Effects of 1-H-indole-3-glyoxamide (A-002) on concentration of secretory phospholipase A_2 (PLASMA study): a phase II double-blind, randomised, placebo-controlled trial

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Summary

Background Secretory phospholipase A_2 ($sPLA_2$) enzymes, produced and secreted in human blood vessels and hepatocytes, contribute to the development of atherosclerosis through mechanisms that are both dependent and independent of lipoprotein. We examined the effects of an $sPLA_2$ inhibitor on enzyme concentration and on plasma lipoproteins and inflammatory biomarkers in patients with coronary heart disease.

Methods Patients aged 18 years and older with stable coronary heart disease from the USA and Ukraine were eligible for enrolment in this phase II, randomised, double-blind, placebo-controlled, parallel-arm, dose-response study. 393 patients were randomly assigned by computer-generated sequence to receive either placebo (n=79) or one of four doses of an $sPLA_2$ inhibitor, A-002 (1-H-indole-3-glyoxamide; 50 mg [n=79], 100 mg [n=80], 250 mg [n=78], or 500 mg [n=77] twice daily), for 8 weeks. The primary endpoint was the change in $sPLA_2$ group IIA ($sPLA_2$-IIA) concentration or activity from baseline to week 8. Analysis was by modified intention to treat (ITT). The ITT population consisted of all patients who received one dose of study treatment; data for patients who dropped out before the end of the study were carried forward from last observation. This trial is registered with ClinicalTrials.gov, number NCT00455546.

Findings All randomised patients received at least one dose and were included in the ITT population. Data for 45 patients were carried forward from last observation (36 in the A-002 group and nine in the placebo group); the main reason for dropout before completion was because of adverse events. 348 patients reached the primary endpoint (A-002 n=278, placebo n=70). Mean $sPLA_2$-IIA concentration fell by 86·7%, from 157 pmol/L to 21 pmol/L, in the overall active treatment group, and by 4·8%, from 157 pmol/L to 143 pmol/L, in the placebo group ($p<0·0001$ treatment *vs* placebo). The reductions in $sPLA_2$-IIA concentration in the A-002 groups were dose dependent (ranging from 69·2% in the 50 mg group to 95·8% in the 500 mg group) and differed significantly from placebo ($p<0·0001$ for all doses). In the 500 mg A-002 treatment group, there was one serious adverse event (exacerbation of underlying chronic obstructive pulmonary disease), but the proportion of patients reporting treatment-emergent adverse events did not differ from placebo. The main side-effects of the drug included headache (n=20), nausea (n=17), and diarrhoea (n=12).

Interpretation The reductions in $sPLA_2$-IIA concentration suggest that A-002 might be an effective anti-atherosclerotic agent.

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Introduction

Secretory phospholipase A_2 ($sPLA_2$) enzymes hydrolyse the ester bond of phospholipid molecules at the *sn*-2 position to produce two potentially bioactive lipids that include non-esterified fatty acids (mainly arachidonic acid) and lysophospholipids.[1] Of the ten-member family of $sPLA_2$ enzymes, groups IIA ($sPLA_2$-IIA),[2–4] V ($sPLA_2$-V),[5,6] and X ($sPLA_2$-X)[7] are highly expressed in human and mouse atherosclerotic lesions in which the various groups contribute differentially to atherogenesis.[5,7,8]

$sPLA_2$-IIA-modified LDL particles have impaired binding affinity to LDL receptors and long residence time in the circulation.[9,10] Compositional changes of apolipoprotein B in $sPLA_2$-modified LDL mediate increased binding to human aortic proteoglycans.[5,9,11,12] The anchoring of $sPLA_2$ to proteoglycans results in further remodelling of intimal LDL particles with eventual formation of LDL aggregates[13] that are rapidly cleared by tissue macrophages, leading to foam cell formation.[7,13] $sPLA_2$-X contributes to foam cell formation in apolipoprotein-E-deficient mice,[7] and expression of macrophage-specific human $sPLA_2$-IIA in LDL-receptor-deficient mice increases atherosclerotic lesion size and enhances collagen deposition.[14] $sPLA_2$-modified lipoproteins result in a highly oxidised LDL particle[13] that activates inflammatory pathways. Through these mechanisms, the various $sPLA_2$ isoenzymes have a

crucial role in lipoprotein modification, retention, and macrophage uptake.[15]

High $sPLA_2$-IIA concentrations can predict coronary heart disease events in patients with stable coronary artery disease[16,17] and unstable angina,[18] and all-cause mortality in patients with acute myocardial infarction.[19] Raised $sPLA_2$-IIA concentration[16] and activity[20] is also associated with increased risk of incident coronary heart disease events in apparently healthy men and women.

A-002 (1-H-indole-3-glyoxamide, varespladib methyl; Anthera Pharmaceuticals, San Mateo, CA, USA), is a novel selective $sPLA_2$ inhibitor in human beings[21] with specificity towards $sPLA_2$-IIA (50% inhibitory concentration [IC_{50}] 9–14 nmol/L), $sPLA_2$-V (IC_{50} 77 nmol/L), and $sPLA_2$-X (IC_{50} 15 nmol/L) enzymes (data on file Anthera Pharmaceuticals, San Mateo, CA, USA). We examined the effects of A-002 on $sPLA_2$ concentration and activity and on plasma lipoproteins and inflammatory biomarkers in patients with coronary heart disease.

