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Effects of Probucol on plasma amyloid-β transport in patients with hyperlipidemia: a 12-week randomized, double-blind, placebo-controlled trial

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Abstract

Background Although dyslipidemia has been acknowledged as a risk factor for Alzheimer's disease (AD), the effects of lipid-lowering drugs on AD have not been determined. The primary pathophysiological hallmark of AD is the deposition of amyloid-β (Aβ) plaques in the brain. Plasma Aβ levels are influenced by the transport of Aβ from the central nervous system to the peripheral blood. This study investigates the effects of Probucol, a lipid-lowering and antioxidant drug, on plasma Aβ transport.

Methods A total of 120 hyperlipidemic patients with normal cognition were randomly assigned (1:1 ratio) to receive either Probucol (1000 mg daily for 12 weeks) or a placebo. Plasma Aβ, soluble receptor of advanced glycation end products (sRAGE), and fasting lipid profiles were measured at baseline and every 6 weeks.

Results A total of 108 participants completed the study, with 55 in the Probucol group. The cohort consisted of 58 (53.7%) women, with a mean age of 58.4 ± 8.0 (range, 45–80) years. After 12 weeks of treatment, the changes in plasma $A\beta_{42}$ and sRAGE levels significantly differed between the Probucol and placebo groups ($\Delta A\beta_{42}$: β = 6.827, $P = 0.030$; ΔsRAGE: β = 98.668, $P = 0.004$). Furthermore, ΔsRAGE was positively correlated with the change in $A\beta_{42}$ (β = 0.018, $P = 0.048$). When adjusted for ΔsRAGE, the effect of Probucol on plasma $A\beta_{42}$ levels was attenuated (β = 5.065, $P = 0.116$). In the Probucol group only, ΔsRAGE was significantly correlated with oxidized low-density lipoproteins (β = 4.27, $P = 0.011$), total cholesterol (β = 67.50, $P = 0.046$), and low-density lipoproteins (β = −91.01, $P = 0.011$).

Conclusions Daily oral administration of Probucol (1000 mg) for 12 weeks significantly increased plasma $A\beta_{42}$ levels, likely through modulation of sRAGE. This effect may be attributed to the antioxidant and lipid-lowering properties of Probucol. These findings suggest that Probucol could potentially serve as a protective agent against the pathological processes of AD.

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Trial registration This study was registered on the Chinese Clinical Trial Registry platform in June 2019 (Trial registration number: ChiCTR-1900023542).

Keywords Alzheimer's disease, Plasma amyloid beta, Hyperlipidemia, Probucol, Soluble receptor of advanced glycation end products (sRAGE)

Introduction

Alzheimer's disease (AD), the leading cause of dementia, is projected to affect 9.83 million individuals in China over the coming decades [1]. The absence of a definitive cure for AD has intensified the focus on disease-modifying therapies and interventions aimed at targeting known AD risk factors [2]. Among these, elevated blood lipids in middle-aged and elderly populations are identified as risk factors for the development of AD [3, 4].

The mechanisms by which dyslipidemia contributes to cognitive impairment may involve the disruption of amyloid-β (Aβ) metabolism [5]. Both the deposition and clearance of Aβ are recognized as pivotal elements in the pathogenesis of AD [6]. Emerging evidence suggests that Aβ accumulation begins years before irreversible neuropathological damage is observed in AD patients [7, 8]. Numerous studies have shown that hypercholesterolemia exacerbates Aβ accumulation and accelerates AD progression [5, 9, 10].

Currently, cerebral amyloidosis is assessed through techniques such as amyloid positron emission tomography [11] and the analysis of cerebrospinal fluid biomarkers [12]. However, the high cost and invasiveness of these methods limit their usefulness. Meanwhile, systemic measurements of soluble Aβ in the peripheral circulation have been found to correlate positively with brain amyloid pathology [13–15]. Soluble low-density lipoprotein receptor-related protein-1 (sLRP1) and soluble receptor of advanced glycation end products (sRAGE) play essential roles in peripheral transport processes [16]. sLRP1 binds peripheral Aβ, acting as a "sink" for Aβ and preventing free Aβ influx into the brain [17]. Additionally, sRAGE serves as a decoy receptor, binding Aβ and inhibiting the formation of the Aβ-RAGE complex, thereby reducing oxidative damage and the associated inflammatory response [18]. Together, sLRP1 and sRAGE facilitate the clearance of Aβ from the central nervous system to peripheral Aβ pools, thereby mitigating the cerebral amyloid burden.

Probucol, a well-established cholesterol-lowering drug, possesses reliable anti-inflammatory and antioxidant capacity [19]. It is known to prevent the oxidation of low-density lipoproteins (LDL) into oxidized low-density lipoproteins (oxLDL) [20] and improve oxidative stress in the brain. Additionally, in murine models, Probucol has been shown to inhibit lipoprotein-Aβ secretion and reduce neurovascular inflammation, which helps maintain the integrity of the blood–brain barrier (BBB) [21]. Previous animal studies have also demonstrated that Probucol treatment can alleviate the deleterious effects of the cerebral Aβ deposition [21, 22]. However, the effects of Probucol on Aβ metabolism in humans are not clear.

Over the last 10 years, the antioxidant effects of Probucol have attracted considerable attention. A recent study showed that Probucol-based nanoparticles exhibited antioxidant properties that protected auditory nerve cells from damage, suggesting a potential therapeutic role in sensorineural hearing loss [23]. Moreover, Probucol has been found to protect pancreatic β-cells from oxidative damage in diabetes mellitus [24]. While these findings are primarily based on animal and in vitro experiments, the present study investigated changes in the levels of oxLDL and plasma Aβ before and after Probucol treatment in patients with hypercholesterolemia. Furthermore, the relationship between AD biomarkers and the antioxidant effects of Probucol is explored, highlighting its potential clinical applications in combating AD.

Given the properties and clinical significance of Probucol, the present study conducted a 12-week randomized, double-blind, placebo-controlled trial to investigate the effects of Probucol on plasma Aβ transport in patients with hypercholesterolemia.

Methods

Participants

This 12-week study was carried out between June and August 2019 in the suburbs of Xi'an, China. The inclusion criteria were as follows: (A) age between 45 and 80 years; (B) fasting blood lipid levels meeting the criteria for hyperlipidemia at baseline (total cholesterol [TC] ≥ 5.18 mmol/L, triglycerides [TG] ≥ 1.70 mmol/L, and/or low-density lipoprotein cholesterol [LDL-c] ≥ 3.37 mmol/L); (C) normal cognitive function, defined by a Mini-Mental State Examination (MMSE) score > 17 for subjects with illiteracy, > 20 for subjects with primary school education, and > 24 for subjects with junior high school education or above; and (D) voluntary participation in the study with signed informed consent. The exclusion criteria included: (A) use of lipid-lowering medications or other drugs that could affect blood lipids or liver function within the three months prior to recruitment; (B) secondary

hyperlipidemia (e.g., due to endocrine disorders, nephrotic syndrome); (C) central nervous system diseases (such as a history of stroke, encephalitis, or epilepsy); (D) severe liver or kidney dysfunction, indicated by elevated creatine phosphokinase, uric acid, or urea nitrogen levels; and (E) known allergy to Probucol (Fig. 1).

