Effect Of The ABCA1 Agonist CS-6253 On Amyloid β And Lipoproteins Metabolism In Cynomolgus Monkeys.

Sassan D. Noveir
University of Southern California
Bilal E. Kerman
University of Southern California
Haotian Xian
University of Southern California
Cristiana Meuret
University of Southern California
Sabrina Smadi
University of Southern California
Ashley E. Martinez
University of Southern California
Johannes Johansson
Artery Therapeutics, Inc.
Henrik Zetterberg
Centers for Disease Control and Prevention
Bryan A. Parks
Centers for Disease Control and Prevention
Zsuzsanna Kuklenyik
Centers for Disease Control and Prevention
Jan O. Johansson
Artery Therapeutics, Inc.
Hussein N. Yassine (hyassine@usc.edu)
University of Southern California

Research Article

Keywords: ABCA1, apolipoprotein E, Alzheimer's disease, CS-6253

Posted Date: February 15th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1353214/v1

Abstract

Background: Inducing brain ATP binding cassette 1 (ABCA1) activity in Alzheimer's disease (AD) mouse models is associated with improvement in AD pathology. The purpose of this study was to investigate the effects of the ABCA1 agonist peptide CS-6253 on amyloid-β peptides (Aβ) and lipoproteins in plasma and cerebrospinal fluid (CSF) of cynomolgus monkeys, a species with amyloid and lipoprotein metabolism similar to humans.

Methods: CS-6253 peptide was injected intravenously to cynomolgus monkeys at various doses in three different studies. Plasma and CSF samples were collected at several time points before and after treatment. Levels of cholesterol, triglyceride (TG), lipoprotein particles, apolipoproteins, and Aβ were measured using ELISA, ion-mobility analysis, and asymmetric-flow field-flow fractionation (AF4). The relationship between the change in levels of these biomarkers was analyzed using multiple linear regression models and linear mixed-effects models.

Results: Following CS-6253 intravenous injection, within minutes, small, plasma high-density lipoprotein (HDL) particles were increased**.** In two independent experiments, plasma TG, apolipoprotein E (apoE), and Aβ42/40 ratio were transiently increased following CS-6253 intravenous injection. This change was associated with a non-significant decrease in CSF Aβ42. Both plasma total cholesterol and HDL-cholesterol levels were reduced following treatment. Using AF4 fractionation, CS-6253 treatment displaced apoE from HDL to intermediate-density- and low density-lipoprotein (IDL/LDL) sized particles in plasma**.** In contrast to plasma, CS-6253 had no effect on the assessed CSF apolipoproteins or lipids.

Conclusions: These findings support that inducing systemic ABCA1 activity by CS-6253 shifts apoE into larger triglyceride-rich lipoprotein (TRL) particles that assist in Aβ clearance from the brain.

Introduction

Deposition of extra-cellular amyloid-β peptides (Aβ) plaques in the brain is a feature of Alzheimer's disease (AD) pathology as Aβ monomers can aggregate, which form fibrils and senile plaques [1-3]. The ratio of Aβ42 to Aβ40 in plasma is a promising biomarker for selecting patients with brain amyloid accumulation. Low plasma Aβ42/40 ratio has been associated with increased risk of dementia, more pronounced decline in cognitive function, and increased fibrillary Aβ deposition in the brain [4-6]. Aβ plaque brain deposition has been linked to cholesterol and lipid metabolism, both in the brain and in the periphery. In the brain, neuronal production of Aβ is controlled by membrane cholesterol content. Cholesterol content of neurons is kept low, inhibiting Aβ accumulation [7]. In the periphery, Aβ-binding ligands can promote Aβ efflux from the brain by sequestering it into the peripheral circulatory system. Examples of Aβ-binding ligands include Aβ antibodies and lipoproteins containing apolipoprotein E (apoE), apolipoprotein A-I (apoA-I) or apolipoprotein C-III (apoC-III) [8-11]. Once in the cerebrospinal fluid (CSF), Aβ can cross into plasma, where it can bind to high density lipoprotein (HDL) and very-low density lipoprotein (VLDL) particles containing apoE and apoC-III [12].

The activation of the ATP binding cassette 1 (ABCA1) participates in the formation of HDL particles and in the clearance of Aβ from the brain [13]. Recently, studies using CS-6253, an alpha-helical peptide designed from the C-terminus of apoE to induce ABCA1 activity, have shown promising results in reducing AD-related pathology in animal models [14]. With a greater binding affinity to ABCA1 than apoE, CS-6253 prevents ABCA1 degradation by stimulating ABCA1 recycling to the cell membrane which is associated with augmented cholesterol efflux to primarily apoE acceptor particles [13, 15]. Consistent with ABCA1 regulating lipidation of apoE, treatment of apoE4-targeted replacement (ApoE4-TR) mice with the ABCA1 agonist, CS-6253, increased apoE4 lipidation. This was accompanied by a reversal of apoE4-related cognitive and brain pathologies, including intraneuronal Aβ42 accumulation [14]. These findings were associated with an increase in plasma apoE concentrations but no change in brain apoE concentrations [16]. Furthermore, in a similar model, CS-6253 decreased apoE4 and ABCA1 aggregation in hippocampal homogenates of ApoE4-TR mice [13], supporting the importance of apoE lipidation in preventing its aggregation.

The effect of CS-6253 on plasma and CSF lipoproteins, together with measures of Aβ in primates, have not yet been studied. We hypothesized that ABCA1 activation by CS-6253 by virtue of reverse cholesterol transport and HDL particle generation would influence lipoprotein dynamics, including that of apoE particles, to promote Aβ clearance. We tested this hypothesis in monkeys, as part of the CS-6253 IND-enabling toxicology studies, in three cynomolgus monkey studies: the preliminary pharmacokinetics (PK) assessment study, the 10-day non-Good Laboratory Practice (GLP) dose-range finding (DRF) study, and the 30-day GLP study.