Methods

Patients

We undertook a phase II, randomised, double-blind, placebo-controlled, dose-response study (Phospholipase Levels and Serological Markers of Atherosclerosis [PLASMA]) in outpatients in the USA and Ukraine from April, 2007, to November, 2007. Eligible patients were men and women aged 18 years or older with stable coronary heart disease, defined as previous myocardial infarction (more than 12 weeks before inclusion), unstable angina (more than 6 weeks before inclusion), objective evidence of atherosclerotic coronary artery disease, or a previous revascularisation procedure (more than 12 weeks before inclusion). Major exclusion criteria included active inflammatory diseases and drugs likely to modulate the inflammatory response; aspirin doses less than 350 mg per day were allowed. All patients provided written informed consent before being enrolled. The study protocol was approved by local and national ethics committees in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Procedures

After screening, enrolled patients were randomised to receive treatment with either placebo or one of four doses of A-002 (50 mg, 100 mg, 250 mg, or 500 mg twice daily) for 8 weeks. The randomisation code was computer generated and patients were allocated treatment via an interactive voice response system (IVRS). The code and IVRS were the work of United Biosource Corporation (San Francisco, CA, USA), who had no other role in the study. Blinding was maintained by use of a matching placebo that did not differ in taste, colour, appearance, or any other physical characteristic from the active drug. We obtained safety and efficacy data at baseline, and at weeks 2, 4, and 8. All blood samples were taken at the time of the study visit.

The primary endpoint was the change from baseline to week 8 in $sPLA_2$ concentration or activity. Secondary endpoints were the change from baseline to week 8 in inflammatory markers, lipid and biochemical indices, lipoprotein subclasses, and oxidised LDL. Data were analysed on a modified intention-to-treat (ITT) basis. The ITT population consisted of all patients who received one dose of study treatment or placebo; data for patients who dropped out before the end of the study were carried forward from last observation.

We used treatment-emergent adverse events, serious adverse events, electrocardiographic and haemodynamic data, clinical chemistry, and haematological measurements to assess safety. Adverse events were coded according to the MedDra dictionary version 10.0 and categorised by body system and preferred term, by intensity, and by causal relation to study agent. All adverse events that occurred from the first dose of study drug until 14 days after the last dose of study drug were assessed. Efficacy and safety data were also examined according to a prespecified subgroup analysis of patients receiving statin therapy.

Plasma lipids, standard biochemical tests, and C-reactive protein were measured by standard procedures (Quest Diagnostics, Van Nuys, CA, USA). LDL-cholesterol concentrations were calculated with the Friedewald equation.[22] We measured lipoprotein subclass profiles with an automated nuclear magnetic resonance spectroscopic assay (LipoScience Inc, Raleigh, NC, USA).[23] sPLA2 concentration was analysed by a quantitative two-site EIA (Cayman Chemical, Ann Arbor, MI, USA). sPLA2-IIA activity was measured by radiometric C14 assay as previously described.[24] Briefly, this method quantifies hydrolysis of fatty acid from the 2-acyl bond of phospholipids.

We measured oxidised LDL by two-site ELISA (Mercodia, Uppsala, Sweden) with the specific antibody 4E6 and a second monoclonal antibody generated against a different epitope. Plasma arachidonic acid concentrations were measured by a modified method of Nyyssönen and colleagues.[25] Leucotriene B4 was measured by competitive EIA with a specific monoclonal antibody (Cayman Chemical, Ann Arbor, MI, USA). LDL particle size was quantified by nuclear magnetic resonance spectroscopy at LipoScience, Raleigh, NC, USA.

Statistical analysis

Primary efficacy analysis was the comparison of changes in $sPLA_2$-IIA concentration from baseline to week 8 between the pooled A-002 and the placebo groups. For testing within treatment groups, the paired *t* test was used to compare each value at week 8 to the value at baseline. If normality assumptions failed, the Wilcoxon signed-rank test was used. Percentage changes were first calculated as individual percentage change for each