This trial was approved by the Medical Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University and registered with the Chinese Clinical Trial Registry (ChiCTR-1900023542). All participants provided written informed consent.

Sample size calculation

The plasma $A\beta_{42}$ level was the primary outcome used to calculate the target sample size [25]. The average plasma $A\beta_{42}$ level in 509 hyperlipidemic patients who met the eligibility criteria was 52.19 ± 9.01 pg/ml. It was assumed that Probucol treatment for 12 weeks would be considered effective if it reduced the plasma $A\beta_{42}$ levels by 10% compared to the placebo group. Based on an analysis of variance for a one-tailed test with $\alpha = 0.05$, and $\beta = 0.1$, 52 participants were required in each group. Taking into account a 10% dropout rate, a total of 116 subjects (58 per group) was required. Ultimately, 120 subjects were enrolled in the trial.

Randomization and blinding

A total of 120 eligible participants with hyperlipidemia were randomly assigned to either the Probucol group or the placebo group using a computer-generated random sequence, with unique three-digit participant identifiers. Except for the research pharmacists and study statisticians, all trial participants and personnel were blinded to the treatment allocation throughout the study.

Intervention

Before formal recruitment, participants were assessed against the eligibility criteria to ensure an adequate follow-up participation rate. Study visits were conducted at baseline (week 0), week 6, and week 12. Each visit included fasting blood collection, drug counting, and completion of questionnaires. During the screening period, all participants underwent a standardized evaluation to collect demographic data.

The participants in both groups received a 6-week study medication provision at baseline and at the week 6 follow-up. Participants in the Probucol group were instructed to take 500 mg of Probucol twice daily after meals. The placebo group received identical instructions. To monitor adherence, the clinical research coordinator contacted participants by phone every two weeks. In cases of missed doses, participants were instructed to record the missed dose on their medication card but

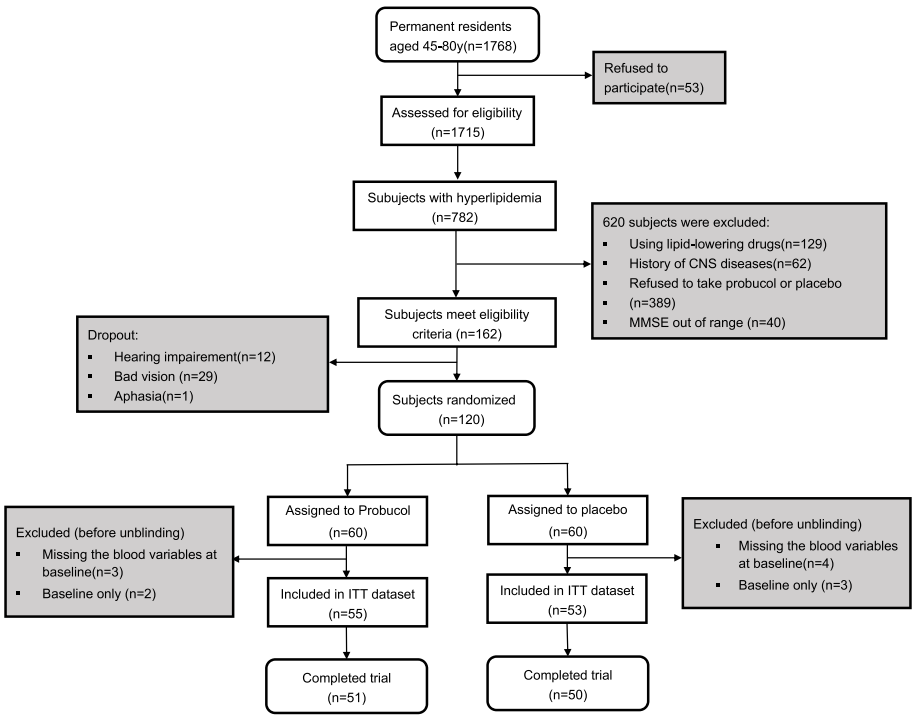

Fig. 1 Flow chart of recruitment, randomization and follow-up. MMSE, Mini-Mental State Examination; ITT, intent-to-treat

were not advised to compensate for the missed medication. Adherence was assessed at each visit using two methods: the self-reported medication logs provided by participants and the pill count, calculated as the number of dispensed pills minus the number of returned pills.

Study medication and safety assessments

Participants were assigned to receive either Probucol or a placebo. Probucol was administered in the form of commercially available tablets (250 mg, Taichang) produced by Jingfukang Pharmaceutical Group Co., Ltd. (state medical permit number H10960161). The placebo was synthesized using starch granules by Cspc Ouyi Pharmaceutical Co., Ltd., in accordance with current Good Manufacturing Practices (cGMP). Both the Probucol and placebo tablets were indistinguishable in terms of packaging, color, taste, and smell, ensuring that treatment allocation remained concealed from both participants and study staff.

The safety of the drug was assessed through physical and neurological examinations, liver and renal function tests, and limb-lead electrocardiograms (including measurements of RR interval, QRS duration, and QT interval) conducted at baseline and at week 12.

Outcome assessment

The primary outcome of the study was the difference in plasma Aβ levels between the Probucol and placebo groups over 12 weeks of treatment. Exploratory outcomes included the effects of Probucol on blood levels of sLRP1 and sRAGE, as well as the association between blood lipids and plasma Aβ levels.

Safety assessments included standard reporting of any adverse events, which encompassed muscle aches, headaches, joint pain, vertigo, fatigue, insomnia, abdominal pain with diarrhea, vomiting, pruritus, and rashes.

Laboratory assay

Eligible participants were required to provide 8 mL of fasting blood samples after an overnight fast of 10–12 h at baseline and during the 6- and 12-week visits.

Four milliliters of each blood sample were used for testing blood lipids (TG, TC, high-density lipoprotein cholesterol (HDL-c), and LDL-c), liver function, and renal function. The remaining 4 mL were used for plasma measurements. Plasma was separated by centrifugation (1,500 rpm, 10 min) and stored at − 80 °C until the analysis of Aβ, sLRP1, sRAGE, and oxLDL levels.

Plasma levels of $A\beta_{40}$, $A\beta_{42}$, sLRP1, sRAGE, and oxLDL were measured using sandwich enzyme-linked immunosorbent assay kits (CEA864Hu, CEA946Hu, SEB010Hu, SEA645Hu, and CEA527Hu, respectively; Cloud-Clone Corp., Wuhan, China). To ensure consistency across the three visits (baseline, 6 weeks, and 12 weeks), all plasma samples were measured in duplicate at the end of the 12-week study. Polymerase chain reaction products were sent to Sangon Co. (Shanghai, China) for APOE genotyping via Sanger sequencing. Participants without the ε4 allele (ε2/2, ε2/3, ε3/3) were classified as APOEε4 (-), while those with the ε4 allele were classified as APOEε4(+).