Methods

Study designs

The preliminary PK study included 2 cynomolgus monkeys, each injected intravenously with a single dose of 25 mg/kg CS-6253. Blood samples were taken pre-injection, baseline, and at 5 min, 30 min, 1 h, 2 h, 4 h, 6 h, 12, 24 h, 48 h, and 72 h post-injection. The DRF study included three active CS-6253 groups: 75mg/kg (low-dose), 150mg/kg (mid-dose), and 225mg/kg (high-dose) and a placebo group (2 male cynomolgus monkeys and 2 female cynomolgus monkeys in each of the 4 groups). Monkeys were dosed every other day for a total of 5 times and blood samples were taken at baseline (2 weeks pre-injection), and at 10 min, 2 h, 4 h, 12 h, 24 h, and 48 h on days 1 and 9. Baseline measurements were used for normalization of the measurements. In the 30-day GLP study dosing study CS-6253 10mg/kg (low-dose), 25mg/kg (mid-dose) and 75mg/kg (high-dose) was injected every other day (QAD). The CS-6253 75mg/kg high-dose and placebo groups consisted of 10 (5 male and 5 female) cynomolgus monkeys each. The CS-6253 10mg/kg and 25mg/kg groups consisted of 6 (3 male and 3 female) monkeys each. Injections were done every other day for 28 days and animals terminated 2 days after the last injection. Blood samples were collected at 5 min post-injection on days 1 and 25 and 48 h post-injection on days 3 and 27 in the placebo group. Blood samples were collected at 5 min and 4 h post-injection on days 1, 9, and 25 and 48 h post-injection on days 3, 11, and 27 for the active treatment groups. The measurements

were normalized to the first reading that was taken at 5 min after the first injection. See also Table S1 for details of each study. All experimental procedures were conducted according to the approved protocols from the relevant institutions: PK study, BTS research, OWAL Assurance ID:D16-00768 (A4519-01) - IACUC: 19-023; DRF study, BASI / Inotiv, OWAL Assurance ID: D16-00571 (A4058-01) - IACUC: 03-MK-2019; GLP study, Altasciences, OWAL Assurance ID: D16-00639 (A4261-01) - IACUC: 147820-01. The experiments at USC were approved by IACUC, protocol #21225.

Amyloid related measurements in plasma and CSF

Plasma and CSF were collected and Ab42 and Ab40 concentrations were measured by sensitive SIMOA ELISA technique (Quanterix Corp., Billerica, MA, USA). CSF APP and AP2B1 concentrations were measured by ELISA [17].

ApoE measurements in plasma

DRF study plasma samples were diluted 1:5000 and GLP study plasma samples were diluted 1:15000. ApoE levels were measured using Sandwich ELISA. The readings were analyzed using Myassays Four Parametric Logistic Curve.

Plasma triglyceride, cholesterol, and pre-β-HDL measurements

Plasma triglyceride levels in the DRF study were measured using the L-Type Triglyceride M test (Fujifilm) according to manufacturer's instructions. Samples were diluted three times before the measurement. Total cholesterol levels in plasma were measured using Cholesterol E kit (Fujifilm). HDL cholesterol levels were measured using HDL-Cholesterol E kit (Fujifilm). Plasma triglyceride, HDL, LDL and total cholesterol levels in the GLP study samples were measured by IDEXX Laboratories. Data was analyzed using linear quantification. The plasma from the monkeys in the PK study were diluted 1:50 and pre-Beta HDL levels were measured using pre-β1 HDL ELISA kits (Daiichi Pure Chemicals, Inc.) according to manufacturer's instructions. The data was analyzed using Myassays Four Parametric Logistic Curve.

Plasma dextran sulfate lipoprotein preparation

In a 96-well round-bottom plate compatible with an accompanying magnetic separator (EpiGentek, Cat. # Q10002-1), an aliquot (30 µL) of plasma from each timepoint was mixed with 70 µL of a primary precipitating solution, then incubated on ice for 15 min. The samples were then centrifuged (2000RCF, 10 min, 4 ℃). The resultant supernatants were mixed in equal proportion with a secondary precipitating solution containing dextran sulfate and incubated at r.t. for 3 min. A volume of 20 µL of magnetic beads (Sigma, Cat. # GE24152105050250) solution [3.5] was added to each suspension, then incubated at r.t. for 3 min. The beads were washed with MQ water (60 µL 2) following a magnetic pulled down. The beads were then washed with 30 µL of releasing buffer (2), subjected to a magnetic pulled down, and the supernatants were pooled for analysis.

Ion-mobility analysis

Monkey plasma samples treated with dextran sulfate were introduced into a charge-reducing electrospray (TSI Inc., model 3482) every 13 min by automated loop injections via an integrated autosampler (Teledyne CETAC Technologies, model MVX-7100). Electrospray settings were as follows: voltage 2.0 kV, CO_2 flow 0.15 slmp, and airflow 1.5 slmp. The differential mobility analyzer (TSI Inc., model 3085), coupled to a condensation particle counter (TSI Inc, model 3788), scanned particles 4.45 to 63.8 nm for 180 s. The generated data of interest was analyzed on Fityk (version 1.3.1), as previously described, and graphed using OriginPro software (version 2021). Voigt probability distribution curves were generated from particle count (#/mL) vs diameter range for LP subclasses and normalized by dividing sub-classes with the sum of peak areas from all LPs present within the spectrum.

Isolation and examination of CSF and plasma lipoprotein fractions using AF4, MRM, and DLS

CSF and plasma samples from the DRF study were sent to the CDC Division of Laboratory Sciences. 50 µL of each plasma sample, at three time points (5 min, 4 h and 12 h) from two monkeys each, was injected into the asymmetric-flow field-flow fractionation (AF4) system, collecting a set of 40 fractions from each sample. The fractions were analyzed by three LC-MS/MS methods using multiple reaction monitoring (MRM) as described elsewhere[18-20], quantifying proteins typically detected in HDL subclasses, main non-polar lipids (free cholesterol and cholesteryl ester), and phospholipid classes (PC, SM, LPC, PE and PI). Particle sizes in the fractions were determined using dynamic light scattering (DLS) as previously described [21]. The moles of analytes in the sized fractions were divided by the volume of plasma injected into the AF4 channel, giving equivalent analyte concentrations in plasma.

Statistical Analysis

We used multiple linear regression models and linear mixed-effects models to analyze the relationship between the change in levels of various biomarkers (on a percentage scale with baseline values of 100%) and the effect of CS-6253 injections over time. Multiple linear regression models were used on those datasets from the DRF study with only two measurement time points per monkey subject. These models were estimated using ordinary least squares fitting. In each model, the main outcome of interest was modeled as a function of dose and treatment, with an interaction term of treatment and dose. Linear mixed-effects models were used on datasets from both the DRF and GLP studies, where each monkey subject had repeated measurements throughout the study. These mixed-effects models were estimated using restricted maximum likelihood fitting. In each mixed-effects model, the main outcome of interest was modeled as a function of fixed effects such as treatment, total time, hours since injection, and an interaction term of treatment and hours since injection, and a random effect of subject as a random intercept. All data were standardized as needed to obtain standardized parameters. Wald approximation was used to obtain p-values and confidence intervals. A p-value of less than 0.05 was considered significant. All models were evaluated for assumptions of normality and homoscedasticity using residual plots. Statistical analyses were done using the lme4 package (Bates, Maechler & Bolker, 2012) in R version 4.0.5 (R Core Team, 2012).