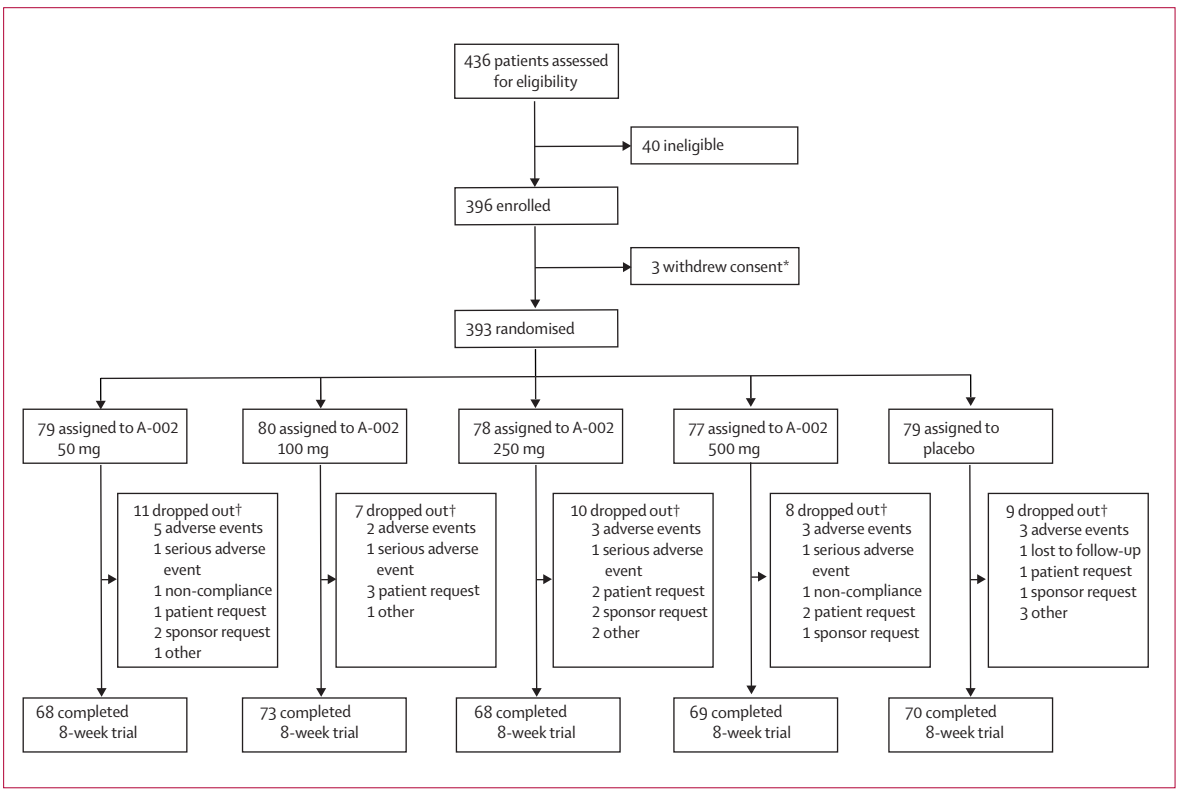

Figure 1: **Trial profile**
*No reasons for withdrawal were given. †Data from last observation carried forward.

participant and then summarised by descriptive statistics. Treatment differences were expressed as least square mean differences with standard deviations. We used SAS software version 9.1.3 for the data analysis.

Secondary efficacy analyses included pair-wise comparisons of each dose of A-002 with all higher doses of the drug, and comparison of changes from baseline to week 2, 4, and 8 between each treatment group and the placebo group. We derived summary descriptive statistics for individual timepoints and for change and percentage change from baseline. C-reactive protein concentrations of 398 nmol/L or more were excluded because of their high association with intercurrent non-cardiovascular illnesses,[26] and the remaining values were log transformed.

Since this was a phase II clinical pharmacology study, no formal sample size or statistical power calculations were undertaken. Assuming a dropout rate of 25%, we estimated that a sample size of 120–160 participants was needed to provide 80% power to detect a difference of 0·68 U/L in change in $sPLA_2$-IIA activity assuming a standard deviation of 1·7 U/L. This trial is registered with ClinicalTrials.gov, number NCT00455546.

Role of the funding source

The sponsor medical doctor (CH) was a member of the steering committee and together with other members provided feedback on study design, analysis, interpretation, and writing of the final report. Statistical analysis was done by an independent organisation (Pharm-Olam International, Houston, TX, USA). The corresponding author (RSR) had full access to all the data in the study, and had final responsibility for the decision to submit the manuscript for publication.

Results

The trial profile is shown in figure 1. 396 patients with stable coronary heart disease were recruited; 275 were from sites in the USA and 121 from sites in Ukraine. 393 were randomly assigned to treatment and received one dose of study treatment or placebo. 348 (89%) patients completed the trial. Data for 45 patients were carried forward from last observation (36 in the A-002 group and nine in the placebo group); the main reason for dropout before completion was because of adverse events. Table 1 shows baseline demographics of patients assigned to the A-002 and placebo groups.

Mean $sPLA_2$-IIA concentration fell by 136 pmol/L (86·7%) from baseline to week 8 in the overall A-002 group, compared with 14 pmol/L (4·8%) in the placebo group ($p<0·0001$ treatment *vs* placebo; table 2). The decreases in $sPLA_2$-IIA concentration in the A-002 groups were dose dependent, ranging from 69·2% in

	A-002					Placebo (N=79)
	50 mg (N=79)	100 mg (N=80)	250 mg (N=78)	500 mg (N=77)	Overall (N=314)	
Age (years)	62 (10)	64 (10)	63 (10)	61 (11)	62 (10)	62 (11)
Male	62 (78%)	58 (73%)	65 (83%)	63 (82%)	248 (79%)	60 (76%)
White*	79 (100%)	76 (95%)	74 (95%)	76 (99%)	305 (97%)	75 (95%)
BMI	30·4 (5·9)	30·6 (6·3)	29·9 (5·4)	30·4 (5·1)	30·3 (5·7)	30·5 (5·3)
Cardiovascular history						
Myocardial infarction	52 (66%)	54 (68%)	47 (60%)	54 (70%)	207 (66%)	53 (67%)
CABG	54 (68%)	56 (70%)	55 (71%)	53 (69%)	218 (69%)	52 (66%)
Hypertension	70 (89%)	64 (80%)	67 (86%)	61 (79%)	262 (83%)	57 (72%)
Diabetes	16 (20%)	28 (35%)	20 (26%)	18 (23%)	82 (26%)	20 (25%)
Medical treatment						
Aspirin	74 (94%)	74 (93%)	68 (87%)	72 (94%)	288 (92%)	67 (85%)
Thienopyridine†	20 (25%)	12 (15%)	26 (33%)	29 (38%)	87 (28%)	22 (28%)
β blockers	52 (66%)	66 (83%)	60 (77%)	54 (70%)	232 (74%)	47 (59%)
ACE inhibitor or ARB	45 (57%)	42 (53%)	47 (60%)	41 (53%)	175 (56%)	43 (54%)
Statin (%)						
Total population	49 (62%)	54 (68%)	53 (68%)	52 (68%)	208 (66%)	51 (65%)
US subgroup	46/52 (88%)	51/56 (91%)	47/54 (87%)	50/55 (91%)	194/217 (89%)	48/56 (86%)

Data are n (%) or mean (SD). ACE=angiotensin-converting enzyme. ARB=angiotensin-receptor blocker. BMI=body-mass index. CABG=coronary artery bypass graft. *Race was established by the investigator. †108 participants were on clopridogrel and one on ticlopidine.