Statistical analysis

Participants included in the per-protocol (PP) population completed the entire intervention. Those enrolled in the intention-to-treat (ITT) group had an adherence rate exceeding 75% and completed at least two observations, including the baseline assessment. To mitigate the impact of missing data on statistical outcomes, the last observation carried forward method was used to impute missing values.

Baseline characteristics were first compared between the Probucol and placebo groups. Subsequently, unpaired Student's t-tests were used to examine changes in the levels of lipids, Aβ, sLRP1, and sRAGE. Multiple linear regression was then employed to adjust for confounding factors, including gender, age, MMSE score, years of education, mean arterial pressure, body mass index, and baseline levels of plasma Aβ, Aβ transporters, and blood lipids.

To explore the relationships between blood lipid levels and Aβ-related biomarkers, generalized estimating equations (GEE) analysis was performed using data from the three observation points (baseline, week 6, and week 12). In the GEE model, the Probucol group was coded as 1, the placebo group as 0, and the following confounders were adjusted for: gender, age, MMSE score, years of education, mean arterial pressure, and body mass index. All statistical analyses were performed using SPSS Statistics software (IBM, USA, version 24.0). A *P*-value (two-sided) of less than 0.05 was considered statistically significant.

Results

Study participants and follow-up

As shown in Fig. 1, 782 participants met the criteria for hyperlipidemia. Among them, 620 were excluded due to severe central nervous system diseases or cognitive dysfunction, a history of lipid-lowering drug use, or refusal to take medication. Ultimately, 120 subjects were included and randomly assigned to either the Probucol group or the placebo group (60 participants in each group). According to the study protocol, 12 participants were excluded from the trial prior to unblinding due to missing baseline blood biomarkers or a lack of post-baseline observations. Of the 108 participants included in the analysis, 55 were in the

Table 1 Baseline characteristics for the Probucol group and the placebo group

	Total (*n* = 108)	Probucol group (*n* = 55)	Placebo group (*n* = 53)	*P*
Demographic information				
Age, y (mean ± SD)	58.4 ± 8.0	60.7 ± 8.6	56.1 ± 6.7	**0.003**
Female, *n* (%)	58 (53.7)	27 (49.1)	31 (58.5)	0.327
Education, y (mean ± SD)	7.0 ± 3.1	6.5 ± 3.2	7.6 ± 2.8	0.051
Lifestyle				
Smoking, *n* (%)	31 (28.7)	16 (29.1)	15 (28.3)	0.928
Alcohol consumption, *n* (%)	20 (18.5)	12 (21.8)	8 (15.1)	0.369
Lack of activity, *n* (%)	15 (13.9)	8 (14.5)	7 (13.2)	0.841
Medical history				
†Cardiovascular disease, *n* (%)	5 (4.6)	4 (7.3)	1 (1.9)	0.382†
Hypertension, *n* (%)	36 (33.3)	19 (34.5)	17 (32.1)	0.785
†Diabetes mellitus, *n* (%)	1 (0.9)	0 (0)	1 (1.9)	0.491†
Physical examination				
MMSE (mean ± SD)	27.7 ± 2.1	27 ± 2.4	28.3 ± 1.4	**0.001**
MAP, mmHg (mean ± SD)	100.5 ± 11.8	100.5 ± 12	100.5 ± 11.8	0.997
BMI, kg/m^2 (mean ± SD)	25.6 ± 2.5	25.4 ± 2.5	25.7 ± 2.4	0.536
Pulse rate, (mean ± SD)	70.6 ± 8.3	70.5 ± 8.2	70.7 ± 8.6	0.897
Biochemical measures				
FBG, mmol/l, median (quartile)	4.94 (4.73, 5.40)	4.92 (4.65, 5.33)	4.95 (4.74, 5.49)	0.437
TG, mmol/L (mean ± SD)	1.74 ± 0.84	1.7 ± 0.84	1.77 ± 0.84	0.686
TC, mmol/L (mean ± SD)	5.95 ± 0.64	6.04 ± 0.64	5.87 ± 0.63	0.160
LDL-c, mmol/L (mean ± SD)	3.49 ± 0.53	3.51 ± 0.55	3.47 ± 0.52	0.712
HDL-c, mmol/L (mean ± SD)	1.51 ± 0.23	1.56 ± 0.21	1.46 ± 0.24	**0.019**
OxLDL, μg/dl (mean ± SD)	12.88 ± 8.43	11.43 ± 7.52	14.37 ± 9.1	0.070
$A\beta_{40}$, pg/ml (mean ± SD)	204.57 ± 68.27	197.25 ± 73.74	212.16 ± 61.86	0.258
$A\beta_{42}$, pg/ml (mean ± SD)	53 ± 22.82	50.41 ± 24.09	55.68 ± 21.33	0.232
$A\beta_{42}/A\beta_{40}$ ratio (mean ± SD)	0.27 ± 0.12	0.27 ± 0.12	0.27 ± 0.11	0.747
sLRP1, ng/ml (mean ± SD)	3443 ± 2250	3408 ± 2394	3481 ± 2111	0.867
sRAGE, pg/ml (mean ± SD)	760.9 ± 218	783.9 ± 212	737 ± 223.6	0.265

Unpaired Student's *t*-test (two tailed) and mean ± SD were used to compare the difference of the approximately normally distributed continuous variables between the Probucol group and the placebo group, and Mann–Whitney test were used for skewness distribution data. A chi-square test and percentage were used for categorical variables († Fisher's exact test)

MMSE Mini-Mental State Examination, *MAP* Mean arterial pressure, *BMI* Body mass index, *FBG* Fasting blood glucose, *SD* Standard deviation, *Aβ* Amyloid-β, *sLRP1* Soluble low-density lipoprotein receptor-related protein-1, *sRAGE* Soluble receptor for advanced glycation end products, *TG* Triglycerides, *TC* Total cholesterol, *LDL-c* Low-density lipoprotein cholesterol, *HDL-c* High-density lipoprotein cholesterol, *oxLDL* oxidized low-density lipoprotein

Probucol group and 53 in the placebo group. The discontinuation rates at 12 weeks were similar between the Probucol group (4/55) and the placebo group (3/53) ($\chi^2 = 0.116$, $P = 0.734$). All participants tolerated Probucol well. At the 12-week follow-up, no adverse events were reported in either the PP or ITT populations in either group. However, two participants in the Probucol group withdrew from the study due to adverse events, specifically, nausea and pruritus.

Table 1 presents the baseline characteristics of the ITT population. The baseline characteristics were generally balanced between the Probucol and placebo groups, with the exception that the placebo group was younger ($P = 0.003$), had higher MMSE scores ($P = 0.001$) and lower HDL-c levels ($P = 0.019$).

Changes in blood lipid levels

At baseline, only the HDL-c level (1.56 ± 0.21 vs. 1.46 ± 0.24 mmol/L, $P = 0.019$) was higher in the Probucol group; the other blood lipid levels were balanced between the Probucol and placebo groups (Table 1).