Results

Increase in plasma amyloid-β 42/40 ratio following treatment with CS-6253

In the case of the 30-day GLP study, an increase in the plasma Aβ42/40 ratio following injection of CS-6253 was observed compared to the placebo group (Fig. 1). The change was statistically significant at 4 h post-injection (p.i.). Although a trend of increase was observed, the increase at 48 h p.i. was not significant (Fig. 1A). Both Aβ42 and Aβ40 levels in plasma were lower in the treatment groups compared to controls while, the decrease was more pronounced for Aβ40 resulting in the observed increase in the ratio (Fig. S1). In the case of the DRF study, Aβ42 and Aβ40 levels increased in plasma 6 hours p.i. compared to pre-treatment (Fig S2A-B). The Aβ42/40 ratio in the treatment groups also increased compared to the controls after 6 h p.i. on day 9 (Fig. S2C). Taken together, these findings suggested that CS-6253 transiently increased the plasma Aβ42/40 ratio within 4 to 6 h p.i.

Changes in CSF Aβ and lipoprotein levels following treatment with CS-6253

Interestingly, the Aβ42/40 ratio in the CSF of monkeys in the DRF study did not change significantly (Fig. S2F), despite accompanied by a dose-response but non-significant decrease in both Aβ42 and Aβ40 levels (Fig S2D-E). Moreover, Amyloid β precursor protein (APP) levels in the CSF of monkeys in the DRF study had a noticeable but statistically non-significant decrease after CS-6253 treatment (Fig. S2G). This change in APP levels correlated with CSF Aβ42 level changes (Fig. S2H). Previously, endo-lysosomal protein AP2B1 was found to increase in AD patients CSF [17]. In our study, similarly to APP, there was a lowering dose-response trend for CSF AP2B1 levels after CS-6253 treatment, but the results were not statistically significant (Fig. S2I), likely due to the small sample size. This change in CSF AP2B1 levels correlated directly with changes in Aβ42 levels (Fig. S2J). CSF lipoprotein and lipid levels did not change (data not shown).

Increase in plasma apoE following treatment with CS-6253

CS-6253 treatment increased apoE levels in the plasma of monkeys in the GLP study. This effect was statistically significant at 48 h p.i. (Fig 2A). In the DRF study with the higher CS-6253 doses, the results were more complex. Initially, apoE levels decreased some in the plasma after CS-6253 treatment but starting at 12 h p.i., apoE increased significantly (Fig 2B). Overall, CS-6253 treatment increased apoE levels in the plasma. In the PDAPP transgenic mouse model of AD, ApoE4-TR mice had lower CSF and plasma levels of apoE compared to ApoE2-TR and ApoE3-TR mice accompanied with increased amyloid deposition in the brain [22]. Similarly, plasma, but not CSF, apoE levels were lower in apoE4 carriers compared to non-carriers [23] and lower apoE in plasma is associated with increased AD risk [24-26]. Thus, increasing plasma apoE levels via CS-6253 may have therapeutic benefit in AD.

Changes in plasma lipid levels following treatment with CS-6253

Induction of cholesterol efflux from macrophages by activation of ABCA1 is rate-limiting for reverse cholesterol transport, i.e. the efflux of excess cholesterol from peripheral tissues to be transported via

plasma to the liver for biliary excretion [27]. Excess cholesterol synthesized in the brain is also removed into the periphery through plasma [28]. Thus, we analyzed if CS-6253 treatment effected cholesterol levels in the plasma. CS-6253 significantly decreased plasma cholesterol levels in both DRF and GLP Studies (Fig. 3A and B). This decrease in total cholesterol (total-C) levels was also reflected by a decrease in HDL-cholesterol (HDL-C) levels (Fig 3C and D). Interestingly, the HDL-C levels followed a periodic pattern decreasing after injection and increasing back at 48 h p.i. (Fig. 3D). Taken together, these results suggested that CS-6253 treatment lowered both total-C and HDL-C in plasma. The relationship between cholesterol lowering drugs and AD is complicated. While in some studies lowering plasma cholesterol levels via statins or other interventions reduced AD risk in others it had no effect [28, 29]. Regardless, in line with our findings, many in vitro and in vivo studies showed that lowering cholesterol is associated with increased apoE and decreased Aβ deposition [28, 29].

Additionally, we analyzed the plasma HDL-C/apoA-I ratio, an indicator of HDL particle size, after CS-6253 treatment in the GLP study. It has been shown that small particles distribute more in the extra-vascular space than larger particles, which may be of interest for tissue penetration of apoA-I, a natural ABCA1 agonist. A sharp decrease in the HDL-C/apoA-I ratio was observed at 4 h p.i. which increased later (Fig S3). Accumulated effect of CS-6253 was also significant. Thus, CS-6253 decreased plasma HDL-C/apoA-I ratio. Interestingly, treatment groups in the GLP study did not appear to have significant differences in plasma LDL cholesterol levels compared to the control group (Fig S4).

Besides cholesterol, apolipoprotein particles carry triglycerides as a part of the tissue lipid homeostasis [30]. Therefore, we analyzed CS-6253's effect on plasma triglyceride levels. The triglycerides in plasma increased significantly following CS-6253 injection and waned at about 24 h p.i. in the DRF study (Fig 4A). This peak in triglycerides was reproduced in the GLP study, although it was less pronounced (Fig 4B). Thus, continued CS-6253 treatment can maintain increased plasma triglyceride levels. Increase in triglycerides may reflect increased plasma apoE levels (Fig. 2). This effect might have been especially more pronounced in cynomolgus monkeys, whose apoE is more similar to human apoE4 than the other alleles [31], because apoE4 has higher affinity for VLDL [30].

Analysis of pre-β HDL and s-, m-, and l-HDL particles

Furthermore, we analyzed pre-β HDL and small- (s-), medium- (m-), and large- (l-) HDL particle numbers in plasma in the PK and DRF Studies. To extract pre-β HDL and s-, m-, and l-HDL particle concentrations from the calibrated ion-mobility analysis, the concentration vs. size profiles were deconvoluted into Voigt probability distribution peaks. In addition, the baseline data was compared to published concentrations (Fig S5, Table S2) [32]. The calculated size distribution and range for each particle are given in Fig S5B. Pre-β HDL is a natural ABCA1 agonist and lipidation of pre-β HDL particles is one of the first steps in reverse cholesterol transport [33]. Pre-β HDL levels in plasma increased following CS-6253 injection but waned down as shown by an ELISA assay (Fig. 5A) and by ion-mobility analysis (Fig 5B and D). The baseline s-, m-, and l-HDL particle concentrations in monkey plasma were similar to human plasma levels [32]. Total HDL particle concentrations for monkeys both in the DRF and the PK studies increased soon

after injection and started going down (Fig 5C and E). There was general trend for decreasing l-HDL particle levels especially in the DRF study monkey plasma; thus, the change in total HDL was largely driven by changes in s- and m-HDL levels (Fig 5C and E and Table S2). Moreover, the trend in decreasing l-HDL in the DRF study is in parallel to the decreasing HDL-C levels given that l-HDL particles are the major carriers of cholesterol [32, 34]. We also, analyzed IDL, LDL, Midzone, and VLDL particles for one sample in both studies (Fig. S55). The VLDL levels in the DRF study followed a similar trend to the plasma triglyceride levels in the same study (compare Fig 4A and Fig S6B). This trend is in line with previous findings [35].