Table 1: **Patient demographics and baseline characteristics**

	A-002					Placebo (N=79)
	50 mg (N=79)	100 mg (N=80)	250 mg (N=78)	500 mg (N=77)	Overall (N=314)	
Baseline	150 (14 to 629)	157 (4 to 1593)	143 (14 to 429)	171 (29 to 386)	157 (4 to 1593)	157 (6 to 486)
2 weeks	64 (3 to 285)	36 (3 to 435)	14 (0 to 314)	11 (3 to 392)	29 (0 to 436)	157 (43 to 407)
Difference from baseline	–62·1% (–95 to 223)	–75·9% (–99 to 5700)	–89·4% (–100 to 59)	–92·1% (–99 to 225)	–82·8% (–100 to 5700)	5·3% (–91 to 488)
4 weeks	50 (4 to 300)	21 (0 to 1293)	21 (4 to 192)	4 (0 to 600)	21 (0 to 1293)	157 (4 to 1136)
Difference from baseline	–67·8% (–95 to 35)	–84·2% (–100 to 723)	–90·0% (99 to 21)	–95·8% (–99 to 150)	–86·4% (–100 to 723)	5·0% (–81 to 775)
8 weeks	50 (4 to 386)	21 (4 to 207)	21 (4 to 336)	6 (0 to 200)	21 (0 to 386)	143 (43 to 421)
Difference from baseline	–69·2% (–99 to 108)	–82·7% (–99 to 1500)	–87·9% (–98 to 57)	–95·8% (–100 to 13)	–86·7% (–100 to 1500)	–4·8% (–65 to 263)

Data are mean in pmol/L (SD). Intention-to-treat population included all patients who received at least one dose of study treatment or placebo; data for patients who dropped out before the end of the study were carried forward from last observation. Absolute and percentage change in $sPLA_2$-IIA concentration from baseline p<0·0001 for all A-002 groups at all timepoints. Absolute and percentage change in $sPLA_2$-IIA concentration from baseline in A-002 groups p<0·0001 compared with placebo for all doses at all timepoints.

Table 2: **Plasma concentrations of $sPLA_2$-IIA at baseline and 2, 4, and 8 weeks after treatment or placebo**

the 50 mg dose group to 95·8% in the 500 mg group. The reductions in $sPLA_2$-IIA concentration in all treatment dose groups differed significantly from placebo at weeks 2, 4, and 8 (p<0·0001 for all doses; table 2); the largest decrease occurred between baseline and week 2.

We were unable to measure the enzyme inhibitory effect of A-002 because the concentration of $sPLA_2$ was less than the threshold (1071 pmol/L) required for quantification of activity by the assay. As a result, only background phospholipase A_2 activity was detected in our samples.

Table 3 and table 4 show the concentrations of lipids and lipoproteins in the treatment and placebo groups. Mean LDL cholesterol was lower at weeks 2, 4, and 8 than at baseline for all A-002 treatment doses (figure 2). A 0·3 mmol/L (8·0%) reduction from baseline to week 8 was seen in the overall treatment group, compared with a 0·1 mmol/L change (1·7% increase) in the placebo group (p=0·0035 treatment *vs* placebo). The reductions in total, LDL, and non-HDL cholesterol from baseline to week 8 differed from placebo in the 50 mg, 250 mg, 500 mg, and the overall treatment groups (table 3).

In A-002-treated patients, median LDL particle concentration fell by 80·0 nmol/L (differences calculated by use of individual datapoints; p=0·021 *vs* placebo), mainly resulting from a reduction in small LDL particle concentration (58·0 nmol/L, p=0·009 *vs* placebo). By contrast, large LDL particle concentration remained unchanged (0·0 nmol/L, p=0·07). Mean LDL particle size remained unchanged in the A-002 group, whereas it

