Biopsies from NPC-1 Patients at Baseline and 14 Weeks Post HPβCD Treatment



Visual inspection of the change in filipin staining in subjects S, B, H, L and G (panels to left) showed a marked reduction in filipin staining after 7 doses of the study drug. Subjects F and T showed a moderate reduction and Subject C showed a mild reduction.

Image analysis using a customized algorithm showed that the areas of liver tissue negative for filipin III staining increased an average of 50.5% (range of 10.6 to 90.6%). See Table and Graph above. As well, tissue staining positively for filipin III showed dramatic changes with an average change of 84% (range 52 to 98%)

Every subject showed a decrease in filipin staining as compared to baseline. These results demonstrate that successive doses of Trappsol® Cyclo™ at as low as 1500 mg/kg when administered intravenously reduces cholesterol in liver tissue of NPC patients.

On unblinding of this study, additional insights will be gained on whether the high or low dose had greater effect on reducing cholesterol in liver tissue, alterations of cholesterol biomarkers, and on clinical outcome measurements.

Conclusions

There is a decrease in filipin binding in liver tissue after 7 doses of intravenous Trappsol®Cyclo™ HPβCD in every subject in both dosing cohorts. It is not known if the variability in patients is related to dosing.

These results are consistent with our prior data showing that Trappsol®Cyclo™ HPβCD decreases cholesterol synthesis and increases cholesterol metabolism (WORLD Poster 2019.) This is consistent with the mechanism of action of Trappsol®Cyclo™ HPβCD in pre-clinical animal studies.

A more complete interpretation of the data will be possible at study unblinding.

Acknowledgements

We are grateful to all of the patients and families who participated in this trial. Without their energy and commitment to the trial, these findings would not have been possible.

Cyclo Therapeutics, Inc.