Shift of apoE from HDL to IDL/LDL particles

Finally, we analyzed how the relative distribution of apoE within lipoprotein particles changes after CS-6253 injection using AF4. The total apoE and triglyceride levels, measured by mass spectrometry, followed a similar pattern to our earlier measurements with ELISA (compare Fig. S7A to Fig. 2B and Fig S7B to Fig 4A). Interestingly, AF4 analysis revealed that at 10 min after CS-6253 injection, apoE was mainly on the s- and m-HDL particles (Fig 6A) but apoE shifted to the LDL and IDL particles at 4 h p.i. and was mostly present in these larger particles at 12 h p.i. (Fig 6A). In contrast, apoA-I's abundance shifted to smaller sized HDL particles. The relative distribution of other apolipoproteins analyzed including apoC-III was not changed among the fractions (data not shown). Relative abundance of triglyceride in the LDL and IDL particles increased at 12 h p.i. (Fig 6B) reflecting shift in apoE distribution, in line with increased triglyceride levels following increased apoE levels (Fig 2B, 4A, and S7). It is possible that the shift of apoE to larger particles in combination with increased triglycerides in these particles can absorb excess Aβ from the CSF and carry it to the liver for clearance [36].

Discussion

In this study, cynomolgus monkeys were treated with CS-6253 as part of IND enabling studies and its effects on lipid metabolism and AD biomarkers were assessed in plasma and CSF. Since aggregation of Aβ in the brain contributes to the pathogenesis of AD, Aβ-related biomarkers are used for selecting the prodromal stages of this neurodegenerative disease [37, 38]. Particularly, recent studies have identified lower plasma Aβ42/40 ratio as a predictor of brain amyloidosis [4-6]. Accordingly, plasma Aβ42/40 ratio were used to test the effectiveness of CS-6253. Indeed, treatment with CS-6253 increased the plasma Aβ42/40 ratio, suggesting CS-6253 was able to facilitate Aβ brain to plasma flux in cynomolgus monkeys. These results are consistent with previous studies in apoE4-targeted replacement mice, which showed that CS-6253 can counteract Aβ42 accumulation in hippocampal neurons and improve behavioral deficits [14].

CS-6253 treatment did not have a significant effect on CSF lipoproteins or lipids. A possible explanation for these findings follows the Peripheral-Sink Hypothesis[39], which postulates that Aβ-binding ligands in the periphery sequester Aβ, promoting efflux of Aβ from the CSF to the periphery. This aligns with the present study's finding that CS-6253 was able to simultaneously increase Aβ42 concentrations in plasma

and decrease them in CSF. Studies have shown support for this hypothesis, showing that increasing peripheral Aβ antibodies and Aβ-binding lipoproteins increase Aβ efflux [8, 9] through LRP1 [10, 11]. As Aβ is highly lipophilic, the majority of Aβ1-40 and Aβ1-42 in the circulation are bound to lipoproteins, particularly triglyceride-rich lipoproteins (TRLs)[40]. Since apoE plays an important role in lipoprotein association with Aβ, with apoE-containing human plasma lipoproteins able to absorb excess Aβ [36]. It is likely then that Aβ may cross into the periphery with an increase in plasma apoE in TRLs. Indeed, the present report found that CS-6253 consistently caused a transient increase in plasma apoE concentrations in TRL particles. These results are favorable, as low plasma apoE levels are associated with increased risk of AD [24-26]. The transient nature of the plasma apoE and Aβ42/40 ratio increase may be explained by liver uptake of Aβ42 containing apoE particles by apoE receptors such as LRP1[41], thus forming a vector from the brain, then to plasma, and finally to the liver for degradation or excretion.

In the periphery, apoE plays an important role in reverse cholesterol transport [42, 43]. In plasma, both apoE and apoA-I receive cholesterol and phospholipids from the plasma membrane of peripheral cells, via ABCA1, a process most pronounced in monocyte-macrophage cells. This reverse cholesterol transport results in the formation of HDL particles, which transport excess cholesterol to the liver for secretion [44]. In addition to demonstrating vasoprotective functions, plasma HDL particles have been implicated in protecting from AD [45, 46]. Low plasma HDL cholesterol levels have been linked to greater cerebral Aβ deposition [47]. Intravenous administration of HDL has been shown to reduce soluble levels of Aβ in the brain [48]. Levels of plasma apoA-I and apoE, which are components of plasma HDL, are lower in AD patients [47, 49-52]. Isolated apoA-I binds to Aβ peptide and can prevent Aβ-induced toxicity and Aβ aggregation [53, 54]. Furthermore, plasma lipoproteins have been linked to the transport and clearance of Aβ from the brain [55]. Interestingly, adding CS-6253 to plasma has been shown to displace apoA-I from alpha-HDL particles, and stimulate the formation of pre-β HDL [15]. CS-6253 mimics apoA-I's ability to interact with ABCA1 to form functional, so-called nascent HDL particles that are actively remodeled in plasma [15]. The capacity of CS-6253 to compete with other apolipoproteins such as apoE remains to be delineated.

The results of the present study validate previous findings in vitro which demonstrate the ability of CS-6253 to induce formation of pre-β HDL in plasma [15]. Treatment with CS-6253 increased plasma pre-β levels as soon as 5 minutes following injection. Plasma pre-β plays an important role in reverse cholesterol transport, as it efficiently stimulates ABCA1-dependent cholesterol efflux. This study also found that CS-6253 decreased HDL-C levels, which may account for the decrease in total-C levels. While low levels of HDL-C have been associated with negative AD outcomes, recent studies suggest that HDL particle functionality is more important than HDL-C levels [56]. It has been shown that plasma HDL-C concentrations divided by apoA-I concentrations may be a better alternative to HDL-C levels alone [57]. This may provide more information about the quality of HDL, as HDL is a dynamic and heterogeneous particle. Particularly, this ratio is thought to represent the amount of cholesterol per HDL particle. Accordingly, lower HDL-C/apoA-I ratios would represent higher number of lipid-poor HDL particles, which are better able to pick up cholesterol from peripheral tissues than cholesterol rich HDL particles. Indeed, it has been shown that individuals with lower HDL-C/apoA-I ratios had a decreased likelihood of subclinical

atherosclerosis and mortality [57]. The present study found that when accounting for apoA-I, CS-6253 was able to decrease the HDL-C/apoA-I ratio, suggesting CS-6253 increases lipid-poor HDL particles. Interestingly, the time of the HDL-C/apoA-I decrease (4 hours after treatment) correlates with the time of the plasma Aβ42/40 ratio increase. The significance of this is unclear and more work needs to be done to understand the plasma HDL-C/apoA-I in relation to AD. It is possible that exchangeable apolipoproteins such as apoE and apoA-I which are present on lipid-poor, s-HDL may enter the brain and become lipidated via ABCA1 [8, 58, 59]. This may allow for the transport of brain lipids and peripheral lipoproteins, which are important for Aβ clearance from the brain. However, we did not detect any changes in CSF lipids or apolipoproteins in this study.