As shown in Table 2 and Fig. 2, in the ITT population, changes in TC, LDL-c, and HDL-c were larger in the Probucol group compared to the placebo group after 12 weeks of treatment (ΔTC: − 2.09 ± 0.85 vs. − 1.16 ± 0.74 mmol/L, $P < 0.001$;

Table 2 Univariate analysis of plasma Aβ, sLRP1, sRAGE, and lipid levels changes from baseline at week 6 and week 12 in the PP and ITT populations

Variables	PP			ITT		
	Probucol (*n* = 51)	Placebo (*n* = 50)	*p*	Probucol (*n* = 55)	Placebo (*n* = 53)	*P*
6W-0W						
$\Delta A\beta_{40}$	34.32 ± 58.15	23.57 ± 54.44	0.340	36.21 ± 56.60	19.20 ± 56.83	0.122
$\Delta A\beta_{42}$	10.25 ± 15.24	10.48 ± 13.49	0.936	10.51 ± 14.90	10.29 ± 13.14	0.935
$\Delta A\beta_{42}/A\beta_{40}$ ratio	0.001 ± 0.089	0.011 ± 0.061	0.538	− 0.001 ± 0.087	0.018 ± 0.071	0.216
ΔsLRP1	− 1797 ± 1834	− 1301 ± 1738	0.166	− 1728 ± 1804	− 1453 ± 1872	0.437
ΔsRAGE	− 92.79 ± 160.36	− 70.59 ± 179.90	0.514	− 106.04 ± 165.19	− 72.33 ± 178.4	0.310
ΔTG	− 0.25 ± 0.89	0.25 ± 1.10	**0.015**	− 0.23 ± 0.86	0.23 ± 1.07	**0.016**
ΔTC	− 2.12 ± 0.82	− 0.91 ± 0.67	**< 0.001**	− 2.04 ± 0.85	− 0.91 ± 0.65	**< 0.001**
ΔLDL-c	− 1.14 ± 0.65	− 0.39 ± 0.54	**< 0.001**	− 1.10 ± 0.67	− 0.41 ± 0.52	**< 0.001**
ΔHDL-c	− 0.60 ± 0.18	− 0.22 ± 0.18	**< 0.001**	− 0.58 ± 0.19	− 0.22 ± 0.17	**< 0.001**
ΔoxLDL	− 0.53 ± 2.81	− 0.78 ± 3.91	0.710	− 1.12 ± 3.98	− 0.64 ± 3.94	0.536
12W-0W						
$\Delta A\beta_{40}$	33.57 ± 62.2	40.00 ± 58.27	0.593	36.78 ± 61.95	34.71 ± 61.56	0.862
$\Delta A\beta_{42}$	11.44 ± 17.48	5.35 ± 14.89	0.063	11.31 ± 16.91	5.45 ± 14.48	0.056
$\Delta A\beta_{42}/A\beta_{40}$ ratio	0.012 ± 0.099	− 0.026 ± 0.084	**0.039**	0.007 ± 0.098	− 0.017 ± 0.094	0.197
ΔsLRP1	− 1565 ± 2127	− 1143 ± 1543	0.257	− 1513 ± 2074	− 1304 ± 1717	0.569
ΔsRAGE	− 58.74 ± 170.71	− 137.11 ± 223.82	**0.050**	− 73.87 ± 177.05	− 135.08 ± 220.35	0.114
ΔTG	0.18 ± 1.16	0.50 ± 1.10	0.160	0.16 ± 1.12	0.45 ± 1.08	0.170
ΔTC	− 2.17 ± 0.81	− 1.17 ± 0.75	**< 0.001**	− 2.09 ± 0.85	− 1.16 ± 0.74	**< 0.001**
ΔLDL-c	− 1.61 ± 0.97	− 1.18 ± 0.95	**0.026**	− 1.53 ± 0.99	− 1.16 ± 0.93	**0.046**
ΔHDL-c	− 0.50 ± 0.50	− 0.06 ± 0.45	**< 0.001**	− 0.49 ± 0.49	− 0.08 ± 0.45	**< 0.001**
ΔoxLDL	− 0.65 ± 2.91	− 0.49 ± 3.77	0.822	− 1.03 ± 3.24	− 0.37 ± 3.81	0.334
AE (n, %)	0	0	1.00	0	0	1.00

In the PP and ITT populations, the variations in plasma Aβ, sLRP1, sRAGE, and lipid levels were compared by unpaired Student's t-test between the Probucol group and the placebo group

PP Per protocol, *ITT* Intention-to-treat, *Aβ* Amyloid-β, *sLRP1* Soluble low-density lipoprotein receptor-related protein-1, *sRAGE* Soluble receptor for advanced glycation end products, *TC* Total cholesterol, *TG* Triglycerides, *LDL-c* Low-density lipoprotein cholesterol, *HDL-c* High-density lipoprotein cholesterol, *oxLDL* oxidized low-density lipoprotein, *AE* Adverse event

ΔLDL-c: − 1.53 ± 0.99 vs. − 1.16 ± 0.93 mmol/L, $P = 0.046$; ΔHDL-c: − 0.49 ± 0.49 vs. − 0.08 ± 0.45 mmol/L, $P < 0.001$). However, this trend was not observed for the changes in TG or oxLDL.

Significant differences in blood lipid levels were also observed within the placebo group before and after treatment. As shown in Table S1, TC and LDL-c levels in the placebo group were significantly reduced at both 6 and 12 weeks compared to baseline (6w-TC: 4.00 ± 0.70 vs. 6.04 ± 0.64 mmol/L, $P < 0.001$; 12w-TC: 3.95 ± 0.73 vs. 6.04 ± 0.64 mmol/L, $P < 0.001$; 6w- LDL-c: 2.41 ± 0.56 vs. 3.51 ± 0.55 mmol/L, $P < 0.001$; 12w-LDL-c: 1.98 ± 0.77 vs. 3.51 ± 0.55 mmol/L, $P < 0.001$). Additionally, the HDL-c level was significantly lower at 6 weeks compared to baseline (0.98 ± 0.20 vs. 1.56 ± 0.21 mmol/L, $P < 0.001$). Overall, the trends in lipid levels, except for oxLDL, were similar between the placebo and Probucol groups.

Changes in the levels of plasma Aβ and Aβ transporters

At baseline, there were no differences in the plasma levels of Aβ, sLRP1, and sRAGE between the Probucol and placebo groups (Table 1).