	A-002					Placebo (N=79)
	50 mg (N=79)	100 mg (N=80)	250 mg (N=78)	500 mg (N=77)	Overall (N=314)	
Total cholesterol (mmol/L)						
Baseline	4·5 (1·4)	4·6 (1·3)	4·5 (1·2)	4·6 (4·6)	4·5 (1·3)	4·5 (1·3)
8 weeks	4·3 (1·4)*†	4·4 (4·4)‡	4·1 (4·1)†§	4·1 (4·1)§¶	4·2 (4·2)§¶	4·5 (4·5)
Difference	−4·5% (0·5)*†	−3·0% (0·5)‡	−6·6% (0·3)†§	−7·6% (0·6)§¶	−5·4% (0·5)§¶	−0·5% (0·4)
LDL cholesterol (mmol/L)						
Baseline	2·4 (1·1)	2·6 (1·1)	2·4 (1·0)	2·5 (1·2)	2·5 (1·1)	2·5 (1·2)
8 weeks	2·2 (1·2)*†	2·4 (1·3)‡	2·2 (0·9)†§	2·2 (1·0)§¶	2·2 (1·1)§¶	2·4 (1·1)
Difference	−7·2% (0·7)†‖	−6·3% (0·6)‡	−8·2% (0·5)*†	−10·2% (0·6)§¶	−8·0% (0·6)§¶	1·7% (0·8)
Non-HDL cholesterol (mmol/L)						
Baseline	3·3 (1·4)	3·4 (1·2)	3·2 (1·1)	3·3 (1·5)	3·3 (1·3)	3·2 (1·3)
8 weeks	3·1 (1·4)*†	3·2 (1·4)‡	2·9 (1·0)§¶	2·9 (1·3)§¶	3·0 (1·3)§¶	3·2 (1·2)
Difference	−3·9% (0·7)†‖	−4·2% (0·6)‡	−7·7% (0·4)*¶	−7·7% (0·7)§¶	−5·9% (0·6)§**	1·4% (0·5)
LDL size (nm)						
Baseline	20·53 (0·10)	20·54 (0·09)	20·54 (0·10)	20·54 (0·09)	20·54 (0·05)	20·70 (0·09)
8 weeks	20·63 (0·09)†	20·39 (0·09)	20·53 (0·08)	20·60 (0·09)†	20·54 (0·04)†	20·45 (0·09)‡
Difference	0·6% (0·36)†	−0·4% (0·30)	0·1% (0·23)	0·2% (0·30)†	0·1% (0·15)†	−0·8% (0·35)‡
LDL particles (nmol/L)						
Total						
Baseline	1076·5 (344 to 2531)	1105·0 (502 to 2443)	1043·5 (451 to 2422)	1076·0 (445 to 2410)	1085·0 (344 to 2531)	1065·0 (509 to 2641)
8 weeks	995·5 (367 to 2544)¶‖	1038·0 (505 to 2582)	1043·0 (500 to 2278)‡	1020·0 (487 to 2425)	1020·0 (367 to 2582)†§	1124·0 (560 to 2853)
Difference	−11·0% (−54 to 123)¶‖	−6·2% (−63 to 100)	−4·6% (−29 to 46)	−5·2% (−75 to 129)	−6·6% (−75 to 129)†‖	0·8% (−51 to 130)
Small						
Baseline	755·0 (16 to 2251)	779·0 (57 to 2143)	741·5 (29 to 2185)	838·0 (48 to 1980)	777·0 (16 to 2251)	785·0 (27 to 2067)
8 weeks	680·0 (2 to 1847)¶‖	798·0 (133 to 2331)	712·0 (239 to 1872)	738·0 (14 to 2025)†	733·0 (2 to 2331)¶‖	844·0 (73 to 2436)
Difference	−14·9% (−88 to 743)‡¶	−5·2% (−66 to 361)	−7·7% (−37 to 584)	−6·9% (−78 to 439)†	−7·3% (−88 to 743)¶	2·4% (−59 to 451)
Large						
Baseline	220·0 (1 to 979)	281·0 (10 to 1122)	200·5 (24 to 896)	226·0 (0 to 814)	226·0 (0 to 1122)	265·0 (0 to 1160)
8 weeks	241·0 (0 to 1056)†	214·0 (0 to 1179)	250·0 (10 to 740)	240 (20 to 882)	229·0 (0 to 1179)	213·0 (0 to 1064)‡
Difference	7·6% (−100 to 333)†	−16·6% (−100 to 304)	2·9% (−86 to 411)†	1·0% (−94 to 1022)†	−0·3% (−100 to 1022)†	−13·3% (−100 to 241)
Apolipoprotein B (μmol/L)						
Baseline	3·72 (0·2)	3·84 (0·2)	3·84 (0·2)	3·92 (0·2)	3·84 (0·1)	3·92 (0·2)
8 weeks	3·44 (0·1)‡	3·68 (0·2)	3·56 (0·1)†‖	3·60 (0·2)†‡	3·56 (0·1)†§	3·96 (0·2)
Difference	−1·7% (0·1)	−0·0% (0·1)	−4·0% (0·1)‡	−1·7% (0·2)†	−1·8% (0·1)*†	3·0% (0·1)
Oxidised LDL (mU/L)						
Baseline	60·8 (22·6)	60·1 (18·6)	59·5 (19·9)	60·2 (19·3)	60·2 (20·0)	61·8 (21·0)
8 weeks	57·5 (19·1)‡	56·5 (20·2)†‖	55·4 (17·6)*†	56·3 (19·4)‖	56·4 (19·0)†§	61·5 (20·3)
Difference	−0·5% (29·2)	−5·1% (21·3)†‖	−5·1% (18·1)*¶	−4·3% (24·3)†‡	−3·8% (23·6)§¶	0·5% (15·3)

Data are least square means (SD) or median (IQR). Intention-to-treat population included all patients who received at least one dose of study treatment or placebo; data for patients who dropped out before the end of the study were carried forward from last observation. Percentage changes were first calculated as individual percentage change for each participant and then summarised by descriptive statistics.
*p<0·001 versus baseline. †p<0·05 versus placebo. ‡p<0·05 versus baseline. §p<0·0001 versus baseline. ¶p<0·01 versus placebo. ||p<0·01 versus baseline. **p<0·001 versus placebo.

Table 3: **Plasma concentrations of LDL at baseline and end of treatment or placebo**

reduced by 0·17 nm in the placebo group (p=0·02 *vs* baseline).

In the overall A-002 group, mean oxidised LDL fell by 3·8 mU/L from baseline to week 8 (p=0·0001; table 3). The absolute reduction in oxidised LDL concentration differed from baseline and from placebo only for treatment doses of 100 mg and 250 mg.

Arachidonic acid concentrations were unchanged in the treatment and placebo groups. Treatment with A-002 significantly reduced leucotriene B4 concentration from baseline in a dose-dependent manner in all dose groups apart from the 50 mg group. However, the reductions did not differ from those with placebo (data not shown).

Mean C-reactive protein concentration at baseline ranged from 14·3 nmol/L to 22·7 nmol/L. At week 8, it was 12·0 nmol/L (SD 0·93) in the overall A-002 group (55·6% reduction, p<0·0001 *vs* baseline) and 17·1 nmol/L (0·91) in the placebo group (24·8% reduction, p=0·288 *vs* baseline; treatment *vs* placebo p=0·477).

In 106 patients in the overall A-002 group who were not receiving statin therapy, mean $sPLA_2$-IIA concentration fell by 78·6% for the overall A-002 group (66·7% for the 50 mg group, 78·3% 100 mg, 85·3% 250 mg, and 96·0% 500 mg).