Limitations

A limitation of this study was the small sample sizes, particularly for CSF studies, which explains why the changes in CSF Aβ after treatment did not reach statistical significance. We also did not characterize apoE-HDL interactions. An initial increase in apoE-HDL levels and later shift to apoE-LDL/IDL, may explain the peak in triglyceride levels following treatment with CS-6253. Higher levels of apoE-HDL have been shown to increase triglyceride levels by inhibiting displacement of hepatic lipase, an enzyme which must be liberated to hydrolyze triglycerides [60, 61]. The increase of apoE and triglycerides found in the present study suggests there may have been a substantial rise in apoE-HDL levels, but further investigation is necessary. The cooperation between apoE and HDL also has an important role in Aβ clearance. It has been shown that injecting apoE into the periphery in the presence of reconstituted HDL promoted the transport of Aβ across bioengineered cerebral blood vessels [62]. This suggests that interactions between apoE and HDL have synergistic effects on the clearance of Aβ across vasculature.

Conclusions

In spite of some limitations of the present study, on the plasma and CSF effects of the ABCA1 agonist CS-6253 in cynomolgus monkeys, the results point to significant treatment response of several lipid and AD biomarkers that, if reproduced in ensuing human studies, will allow generation of useful pharmacokinetic and pharmacodynamic relationships to guide CS-6253 drug development. The findings of study reveal for the first time, how generation of apolipoproteins by activating ABCA1 in the periphery participate in Aβ clearance.

List Of Abbreviations

ABCA1: ATP binding cassette 1

AD: Alzheimer's disease

Aβ: Amyloid-β peptides

APP: Amyloid β precursor protein

AF4: Asymmetric-flow field-flow fractionation

apoA-I: apolipoprotein A-I

apoC-III: apolipoprotein C-III

apoE: Apolipoprotein E

ApoE4-TR: apoE4-targeted replacement mice

CSF: Cerebrospinal fluid

DLS: Dynamic light scattering

DRF: Dose-range finding

GLP: Good Laboratory Practice

h: Hour

HDL: High-density lipoprotein

s-HDL: small-HDL

m-HDL: medium-HDL

l-HDL: large-HDL

HDL-C: HDL-cholesterol

IACUC: Institutional Animal Care and Use Committee

IDL: Intermediate-density lipoprotein

min: Minute

MRM: Multiple reaction monitoring

LDL: Low-density lipoprotein

p.i.: post-injection

PK: pharmacokinetics

TG: triglyceride

total-C: Total-cholesterol

TRLs: triglyceride-rich lipoproteins

VLDL: Very-low-density lipoprotein

Declarations

Ethics declarations

Ethics approval and consent to participate

This study uses monkeys and experimental protocols that have been approved by IACUC of USC (see the “Materials and Methods” section for details).

Consent for publication

Not applicable.

Availability of data and material

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study. All other data are available from the corresponding author on reasonable request.

Competing interests

JOJ, is founder and CEO of Artery Therapeutics, Inc. JOJ and JJ receive salary from and hold stock in Artery Therapeutics. JOJ is a co-inventor of CS6253 composition of matter and method of use patents. The relevant United States patent is United States 9416162 B2 with JOJ as inventor. This does not alter the authors' adherence to all the publications policies on sharing data and materials. HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, Annexon, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Pinteon Therapeutics, Red Abbey Labs, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). The authors declare no other competing financial interests.

Funding

Research reported in this publication was supported by the National Institute on Aging of the National Institutes of Health under Award Number R44AG060826 (JJ). HNY was supported by R01AG055770, R01AG054434, R01AG067063 from the National Institute on Aging. This work was also supported by P50AG05142. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. HZ is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532), the European Research Council (#681712), Swedish State Support for Clinical Research (#ALFGBG-720931), the Alzheimer Drug Discovery Foundation

(ADDF), USA (#201809-2016862), the AD Strategic Fund and the Alzheimer's Association (#ADSF-21-831376-C, #ADSF-21-831381-C and #ADSF-21-831377-C), the Olav Thon Foundation, the Erling-Persson Family Foundation, Stiftelsen för Gamla Tjänarinnor, Hjärnfonden, Sweden (#FO2019-0228), the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860197 (MIRIADE), and the UK Dementia Research Institute at UCL.

Author contributions: "S.N. and B.K. wrote the main manuscript text and B.K. prepared figures. HX analyzed the data. S.N., C.M., S.S., A.M., Z.K., B.P., H.Z. ran experiments. J.J. and H.Y. designed the study and wrote the manuscript. Jo.J. critically reviewed the literature. All authors reviewed the manuscript."

Acknowledgements

We thank Arthur Ter-Zekarian for his technical support in running the prebeta HDL assays. We thank Kevin P Bierbaum for technical assistance in performing AF4 and Michael S Gardner for support in mass spectrometry of lipids.

References

1. Haass C, Selkoe DJ: **Cellular processing of beta-amyloid precursor protein and the genesis of amyloid beta-peptide**. *Cell* 1993, **75**(6):1039-1042.

2. Glenner GG, Wong CW: **Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein**. *Biochem Biophys Res Commun* 1984, **120**(3):885-890.

3. Selkoe DJ, Hardy J: **The amyloid hypothesis of Alzheimer's disease at 25 years**. *EMBO Mol Med* 2016, **8**(6):595-608.

4. Fandos N, Perez-Grijalba V, Pesini P, Olmos S, Bossa M, Villemagne VL, Doecke J, Fowler C, Masters CL, Sarasa M *et al*: **Plasma amyloid beta 42/40 ratios as biomarkers for amyloid beta cerebral deposition in cognitively normal individuals**. *Alzheimers Dement (Amst)* 2017, **8**:179-187.

5. Giudici KV, de Souto Barreto P, Guyonnet S, Li Y, Bateman RJ, Vellas B, Group MD: **Assessment of Plasma Amyloid-beta42/40 and Cognitive Decline Among Community-Dwelling Older Adults**. *JAMA Netw Open* 2020, **3**(12):e2028634.

6. Perez-Grijalba V, Romero J, Pesini P, Sarasa L, Monleon I, San-Jose I, Arbizu J, Martinez-Lage P, Munuera J, Ruiz A *et al*: **Plasma Abeta42/40 Ratio Detects Early Stages of Alzheimer's Disease and Correlates with CSF and Neuroimaging Biomarkers in the AB255 Study**. *J Prev Alzheimers Dis* 2019, **6**(1):34-41.