In the PP population, significant differences were observed in the changes of the plasma $A\beta_{42}/A\beta_{40}$ ratio and sRAGE levels from baseline to 12 weeks between the Probucol and placebo groups ($\Delta A\beta_{42}/A\beta_{40}$ ratio: 0.012 ± 0.099 vs. − 0.026 ± 0.084, $P = 0.039$; ΔsRAGE: − 58.74 ± 170.71 vs. − 137.11 ± 223.82 pg/ml, $P = 0.050$). The change in plasma $A\beta_{42}$ levels from baseline to 12 weeks was also more pronounced in the Probucol group, although the difference did not reach statistical significance (11.44 ± 17.48 vs. 5.35 ± 14.89 pg/ml, $P = 0.063$). In contrast, no significant differences were found between the Probucol and placebo groups regarding the changes in plasma $A\beta_{40}$ and sLRP1 levels from baseline to 6 and 12 weeks in both the ITT and PP populations (Table 2 and Fig. 3).

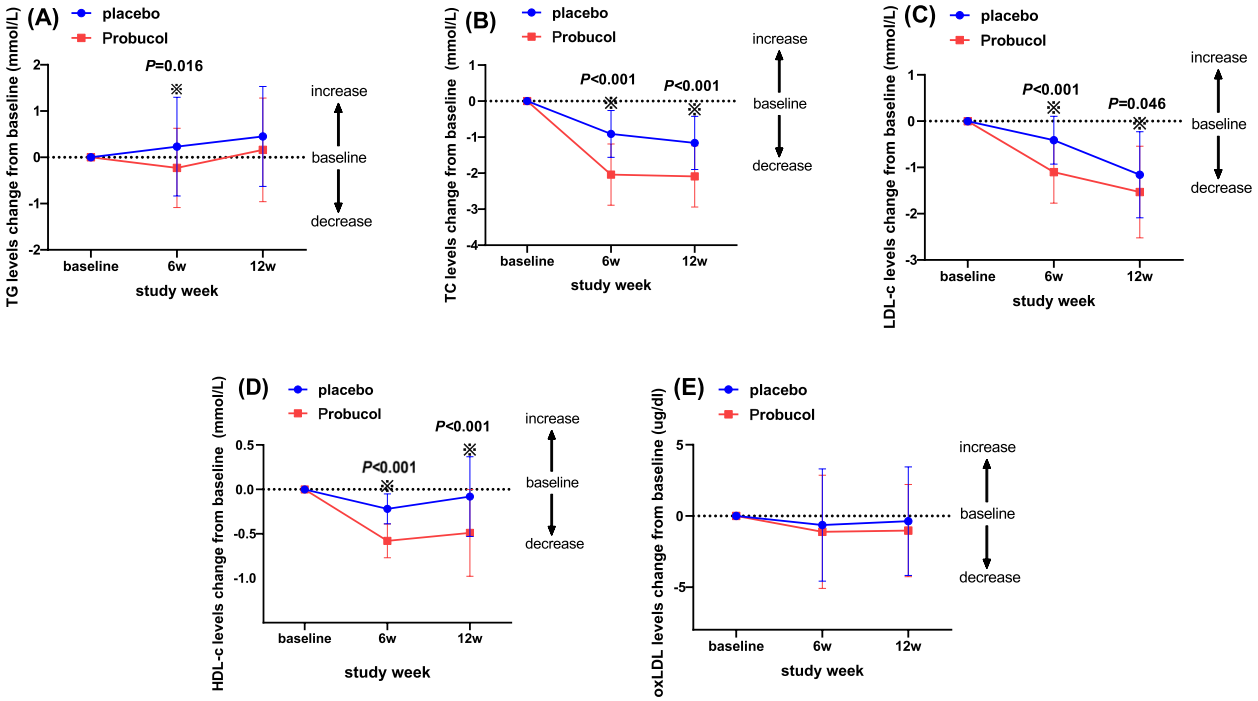

Fig. 2 Changes in blood TG (**A**), TC (**B**), LDL-c (**C**), HDL-c (**D**), and oxLDL (**E**) levels. The changes in blood lipid levels from baseline at week 6 and week 12 in the Probucol group and the placebo group in the ITT population. Missing data were replaced using the last observation carried forward method. Mean blood lipid levels are indicated by squares and dots. TG, triglycerides; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; oxLDL, oxidized low-density lipoprotein

Multiple linear regression analysis of the changes in the levels of plasma $A\beta_{40}$, $A\beta_{42}$, sLRP1, and sRAGE

After adjusting for confounding factors, no significant differences were observed in the changes in Aβ and transporters levels from baseline to 6 weeks between the Probucol and placebo groups. However, as shown in Table 3, the changes in plasma $A\beta_{42}$, the $A\beta_{42}/A\beta_{40}$ ratio, and sRAGE levels at week 12 significantly differed between the Probucol and placebo groups ($\Delta A\beta_{42}$: $\beta = 6.827$, $P = 0.030$; $\Delta A\beta_{42}/A\beta_{40}$ ratio: $\beta = 0.032$, $P = 0.050$; ΔsRAGE: $\beta = 98.668$, $P = 0.004$). In contrast, the variations in $A\beta_{40}$ and sLRP1 levels were similar between the Probucol group and placebo groups.

Relationships between plasma ΔAβ (12W-0W) and ΔsRAGE (12W-0W)

To further explore whether Probucol affected the plasma $A\beta_{42}$ level and the $A\beta_{42}/A\beta_{40}$ ratio through its effect on sRAGE, multiple linear regression analysis was performed (Table 4). After 12 weeks of treatment, changes in plasma $A\beta_{42}$ levels (Model 2: $\beta = 6.827$, $P = 0.030$) and the $A\beta_{42}/A\beta_{40}$ ratio (Model 2: $\beta = 0.032$, $P = 0.050$) significantly differed between the Probucol and placebo groups in Model 2. However, in Model 3, the relationships between the Probucol group and $\Delta A\beta_{42}$ (Model 3: $\beta = 5.065$, $P = 0.116$) and $\Delta A\beta_{42}/A\beta_{40}$ ratio (Model 3: $\beta = 0.021$, $P = 0.210$) were no longer significant after adjusting for ΔsRAGE and baseline sRAGE level. Additionally, ΔsRAGE(12W-0W) was positively correlated with both $\Delta A\beta_{42}$ ($\beta = 0.018$, $P = 0.048$) and the $\Delta A\beta_{42}/A\beta_{40}$ ratio ($\beta < 0.001$, $P = 0.023$).

Associations between plasma $A\beta_{42}$, sRAGE, and blood lipids

A GEE analysis was performed to investigate whether Probucol affects peripheral Aβ levels through its lipid-lowering and antioxidant effects. In all subjects, TG levels were negatively correlated with $A\beta_{42}$ ($\beta = -2.50$, $P = 0.028$), and oxLDL levels were positively correlated with sRAGE ($\beta = 3.08$, $P = 0.042$). No other lipid parameters showed significant associations with plasma Aβ or sRAGE levels (Table 5).

In the Probucol group, the overall $A\beta_{42}$ level was negatively associated with oxLDL ($\beta = -0.57$, $P = 0.047$), while the overall sRAGE level was positively correlated with oxLDL ($\beta = 4.27$, $P = 0.011$) and TC ($\beta = 67.50$, $P = 0.046$) but negatively associated with LDL-c ($\beta = -91.01$, $P = 0.011$). In contrast, only a negative correlation was found between the overall levels of $A\beta_{42}$ and TG in the placebo group ($\beta = -3.39$, $P = 0.042$; Table 5).