Although the reduction in mean LDL cholesterol was different from placebo for treatment doses of 50 mg (difference from placebo –4·8%, p=0·041) and 500 mg (–12·2%, p=0·031), the reduction in the overall treatment group did not differ from placebo (–4·3%, p=0·075). No

	A-002					Placebo (N=79)
	50 mg (N=79)	100 mg (N=80)	250 mg (N=78)	500 mg (N=77)	Overall (N=314)	
IDL particles (nmol/L)						
Baseline	23·0 (0 to 236)	31·0 (0 to 198)	33·5 (0 to 193)	23·0 (0 to 376)	28·0 (0 to 376)	34·0 (0 to 262)
8 weeks	22·5 (0 to 257)	31·0 (0 to 195)	27·0 (0 to 126)*	21·0 (0 to 138)	25·0 (0 to 257)*	23·0 (0 to 177)
Difference	–30·3% (–100 to 3000)	–24·2% (–100 to 1386)	–35·7% (–100 to 1100)	–43·6% (–100 to 729)	–33·1% (–100 to 3000)†	–22·2% (–100 to 950)
VLDL cholesterol (mmol/L)						
Baseline	0·81 (0·4)	0·76 (0·3)	0·80 (0·4)	0·77 (0·4)	0·79 (0·4)	0·70 (0·3)
8 weeks	0·78 (0·4)	0·76 (0·3)	0·75 (0·3)	0·73 (0·4)‡	0·75 (0·3)	0·76 (0·4)
Difference	5·4% (1·2)	8·2% (1·1)	0·4% (0·8)	2·0% (1·1)	4·0% (1·0)	9·8% (0·8)†
VLDL particles (nmol/L)						
Total						
Baseline	73·1 (0·0 to 321·0)	77·5 (10·0 to 167·8)	68·7 (9·0 to 220·2)	73·3 (4·8 to 334·4)	73·1 (0·0 to 334·4)	60·7 (7·3 to 190·2)
8 weeks	75·2 (3·3 to 227·6)	70·60 (2·8 to 318·4)‡	70·7 (11·9 to 211·7)‡	58·3 (5·6 to 189·8)§¶	68·7 (2·8 to 318·4)§‖	66·2 (6·3 to 235·4)
Difference	–4·9% (–94 to 341)	–6·4% (–73 to 641)	–9·9% (–75 to 221)‡	–24·1% (–82 to 213)‖	–9·9% (–94 to 641)‖	11·8% (–70 to 788)†
Small						
Baseline	36·5 (0·0 to 113·1)	38·6 (2·6 to 95·0)	37·6 (8·5 to 79·8)	37·7 (0·0 to 130·3)	37·6 (0·0 to 130·3)	28·8 (0·0 to 91·2)
8 weeks	35·3 (1·1 to 96·3)	35·0 (2·2 to 90·7)‡	32·6 (1·2 to 75·9)*‖	31·3 (0·0 to 83·8)*‖	33·5 (0·0 to 96·3)‖**	33·8 (0·0 to 112·9)
Difference	–11·7% (–94 to 8700)	–14·6% (–94 to 283)	–17·4% (–95 to 140)*‖	–22·6% (–100 to 5200)‡	15·2% (–100 to 8700)*‖	9·6% (–87 to 1256)
Large						
Baseline	1·7 (0·0 to 34·1)	1·8 (0·0 to 21·7)	2·7 (0·0 to 30·3)	2·2 (0·0 to 48·8)	1·9 (0·0 to 48·8)	1·5 (0·0 to 31·3)
8 weeks	1·6 (0·0 to 60·2)	2·2 (0·0 to 112·0)	1·9 (0·0 to 24·5)	1·8 (0·1 to 43·4)	1·9 (0·0 to 112·0)	1·6 (0·0 to 26·5)
Difference	–9·0% (–100 to 3400)	0·0% (–100 to 1700)†	–14·3% (–100 to 2200)	0·0% (–97 to 6700)	–0·8% (–100 to 6700)	0·0% (–100 to 7100)
HDL cholesterol (mmol/L)						
Baseline	1·2 (0·4)	1·2 (0·3)	1·2 (0·4)	1·3 (0·4)	1·2 (0·4)	1·3 (0·3)
8 weeks	1·2 (0·4)	1·2 (0·3)	1·2 (0·4)	1·2 (0·3)†	1·2 (0·3)*	1·2 (0·3)
Difference	–1·5% (0·4)	1·7% (0·5)	–2·5% (0·3)	–3·9% (0·4)†	–1·5% (0·4)*	–3·8% (0·3)
HDL particles (µmol/L)						
Total						
Baseline	30·9 (15·2 to 46·0)	31·0 (18·4 to 42·6)	30·4 (20·2 to 47·1)	31·3 (22·9 to 44·2)	30·8 (15·2 to 47·1)	31·9 (15·6 to 44·8)
8 weeks	30·1 (12·2 to 44·6)†	31·3 (16·6 to 43·8)	29·0 (19·9 to 43·7)*	29·9 (20·7 to 38·8)	30·1 (12·2 to 44·6)§	29·8 (17·6 to 44·5)†
Difference	–3·0% (–36 to 23)†	0·3% (–27 to 39)	–3·6% (–37 to 17)*	–1·2% (–42 to 32)	–2·3% (–42 to 39)§	–1·6% (–29 to 35)†
Small						
Baseline	23·(7·4 to 37·7)	24·1 (10·6 to 33·2)	21·6 (5·7 to 36·7)	23·2 (7·4 to 34·2)	23·1 (5·7 to 37·7)	23·2 (8·1 to 35·0)
8 weeks	21·5 (5·1 to 37·1)†	23·7 (10·1 to 33·8)	21·6 (11·9 to 31·2)	22·5 (3·0 to 32·6)	22·4 (3·0 to 37·1)*	22·5 (10·9 to 35·1)
Difference	–4·0% (–51 to 53)	–2·3% (–30 to 35)	–3·5% (–46 to 230)	–5·0% (–59 to 130)	–3·5% (–59 to 230)†	–0·4% (–53 to 101)
Large						
Baseline	4·7 (0·5 to 14·4)	4·9 (0·3 to 11·5)	4·6 (1·0 to 21·5)	5·3 (0·0 to 15·0)	4·8 (0·0 to 21·5)	6·0 (0·2 to 15·8)
8 weeks	5·1 (0·6 to 14·7)‡	4·4 (0·4 to 11·6)	4·8 (0·7 to 19·6)	4·6 (0·1 to 14·0)	4·8 (0·1 to 19·6)	5·2 (0·6 to 15·8)
Difference	9·1% (–60 to 395)†‡	4·1% (–71 to 1175)†	–4·4% (–72 to 247)	2·0% (–73 to 611)	3·7% (–73 to 1175)*	–1·3% (–75 to 500)
Triglycerides (mmol/L)						
Baseline	1·9 (1·2)	1·8 (1·0)	1·8 (1·1)	1·9 (1·8)	1·9 (1·3)	1·7 (1·1)
8 weeks	2·0 (1·5)	1·8 (1·5)	1·7 (1·0)	1·8 (1·4)‡	1·8 (1·4)	1·7 (1·1)
Difference	9·9% (0·6)	8·0% (0·5)	3·0% (0·5)	4·4% (0·6)	6·3% (0·6)	8·6% (0·4)