7. Wang H, Kulas JA, Wang C, Holtzman DM, Ferris HA, Hansen SB: **Regulation of beta-amyloid production in neurons by astrocyte-derived cholesterol**. *Proceedings of the National Academy of Sciences* 2021, **118**(33):e2102191118.

8. Deane R, Bell RD, Sagare A, Zlokovic BV: **Clearance of amyloid-beta peptide across the blood-brain barrier: implication for therapies in Alzheimer's disease**. *CNS Neurol Disord Drug Targets* 2009, **8**(1):16-30.

9. Lemere CA, Spooner ET, LaFrancois J, Malester B, Mori C, Leverone JF, Matsuoka Y, Taylor JW, DeMattos RB, Holtzman DM *et al*: **Evidence for peripheral clearance of cerebral Abeta protein following chronic, active Abeta immunization in PSAPP mice**. *Neurobiol Dis* 2003, **14**(1):10-18.

10. Shibata M, Yamada S, Kumar SR, Calero M, Bading J, Frangione B, Holtzman DM, Miller CA, Strickland DK, Ghiso J *et al*: **Clearance of Alzheimer's amyloid-ss(1-40) peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier**. *J Clin Invest* 2000, **106**(12):1489-1499.

11. Kang DE, Pietrzik CU, Baum L, Chevallier N, Merriam DE, Kounnas MZ, Wagner SL, Troncoso JC, Kawas CH, Katzman R *et al*: **Modulation of amyloid beta-protein clearance and Alzheimer's disease susceptibility by the LDL receptor-related protein pathway**. *J Clin Invest* 2000, **106**(9):1159-1166.

12. Bell RD, Winkler EA, Singh I, Sagare AP, Deane R, Wu Z, Holtzman DM, Betsholtz C, Armulik A, Sallstrom J *et al*: **Apolipoprotein E controls cerebrovascular integrity via cyclophilin A**. *Nature* 2012, **485**(7399):512-516.

13. Rawat V, Wang S, Sima J, Bar R, Liraz O, Gundimeda U, Parekh T, Chan J, Johansson JO, Tang C *et al*: **ApoE4 Alters ABCA1 Membrane Trafficking in Astrocytes**. *J Neurosci* 2019, **39**(48):9611-9622.

14. Boehm-Cagan A, Bar R, Liraz O, Bielicki JK, Johansson JO, Michaelson DM: **ABCA1 Agonist Reverses the ApoE4-Driven Cognitive and Brain Pathologies**. *J Alzheimers Dis* 2016, **54**(3):1219-1233.

15. Hafiane A, Bielicki JK, Johansson JO, Genest J: **Novel Apo E-Derived ABCA1 Agonist Peptide (CS-6253) Promotes Reverse Cholesterol Transport and Induces Formation of prebeta-1 HDL In Vitro**. *PLoS One* 2015, **10**(7):e0131997.

16. Boehm-Cagan A, Bar R, Harats D, Shaish A, Levkovitz H, Bielicki JK, Johansson JO, Michaelson DM: **Differential Effects of apoE4 and Activation of ABCA1 on Brain and Plasma Lipoproteins**. *PLoS One* 2016, **11**(11):e0166195.

17. Sjödin S, Brinkmalm G, Öhrfelt A, Parnetti L, Paciotti S, Hansson O, Hardy J, Blennow K, Zetterberg H, Brinkmalm A: **Endo-lysosomal proteins and ubiquitin CSF concentrations in Alzheimer's and Parkinson's disease**. *Alzheimers Res Ther* 2019, **11**(1):82.

18. Toth CA, Kuklenyik Z, Jones JI, Parks BA, Gardner MS, Schieltz DM, Rees JC, Andrews ML, McWilliams LG, Pirkle JL *et al*: **On-column trypsin digestion coupled with LC-MS/MS for quantification of apolipoproteins**. *Journal of Proteomics* 2017, **150**:258-267.

19. Gardner MS, Kuklenyik Z, Lehtikoski A, Carter KA, McWilliams LG, Kusovschi J, Bierbaum K, Jones JI, Rees J, Reis G *et al*: **Development and application of a high throughput one-pot extraction protocol for quantitative LC-MS/MS analysis of phospholipids in serum and lipoprotein fractions in normolipidemic**

and dyslipidemic subjects. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences* 2019, **1118-1119**:137-147.

20. Gardner MS, McWilliams LG, Jones JI, Kuklenyik Z, Pirkle JL, Barr JR: **Simultaneous Quantification of Free Cholesterol, Cholesteryl Esters, and Triglycerides without Ester Hydrolysis by UHPLC Separation and In-Source Collision Induced Dissociation Coupled MS/MS**. *Journal of The American Society for Mass Spectrometry* 2017.

21. Kuklenyik Z, Jones JI, Gardner MS, Schieltz DM, Parks BA, Toth CA, Rees JC, Andrews ML, Carter K, Lehtikoski AK *et al*: **Core lipid, surface lipid and apolipoprotein composition analysis of lipoprotein particles as a function of particle size in one workflow integrating asymmetric flow field-flow fractionation and liquid chromatography-tandem mass spectrometry**. *PLOS ONE* 2018, **13**(4):e0194797.

22. Bales KR, Liu F, Wu S, Lin S, Koger D, DeLong C, Hansen JC, Sullivan PM, Paul SM: **Human APOE isoform-dependent effects on brain beta-amyloid levels in PDAPP transgenic mice**. *J Neurosci* 2009, **29**(21):6771-6779.

23. Martinez-Morillo E, Hansson O, Atagi Y, Bu G, Minthon L, Diamandis EP, Nielsen HM: **Total apolipoprotein E levels and specific isoform composition in cerebrospinal fluid and plasma from Alzheimer's disease patients and controls**. *Acta Neuropathol* 2014, **127**(5):633-643.

24. Rasmussen KL, Tybjaerg-Hansen A, Nordestgaard BG, Frikke-Schmidt R: **Plasma levels of apolipoprotein E and risk of dementia in the general population**. *Ann Neurol* 2015, **77**(2):301-311.

25. Rasmussen KL, Tybjaerg-Hansen A, Nordestgaard BG, Frikke-Schmidt R: **Plasma apolipoprotein E levels and risk of dementia: A Mendelian randomization study of 106,562 individuals**. *Alzheimers Dement* 2018, **14**(1):71-80.

26. Wolters FJ, Koudstaal PJ, Hofman A, van Duijn CM, Ikram MA: **Serum apolipoprotein E is associated with long-term risk of Alzheimer's disease: The Rotterdam Study**. *Neurosci Lett* 2016, **617**:139-142.