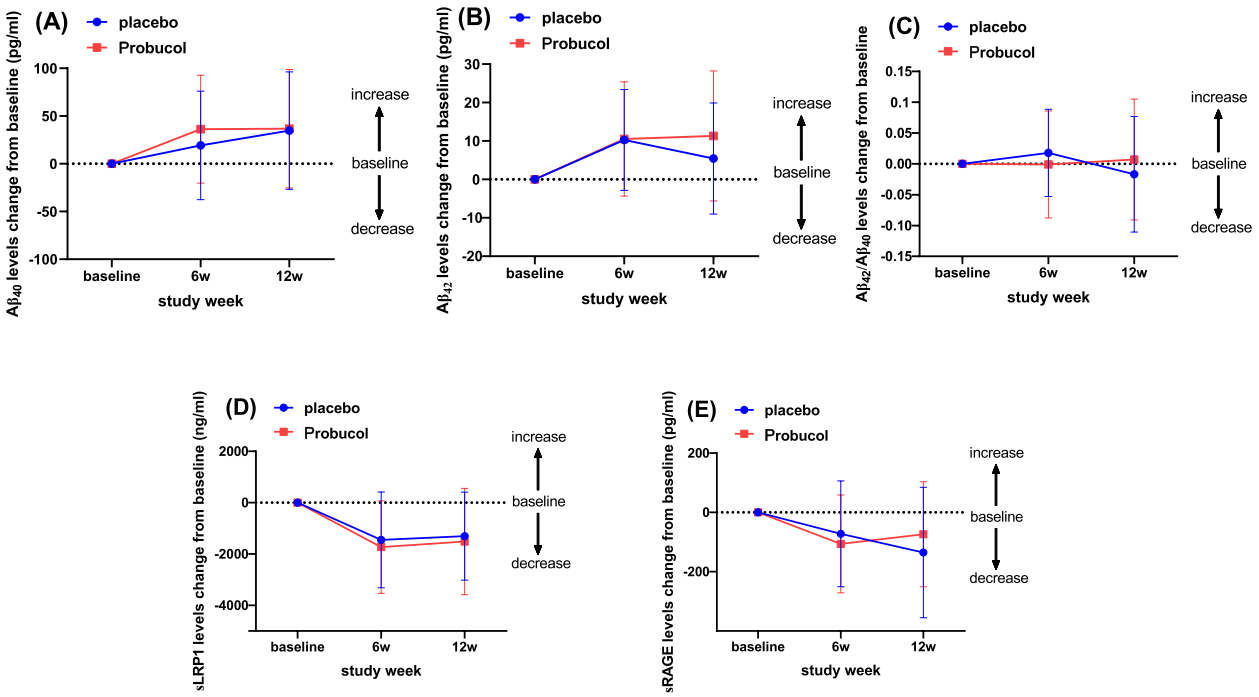

Fig. 3 Changes in plasma $A\beta_{40}$ (**A**), $A\beta_{42}$ (**B**), $A\beta_{42}/A\beta_{40}$ ratio (**C**), sLRP1 (**D**), and sRAGE (**E**) levels. The changes in plasma Aβ levels from baseline at week 6 and week 12 in the Probucol group and the placebo group in the ITT population. Missing data were replaced using the last observation carried forward method. Mean plasma Aβ levels are indicated by squares and dots, respectively. Aβ, amyloid beta; sLRP1, soluble low-density lipoprotein receptor-related protein-1; sRAGE, soluble receptor for advanced glycation end product

Table 3 Multiple linear regression analysis of the changes in plasma Aβ levels from baseline at week 6 and 12 in the ITT population

Variables	β	95% CI		T	*P*
		Lower	Upper		
6W-0W					
$\Delta A\beta_{40}$	7.320	−15.291	29.932	0.642	0.522
$\Delta A\beta_{42}$	1.304	−4.665	7.272	0.433	0.666
$\Delta A\beta_{42}/A\beta_{40}$ ratio	−0.009	−0.038	0.020	−0.585	0.560
ΔsLRP1	−341.318	−770.885	88.249	−1.577	0.118
ΔsRAGE	−29.411	−90.041	31.219	−0.963	0.338
12W-0W					
$\Delta A\beta_{40}$	−0.464	−24.741	23.813	−0.038	0.970
$\Delta A\beta_{42}$	6.827	0.677	12.977	2.203	**0.030**
$\Delta A\beta_{42}/A\beta_{40}$ ratio	0.032	0.000	0.063	1.987	**0.050**
ΔsLRP1	−243.128	−710.462	224.206	−1.032	0.304
ΔsRAGE	98.668	31.415	165.921	2.911	**0.004**

In the multiple linear regression analysis, the changes in plasma Aβ levels from baseline at week 6 (Δ6W-0W) and at week 12 (Δ12W-0W) were the dependent variables and grouping (dummy coded with Probucol = 1, placebo = 0) was the independent variable, and the potential confounders were age, sex, education years, MMSE score, MAP, BMI, and the baseline Aβ, sLRP1, sRAGE

ITT Intention-to-treat, *Aβ* Amyloid-β, *sLRP1* Soluble low-density lipoprotein receptor-related protein-1, *sRAGE* Soluble receptor for advanced glycation end products, *MMSE* Mini-Mental State Examination, *MAP* Mean arterial pressure, *BMI* Body mass index

Discussion

The effects of Probucol on the levels of plasma Aβ and transporters in hyperlipidemic patients were investigated in this placebo-controlled trial. After 12 weeks of treatment, both plasma $A\beta_{42}$ and sRAGE levels were higher in the Probucol group compared to the placebo group. Multiple linear regression analysis revealed that the increase in plasma $A\beta_{42}$ levels was positively associated with the change in sRAGE levels. Moreover, significant associations between sRAGE and the levels of TC, LDL-c, and oxLDL were observed in the Probucol group but not in the placebo group.

Dyslipidemia has been recognized as a risk factor for AD. Kim et al. reported that hypercholesterolemia leads to an accumulation of cholesterol in lipid rafts in the brain, and lipid oxidation products exacerbate the cytotoxic effects of Aβ peptides in apoptotic neurons, compromising the integrity of BBB endothelial cells [26]. A high-cholesterol diet has been shown to aggravate the brain Aβ burden and accelerate the pathological progression of AD [27]. Furthermore, the use of lipid-lowering drugs has been associated with a reduced risk of dementia [28] and a lower likelihood of Aβ plaque formation [21, 29].