Data are least square means (SD) or median (IQR). Intention-to-treat population included all patients who received at least one dose of study treatment or placebo; data for patients who dropped out before the end of the study were carried forward from last observation. Percentage changes were first calculated as individual percentage change for each participant and then summarised by descriptive statistics.
IDL=intermediate-density lipoprotein. *p<0·01 versus baseline. †p<0·05 versus baseline. ‡p<0·05 versus placebo. §p<0·001 versus baseline. ¶p<0·001 versus placebo. ‖p<0·01 versus placebo. **p<0·0001 versus baseline.

Table 4: **Plasma concentrations of VLDL, IDL, and HDL subclasses at baseline and end of treatment or placebo**

difference was recorded in LDL particle concentration (p=0·166) or LDL size (p=0·282) between the overall A-002 and placebo groups in patients not taking a statin.

259 patients were taking a statin (USA, 242 of 273; Ukraine, 17 of 120). In these patients, A-002 treatment reduced mean $sPLA_2$-IIA concentration by 88·6% for the overall group (ranging from 71·0% for the lowest dose to 95·8% for the highest dose). Decreases of $sPLA_2$-IIA concentration differed from placebo for all doses (p<0·0001). Baseline LDL cholesterol in this subgroup was lower (range 1·9–2·0 mmol/L) than in the total study population (0·7–4·7 mmol/L). In general, the mean reduction in LDL cholesterol in patients taking a statin was greater than or similar to that seen in the total study population. At week 8, LDL cholesterol fell by 9·7% (p=0·018 *vs* placebo) in the 500 mg treatment group, by 9·8% in the overall treatment group (p=0·022 *vs* placebo), and by 0·8% in the placebo group (figure 3). In statin-treated patients, mean oxidised LDL fell by 4·1% in the A-002 group compared with 0·1% in the placebo group (p=0·036 treatment *vs* placebo).

As noted in the total study population, the treatment-related reductions in LDL cholesterol were mainly caused by reductions in the concentration of small LDL particles. Small LDL particle concentration fell by 64·0 nmol/L (10·6%) in the overall A-002 treatment group, but rose by 2·0 nmol/L (0·3%) in the placebo group (p=0·026 treatment *vs* placebo). Mean LDL particle size rose from 20·49 nm (SE 0·05) to 20·51 nm (SE 0·05; p=0·402 *vs* baseline) in the A-002 group, compared with a decrease from 20·67 nm (SE 0·10) to 20·41 nm (SE 0·09; p=0·073 *vs* baseline) in the placebo group (p=0·027 treatment *vs* placebo).

Changes in LDL cholesterol were also examined in statin-treated patients in the A-002 group who had a baseline LDL-cholesterol concentration of more than 1·8 mmol/L, which is the US National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) and American Heart Association/American College of Cardiology target for those at highest risk of coronary heart disease.[27] After 8 weeks of treatment, the mean reduction in LDL cholesterol was 0·43 mmol/L (16·9%, p=0·006 *vs* baseline) for the overall A-002 group (50 mg, 0·59 mmol/L [20·2%], p=0·013; 100 mg, 0·39 mmol/L [16·6%], p=0·044; 250 mg, 0·30 mmol/L, [11·2%], p=0·17; 500 mg, 0·54 mmol/L [22·1%], p=0·002). In this subgroup, a larger reduction in oxidised LDL was seen in the treatment group than in the placebo group (8·6% *vs* 2·2%, p=0·035).

In statin-treated patients, arachidonic acid concentrations fell by 75·3% in the 500 mg A-002 group compared with 59·4% in the placebo group (p=0·0390). For statin-treated patients with LDL-cholesterol concentration of more than 1·8 mmol/L, a larger reduction in arachidonic acid was seen in the 500 mg

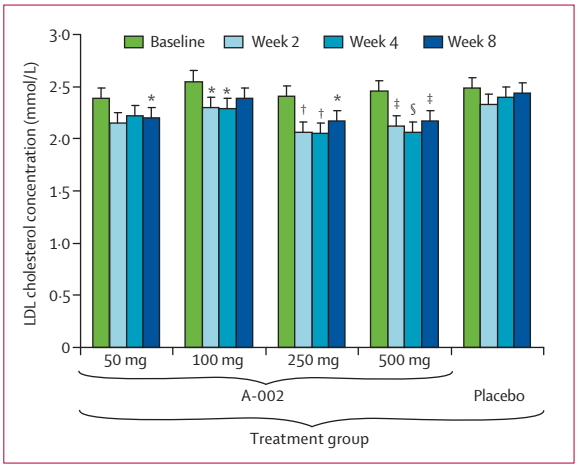

Figure 2: **LDL-cholesterol concentration during treatment and placebo**
Data are least square means (SD). Non-significant p values are not shown. *p<0·05. †p<0·001. ‡p<0·005. §p=0·0005 (all *vs* placebo).