27. Xia M, Hou M, Zhu H, Ma J, Tang Z, Wang Q, Li Y, Chi D, Yu X, Zhao T *et al*: **Anthocyanins induce cholesterol efflux from mouse peritoneal macrophages: the role of the peroxisome proliferator-activated receptor {gamma}-liver X receptor {alpha}-ABCA1 pathway**. *J Biol Chem* 2005, **280**(44):36792-36801.

28. Panza F, D'Introno A, Colacicco AM, Capurso C, Pichichero G, Capurso SA, Capurso A, Solfrizzi V: **Lipid metabolism in cognitive decline and dementia**. *Brain Res Rev* 2006, **51**(2):275-292.

29. Poirier J: **Apolipoprotein E and cholesterol metabolism in the pathogenesis and treatment of Alzheimer's disease**. *Trends Mol Med* 2003, **9**(3):94-101.

30. Yassine HN, Finch CE: **APOE Alleles and Diet in Brain Aging and Alzheimer's Disease**. *Front Aging Neurosci* 2020, **12**:150.

31. Poduri A, Gearing M, Rebeck GW, Mirra SS, Tigges J, Hyman BT: **Apolipoprotein E4 and beta amyloid in senile plaques and cerebral blood vessels of aged rhesus monkeys**. *Am J Pathol* 1994, **144**(6):1183-1187.

32. Hutchins PM, Ronsein GE, Monette JS, Pamir N, Wimberger J, He Y, Anantharamaiah GM, Kim DS, Ranchalis JE, Jarvik GP *et al*: **Quantification of HDL particle concentration by calibrated ion mobility analysis**. *Clin Chem* 2014, **60**(11):1393-1401.

33. Rye KA, Barter PJ: **Formation and metabolism of prebeta-migrating, lipid-poor apolipoprotein A-I**. *Arterioscler Thromb Vasc Biol* 2004, **24**(3):421-428.

34. Mackey RH, Greenland P, Goff DC, Jr., Lloyd-Jones D, Sibley CT, Mora S: **High-density lipoprotein cholesterol and particle concentrations, carotid atherosclerosis, and coronary events: MESA (multi-ethnic study of atherosclerosis)**. *J Am Coll Cardiol* 2012, **60**(6):508-516.

35. Wahl PW, Warnick GR, Albers JJ, Hoover JJ, Walden CE, Bergelin RO, Ogilvie JT, Hazzard WR, Knopp RH: **Distribution of lipoproteins triglyceride and lipoprotein cholesterol in an adult population by age, sex, and hormone use- The Pacific Northwest Bell Telephone Company health survey**. *Atherosclerosis* 1981, **39**(1):111-124.

36. LaDu MJ, Munson GW, Jungbauer L, Getz GS, Reardon CA, Tai LM, Yu C: **Preferential interactions between ApoE-containing lipoproteins and Abeta revealed by a detection method that combines size exclusion chromatography with non-reducing gel-shift**. *Biochim Biophys Acta* 2012, **1821**(2):295-302.

37. Jack CR, Jr., Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, Petersen RC, Trojanowski JQ: **Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade**. *Lancet Neurol* 2010, **9**(1):119-128.

38. Villemagne VL, Burnham S, Bourgeat P, Brown B, Ellis KA, Salvado O, Szoeke C, Macaulay SL, Martins R, Maruff P *et al*: **Amyloid beta deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study**. *Lancet Neurol* 2013, **12**(4):357-367.

39. Van Valkenburgh J, Meuret C, Martinez AE, Kodancha V, Solomon V, Chen K, Yassine HN: **Understanding the Exchange of Systemic HDL Particles Into the Brain and Vascular Cells Has Diagnostic and Therapeutic Implications for Neurodegenerative Diseases**. *Frontiers in physiology* 2021, **12**:700847-700847.

40. Matsubara E, Sekijima Y, Tokuda T, Urakami K, Amari M, Shizuka-Ikeda M, Tomidokoro Y, Ikeda M, Kawarabayashi T, Harigaya Y *et al*: **Soluble Abeta homeostasis in AD and DS: impairment of anti-amyloidogenic protection by lipoproteins**. *Neurobiol Aging* 2004, **25**(7):833-841.

41. Tamaki C, Ohtsuki S, Iwatsubo T, Hashimoto T, Yamada K, Yabuki C, Terasaki T: **Major involvement of low-density lipoprotein receptor-related protein 1 in the clearance of plasma free amyloid beta-peptide by**

the liver. *Pharm Res* 2006, **23**(7):1407-1416.

42. Shelburne F, Hanks J, Meyers W, Quarfordt S: **Effect of apoproteins on hepatic uptake of triglyceride emulsions in the rat**. *J Clin Invest* 1980, **65**(3):652-658.

43. Yamada N, Murase T: **Modulation, by apolipoprotein E, of lipoprotein lipase activity**. *Biochem Biophys Res Commun* 1980, **94**(2):710-715.

44. Oram JF, Vaughan AM: **ATP-Binding cassette cholesterol transporters and cardiovascular disease**. *Circ Res* 2006, **99**(10):1031-1043.

45. Kingwell BA, Chapman MJ, Kontush A, Miller NE: **HDL-targeted therapies: progress, failures and future**. *Nat Rev Drug Discov* 2014, **13**(6):445-464.

46. Vitali C, Wellington CL, Calabresi L: **HDL and cholesterol handling in the brain**. *Cardiovasc Res* 2014, **103**(3):405-413.

47. Harr SD, Uint L, Hollister R, Hyman BT, Mendez AJ: **Brain expression of apolipoproteins E, J, and A-I in Alzheimer's disease**. *J Neurochem* 1996, **66**(6):2429-2435.

48. Robert J, Stukas S, Button E, Cheng WH, Lee M, Fan J, Wilkinson A, Kulic I, Wright SD, Wellington CL: **Reconstituted high-density lipoproteins acutely reduce soluble brain Abeta levels in symptomatic APP/PS1 mice**. *Biochim Biophys Acta* 2016, **1862**(5):1027-1036.

49. Kawano M, Kawakami M, Otsuka M, Yashima H, Yaginuma T, Ueki A: **Marked decrease of plasma apolipoprotein AI and AII in Japanese patients with late-onset non-familial Alzheimer's disease**. *Clin Chim Acta* 1995, **239**(2):209-211.

50. Liu HC, Hu CJ, Chang JG, Sung SM, Lee LS, Yuan RY, Leu SJ: **Proteomic identification of lower apolipoprotein A-I in Alzheimer's disease**. *Dement Geriatr Cogn Disord* 2006, **21**(3):155-161.

51. Merched A, Xia Y, Visvikis S, Serot JM, Siest G: **Decreased high-density lipoprotein cholesterol and serum apolipoprotein AI concentrations are highly correlated with the severity of Alzheimer's disease**. *Neurobiol Aging* 2000, **21**(1):27-30.