During the asymptomatic phase of AD, several studies have reported a decline in plasma $A\beta_{42}$ levels. In contrast,

Table 4 Relationships between plasma ΔAβ and ΔsRAGE after 12 weeks of treatment with Probucol and placebo in the ITT populations

Variables	Model	Group			ΔsRAGE(12W-0W)		
		β	T	*P*	β	T	*P*
$\Delta A\beta_{40}$ (12W-0W)	1	2.070	0.174	0.862			
	2	−0.464	−0.038	0.970			
	3	2.522	0.196	0.845	−0.025	−0.687	0.494
$\Delta A\beta_{42}$ (12W-0W)	1	5.864	1.932	0.056			
	2	6.827	2.203	**0.030**			
	3	5.065	1.587	0.116	0.018	2.001	**0.048**
$\Delta A\beta_{42}/A\beta_{40}$ (12W-0W)	1	0.024	1.298	0.197			
	2	0.032	1.987	**0.050**			
	3	0.021	1.263	0.210	< 0.001	2.307	**0.023**

In the multiple linear regression analysis, the grouping (dummy coded with Probucol = 1, placebo = 0) was the independent variable and changes in plasma $A\beta_{40}$, $A\beta_{42}$ levels and $A\beta_{42}/A\beta_{40}$ ratio were the dependent variables. Model 1 was non-adjusted. Model 2 was adjusted for age, gender, education years, MMSE score, MAP, BMI, baseline plasma $A\beta_{40}$, $A\beta_{42}$ levels, and $A\beta_{42}/A\beta_{40}$ ratio. Model 3 was adjusted for variables in Model 2 plus the change in sRAGE level from baseline at 12 weeks (Δ 12W-0W) and baseline sRAGE level

Aβ Amyloid-β, *sRAGE* Soluble receptor for advanced glycation end products

persistently low plasma $A\beta_{42}$ level is often observed in the advanced stages of AD [30–32]. A longitudinal study conducted over nine years suggested that cognitively normal elderly individuals with lower plasma $A\beta_{42}/A\beta_{40}$ ratios experience more severe cognitive decline compared to those with higher ratios [33]. In 2018, Nakamura et al. demonstrated that plasma levels of Aβ biomarkers are closely correlated with brain Aβ deposition [15]. The authors found that plasma $A\beta_{42}$ levels were significantly lower in patients with AD and mild cognitive impairment exhibiting brain Aβ-positive deposition compared to cognitively normal individuals. Moreover, they found

Table 5 GEE analysis of the relationships between blood lipid levels and plasma Aβ levels in the ITT populations

	$A\beta_{42}$		$A\beta_{42}/A\beta_{40}$ ratio		sRAGE	
	β	*P*	β	*P*	β	*P*
All subjects						
TG	−2.50	**0.028**	−0.004	0.460	8.96	0.525
TC	−0.86	0.735	−0.002	0.844	50.97	0.166
LDL-c	−1.74	0.473	−0.009	0.423	−40.63	0.167
HDL-c	−6.36	0.253	0.002	0.935	−93.72	0.274
oxLDL	−0.32	0.079	0.001	0.469	3.08	**0.042**
Probucol						
TG	−1.67	0.366	−0.012	0.173	22.74	0.083
TC	−1.12	0.698	0.000	0.994	67.50	**0.046**
LDL-c	−3.52	0.318	−0.017	0.235	−91.01	**0.011**
HDL-c	−1.58	0.683	0.024	0.208	−52.67	0.270
oxLDL	−0.57	**0.047**	0.001	0.658	4.27	**0.011**
Placebo						
TG	−3.39	**0.042**	0.002	0.630	7.02	0.723
TC	0.24	0.950	0.003	0.850	60.57	0.275
LDL-c	−2.12	0.571	−0.010	0.582	−24.73	0.583
HDL-c	−14.08	0.150	−0.033	0.435	−201.40	0.188
oxLDL	−0.23	0.350	0.001	0.573	1.94	0.425

GEE analysis between an overall estimate of baseline, 6-week and 12-week plasma $A\beta_{42}$ or sRAGE levels and the overall estimate of baseline, 6-week and 12-week blood TC, TG, LDL-c, HDL-c or oxLDL levels. The potential confounders in GEE analysis were age, gender, education years, MMSE score, MAP, BMI, grouping in all subjects (dummy coded with Probucol = 1, placebo = 0), follow-up (dummy coded with baseline = 0, 6W = 1, 12W = 2)

GEE Generalized estimation equation, *MMSE* Mini-Mental State Examination, *TG* Triglycerides, *TC* Total cholesterol, *LDL-c* Low-density lipoprotein cholesterol, *HDL-c* High-density lipoprotein cholesterol, *oxLDL* Oxidized low-density lipoprotein

that the brain amyloid burden could be predicted at the individual level by the combination of a decreased $A\beta_{42}$/$A\beta_{40}$ ratio and $A\beta_{42/APP669-711}$ levels [15]. These findings suggest that increasing peripheral $A\beta_{42}$ levels may lead to a reduction in brain Aβ deposition.

The effects of lipid-lowering drugs on AD have not yet been clearly elucidated. Given the correlation between Aβ metabolism in peripheral blood and brain Aβ deposition, numerous studies have focused on the relationship between lipid-lowering drugs and plasma Aβ metabolism. In a placebo-controlled trial, serum Aβ levels were found to decrease after 12 weeks of lovastatin treatment in patients with elevated LDL-c levels [34]. However, another study reported that although statin treatment reduced TC levels in hypercholesterolemic patients, plasma Aβ levels remained unaffected [35].

Probucol has been shown to modulate the Aβ metabolism in both the brain and peripheral circulation [21]. Takechi et al. demonstrated that Probucol could mitigate cholesterol-induced elevations of Aβ in the brain and prevent dysfunction of cerebral capillary by inhibiting Aβ secretion [36]. In the present study, it was discovered that the change in $A\beta_{42}$ levels from baseline to the 6-week did not differ between the groups receiving Probucol and those receiving a placebo. However, after 12 weeks of treatment, the elevation of plasma $A\beta_{42}$ levels from baseline was more pronounced in participants treated with Probucol compared to those receiving the placebo. These findings suggest that daily oral administration of Probucol for 12 weeks significantly elevates the plasma $A\beta_{42}$ level.

sRAGE, a transport protein of Aβ in the peripheral circulation, plays a major role in reducing Aβ deposition and facilitating the metabolism of plasma Aβ. As a "decoy receptor", sRAGE prevents soluble Aβ in the blood from interacting with RAGE, thereby reducing the backflow of peripheral Aβ into the central nervous system [17]. Furthermore, sRAGE enhances the degradation of blood Aβ by binding to it [18]. In the present study, the effect of Probucol on plasma $A\beta_{42}$ levels diminished substantially when adjusted for the change in sRAGE levels, suggesting that the effect of Probucol on plasma $A\beta_{42}$ levels is largely mediated by alterations in sRAGE.

Although existing studies have indicated that low-density lipoprotein receptor-related proteins on the BBB play a critical role in the clearance of free Aβ from the brain into the peripheral blood [37], no significant association between sLRP1 and plasma Aβ was observed in the present study. This discrepancy may be related to the diverse forms of sLRP1. Oxidized sLRP1, as opposed to simply sLRP1, may be a key determinant of plasma Aβ levels. Unlike sLRP1, which binds approximately 70% of free Aβ in the peripheral circulation, oxidized sLRP1 has extremely low affinity for circulating Aβ, thereby diminishing the normal sink effect of sLRP1 on Aβ clearance [38]. Consequently, future investigations into Aβ clearance mechanisms should focus on quantifying oxidized sLRP1 to better understand the potential role of antioxidants, such as Probucol, in mitigating AD pathology.