52. Gupta VB, Wilson AC, Burnham S, Hone E, Pedrini S, Laws SM, Lim WL, Rembach A, Rainey-Smith S, Ames D *et al*: **Follow-up plasma apolipoprotein E levels in the Australian Imaging, Biomarkers and Lifestyle Flagship Study of Ageing (AIBL) cohort**. *Alzheimers Res Ther* 2015, **7**(1):16.

53. Koldamova RP, Lefterov IM, Lefterova MI, Lazo JS: **Apolipoprotein A-I directly interacts with amyloid precursor protein and inhibits A beta aggregation and toxicity**. *Biochemistry* 2001, **40**(12):3553-3560.

54. Paula-Lima AC, Tricerri MA, Brito-Moreira J, Bomfim TR, Oliveira FF, Magdesian MH, Grinberg LT, Panizzutti R, Ferreira ST: **Human apolipoprotein A-I binds amyloid-beta and prevents Abeta-induced**

neurotoxicity. *Int J Biochem Cell Biol* 2009, **41**(6):1361-1370.

55. Sagare A, Deane R, Bell RD, Johnson B, Hamm K, Pendu R, Marky A, Lenting PJ, Wu Z, Zarcone T *et al*: **Clearance of amyloid-beta by circulating lipoprotein receptors**. *Nat Med* 2007, **13**(9):1029-1031.

56. Rosenson RS, Brewer HB, Jr., Ansell BJ, Barter P, Chapman MJ, Heinecke JW, Kontush A, Tall AR, Webb NR: **Dysfunctional HDL and atherosclerotic cardiovascular disease**. *Nat Rev Cardiol* 2016, **13**(1):48-60.

57. Rhee EJ, Byrne CD, Sung KC: **The HDL cholesterol/apolipoprotein A-I ratio: an indicator of cardiovascular disease**. *Current opinion in endocrinology, diabetes, and obesity* 2017, **24**(2):148-153.

58. Fujiyoshi M, Ohtsuki S, Hori S, Tachikawa M, Terasaki T: **24S-hydroxycholesterol induces cholesterol release from choroid plexus epithelial cells in an apical- and apoE isoform-dependent manner concomitantly with the induction of ABCA1 and ABCG1 expression**. *J Neurochem* 2007, **100**(4):968-978.

59. Cavelier C, Lorenzi I, Rohrer L, von Eckardstein A: **Lipid efflux by the ATP-binding cassette transporters ABCA1 and ABCG1**. *Biochim Biophys Acta* 2006, **1761**(7):655-666.

60. Young EK, Chatterjee C, Sparks DL: **HDL-ApoE content regulates the displacement of hepatic lipase from cell surface proteoglycans**. *Am J Pathol* 2009, **175**(1):448-457.

61. Connelly PW: **The role of hepatic lipase in lipoprotein metabolism**. *Clin Chim Acta* 1999, **286**(1-2):243-255.

62. Robert J, Button EB, Yuen B, Gilmour M, Kang K, Bahrabadi A, Stukas S, Zhao W, Kulic I, Wellington CL: **Clearance of beta-amyloid is facilitated by apolipoprotein E and circulating high-density lipoproteins in bioengineered human vessels**. *Elife* 2017, **6**.

Figures

Figure 1

Plasma Aβ42/40 levels were increased following CS-6253 injection in the GLP study. The increase at 4 h p.i. and the effect of accumulated treatment over time were statistically significant ($p < 0.001$ and $p = 0.007$, respectively). The values were shown as percent change from the first measurement point at 5 m p.i. on day 1.

Figure 2

Plasma apoE levels increased overtime in both studies. A) In the GLP study, CS-6253 treatment significantly increased plasma apoE levels 48 h p.i. ($p = 0.008$). B) In the DRF study, at 10 min and 2 h p.i. apoE decreased significantly ($p < 0.001$ and $p = 0.020$, respectively). Then, apoE significantly increased at 12 h, 24 h, 48 h p.i. ($p < 0.001$ for all 3) and accumulated treatment over time ($p = 0.040$). The values for the GLP study were shown as percent change from the first measurement point at 5 m p.i. on day 1 while the values for the DRF study were shown as percent change from the baseline measurement.

Figure 3

Plasma total cholesterol and HDL-cholesterol levels decreased after CS6253 treatment A) In the DRF study decrease in plasma total cholesterol levels was significant for treatment and for accumulated treatment over time ($p = 0.027$ and $p < 0.001$, respectively). B) In the GLP study decrease in plasma total cholesterol levels was significant for treatment and for accumulated treatment over time ($p < 0.001$ for both). C) In the DRF study decrease in plasma HDL cholesterol levels was significant for treatment and for accumulated treatment over time ($p = 0.002$ and $p < 0.001$, respectively). D) In the GLP study decrease in plasma HDL cholesterol levels was significant for treatment and for accumulated treatment over time ($p < 0.001$ for both). Additionally, the time after injection was significantly correlated with HDL cholesterol levels in a quadratic model ($p < 0.001$). The values for the DRF study were shown as percent change from the baseline measurement while the values for the GLP study were shown as percent change from the first measurement point at 5 m p.i. on day 1.

Figure 4

Plasma triglyceride levels increased after CS-6253 treatment. A) In the DRF study increase in plasma triglyceride levels was significant at 2 h, 4 h, 24 h p.i. ($p < 0.001$ for all 3) and for accumulated treatment over time ($p = 0.016$). B) Decrease in plasma triglyceride levels was significant at 2 h, 4 h, and 12 h p.i. in the GLP study ($p < 0.001$, $p < 0.001$, and $p = 0.050$, respectively). The values for the DRF study were shown as percent change from the baseline measurement while the values for the GLP study were shown as percent change from the first measurement point at 5 m p.i. on day 1.

Figure 5

Analysis of pre-β HDL and s-, m-, and l-HDL particles in plasma. A) Plasma pre-β HDL levels in the PK study were measured by ELISA assay (n=2). B) Plasma pre-β1 HDL levels were determined using ion-mobility analysis and Voigt probability distribution (see also Fig S5) for one monkey in the PK study. C) For the same monkey, plasma s-, m-, and l-HDL particle concentrations were calculated using ion-mobility

analysis and Voigt probability distribution. D) For one monkey in the DRF study, plasma pre-β HDL levels were determined using ion-mobility analysis and Voigt probability distribution. E) For the same monkey, plasma s-, m-, and l-HDL particle concentrations were calculated using ion-mobility analysis and Voigt probability distribution.

Figure 6

Analysis of abundance of apoE and triglycerides in HDL, IDL and LDL particles using AF4 for two monkeys in the DRF study. A) Following CS-6253 injection, apoE shifted to larger lipoprotein particles. B) The triglycerides distribution among lipoproteins shifted towards IDL and LDL.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SuppData091321.docx