Emerging evidence suggests that extensive lipid peroxidation occurs prior to cognitive decline and Aβ deposition, contributing to the pre-clinical stages of AD. Elevated levels of oxLDL have been shown to significantly increase the risk of AD, particularly in males with cardiovascular disease [39]. Meanwhile, a pilot study demonstrated that blood oxLDL levels were significantly elevated in AD patients compared to matched controls [40]. On the one hand, oxLDL may promote Aβ production via lipid raft formation and disrupt proteasomal activity, thereby preventing cells from degrading Aβ [41]. Moreover, membrane-associated oxidative stress further perturbs cholesterol metabolism, initiating a neurodegenerative cascade that results in additional Aβ accumulation [42]. On the other hand, the translocation of Aβ from the brain into the peripheral circulation is influenced by both the structural integrity and functional capacity of the BBB [43]. Endothelial cells of the BBB are particularly susceptible to damage induced by oxidative stress, which further compromises Aβ clearance [44].

Probucol has been found to prevent the oxidation of low-density lipoproteins to oxLDL during lipid metabolism [20], thereby mitigating oxidative stress in the central nervous system. The antioxidant and anti-inflammatory properties of Probucol can help preserve the integrity of the BBB in wild-type mice fed high-cholesterol diets [21]. This protective effect appears to result from the direct inhibition of inflammatory and oxidative stress pathways rather than from decreased exposure to plasma lipids.

Kotani et al. reported that sRAGE in blood exhibits an inverse correlation with oxLDL in cognitively normal individuals [45]. The most widely accepted theory is that oxLDL acts as a ligand for RAGE, and increased production of oxLDL may lead to the consumption of sRAGE in patients experiencing elevated oxidative stress and inflammation. In the current study, the oxLDL level was decreased more significantly from baseline in the Probucol group, while the reduction in sRAGE from baseline was less pronounced. Furthermore, GEE analysis revealed a correlation between the levels of oxLDL and sRAGE. These findings suggest that Probucol may exert its antioxidant effects by lowering oxLDL, thereby reducing sRAGE consumption and, consequently, improving plasma Aβ transport.

The trends observed in plasma Aβ and blood lipid levels in the present study are noteworthy. First, the decrease in lipid levels and the increase in plasma Aβ levels were more pronounced during the first 6 weeks of treatment compared to

the subsequent 6 weeks. The observed increase in Aβ levels during the first 6 weeks could be attributed to the marked lipid-lowering effect of Probucol during this period. Second, trends in lipid levels were similar between the placebo and Probucol groups, with the exception of oxLDL. This phenomenon can be attributed to the placebo effect. In previous randomized trials of lipid-lowering therapies, placebo recipients were equally concerned about their lipid levels, which may have prompted increased physical activity and reduced consumption of high-cholesterol foods [46]. These positive lifestyle modifications could account for the similar trends in blood lipid levels observed between the two groups.

Study strengths and limitations

To our knowledge, this is the first study in humans to investigate the effects of Probucol on peripheral Aβ metabolism. Utilizing a double-blind, placebo-controlled design within a middle-aged and elderly cohort, we aimed to confirm the modulation of Aβ clearance by Probucol, a phenomenon previously demonstrated in animal models. Secondly, to elucidate a potential antioxidative role of Probucol in Aβ transport as a mechanistic pathway for preventing AD, the oxidative stress marker oxLDL was specifically measured, in addition to two major Aβ transporters. Thirdly, plasma biomarkers were measured uniformly in duplicate across three visits to minimize batch effects and ensure the homogeneity of plasma measurements. Fourthly, multiple linear regression analysis, GEE analysis, and subgroup analysis were employed to adjust for potential covariates and enhance the robustness of the results.

Several limitations of this study need to be mentioned. First, the effects of Probucol on Aβ metabolism were observed only in a cognitively normal population. Given the potential correlation between plasma Aβ levels and cognitive function, the effects of Probucol on peripheral Aβ transport should be further explored in elderly individuals with cognitive dysfunction. In addition, amyloid plaque deposition in the central nervous system was not assessed in this study. Since plasma Aβ levels are influenced by various factors, the conclusion that Probucol may elevate plasma Aβ levels by facilitating the transport of Aβ from the brain to the peripheral circulation should be corroborated by direct measures of cerebral amyloid burden. Third, owing to the relatively small sample size and the short follow-up duration, the observed effects of Probucol on plasma Aβ were modest. Further investigations with larger sample sizes and extended follow-up periods are required to fully assess the role of Probucol in Aβ metabolism.

Conclusion

Daily oral administration of Probucol (1000 mg) for 12 weeks significantly elevated plasma $A\beta_{42}$ levels by influencing sRAGE in hyperlipidemic patients, and this effect was associated with reductions in both LDL-c and oxLDL. These findings suggest that Probucol may modulate plasma Aβ transport through its antioxidant and lipid-lowering effects. Additionally, the observed correlation between plasma Aβ and blood lipids in hyperlipidemic patients warrants further investigation, as this relationship may be influenced by lipid-lowering therapies. Overall, Probucol may have the potential as a therapeutic agent that could mitigate the pathological processes associated with AD.

Abbreviations

AD	Alzheimer's disease
Aβ	Amyloid-β
sLRP1	Soluble low-density lipoprotein receptor-related protein-1
sRAGE	Soluble receptor of advanced glycation end products
oxLDL	Oxidized low-density lipoprotein
BBB	Blood–brain barrier
TC	Total cholesterol
TG	Triglyceride
LDL-c	Low-density lipoprotein cholesterol
MMSE	Mini-mental state examination
HDL-c	High-density lipoprotein cholesterol
ITT	Intention-to-treat
PP	Per protocol
GEE	Generalized estimation equation

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12944-024-02398-1.

Supplementary Material 1: Table S1. Comparison of blood lipid levels before and after treatment in the Probucol and placebo groups.

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Authors' contributions

DLJ took part in the survey, did the statistical analysis and wrote the manuscript. WS contributed to the treatment of the blood specimens and performed data acquisition. WJ and GF designed the study, collected and took part in the statistical analysis. ZY, ZR SSH, and WJY took part in the survey and collected samples. QQM provided technical guidance in all stages of the study. All authors have read and approved the final article.

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Data availability

The data used in this study are available from the corresponding author if needed.

Declarations

Ethics approval and consent to participate

The study complied with the principles of Declaration of Helsinki as revised in 1989. The protocol was approved by the Medical Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University. All participants were aware of the research purpose and signed written informed consent forms prior to study initiation.

Consent for publication

All the participants provided written informed consent for the publication of the results of this study.

Competing interests
The authors declare no competing interests.

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