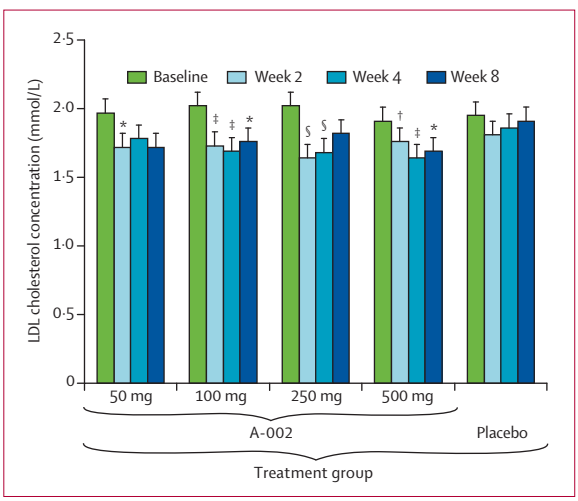

Figure 3: **Concentration of LDL-cholesterol during treatment and placebo in statin-treated patients**
Data are means (SD). Non-significant p values are not shown. *p<0·05. †p<0·001. ‡p<0·0005. §p<0·02 (all *vs* placebo).

A-002 group than in the placebo group (114·8% *vs* 129·2%, p=0·0009). At A-002 doses of 100 mg and higher, leucotriene B4 concentrations reduced in a dose-dependent manner; however, these results did not differ from those with placebo (27·2% reduction in the overall A-002 group *vs* 36·5% in the placebo group, p=0·671). The percentage reductions in leucotriene B4 in the statin subgroup with LDL-cholesterol concentration more than 1·8 mmol/L did not differ from placebo (33·3% reduction in the overall treatment group *vs* 44·7% in the placebo group, p=0·562).

Treatment with A-002, alone or in combination with statin therapy, resulted in no clinically significant changes in vital signs, serum indices, or haematological values. There was one serious adverse event in the 500 mg treatment group (exacerbation of underlying chronic obstructive pulmonary disease, causing cough

and increased shortness of breath), which was judged to be related to the study drug and resulted in discontinuation of the participant from the study. No deaths were reported.

The proportion of patients reporting treatment-emergent adverse events in the A-002 group (47%) did not differ from placebo (44%). In general, the treatment-emergent adverse events were mild and not judged to be related to study drug or dose of the study drug. 17 treatment-emergent adverse events resulted in discontinuation of study drug and no clearly defined dose-response relation was apparent within the data.

The most frequent adverse events in the treatment and placebo groups were headache (20 [6%] *vs* none), nausea (17 [5%] *vs* two [3%]), diarrhoea (12 [4%] *vs* three [4%]), rise in alanine transaminase (ten [3%] *vs* none), dizziness (nine [3%] *vs* none), increase in aspartate aminotransferase (seven [2%] *vs* none), and back pain (seven [2%] *vs* one [1%]). A-002 was safe and well tolerated in patients taking statins, with no evidence of enhanced hepatotoxicity.

Discussion

This study showed that A-002 treatment reduced $sPLA_2$-IIA concentration in patients with coronary heart disease in a dose-dependent manner; similar results were seen in a subgroup analysis of statin-treated patients. Although the mechanism for the reduction in $sPLA_2$-IIA concentration needs further investigation, we postulate that A-002 interferes with the autocrine pathway in which $sPLA_2$ augments its own production.[28]

LDL-cholesterol and LDL-particle concentrations were lower after A-002 treatment than at baseline, mainly as a result of a reduction in small LDL particles. This finding contrasts with a recent report in which darapladib-mediated inhibition of lipoprotein-associated phospholipase A2 (also known as group VII phospholipase A_2 or platelet-activating factor acetylhydolase) had no effect on LDL cholesterol.[29]

In the NCEP-ATP III guidelines,[27] an LDL-cholesterol concentration of less than 2·6 mmol/L was set as a minimum acceptable target for patients with coronary heart disease, and a concentration of less than 1·8 mmol/L was recommended as an optional target for certain subsets of patients at very high risk of coronary heart disease. The results of statin dose-ranging studies indicate that this more aggressive target is difficult to achieve in many very high-risk patients, even with high dose.[30] The effects of A-002 on LDL cholesterol might allow more very high and high-risk patients to achieve their LDL cholesterol target, and have a statin-sparing effect in statin-intolerant patients.

The mechanism for the LDL-cholesterol-lowering effect of A-002 is suggested in part by reduction of $sPLA_2$, which in turn mitigates adverse lipoprotein remodelling. This might explain the enhanced LDL-cholesterol-lowering seen in statin-treated patients who are known to have increased LDL-receptor activity. $sPLA_2$ reduces the phospholipid content of LDL particles,[7,8,11] resulting in smaller particles.[11] In this study, all patients, including those treated with a statin, had significant dose-dependent reductions in leucotriene B4 from baseline values; however, these results did not differ from those for placebo.

The role of A-002 in atherosclerosis has been investigated in three animal models with apolipoprotein-E-null mice. In one study, apolipoprotein-E-null mice that were fed a high-fat diet for 2 weeks and treated with A-002 or placebo for 16 weeks had a 50% greater reduction in aortic plaque coverage than untreated control animals ($p<0·01$).[31] In the second study of accelerated atherosclerosis induced by a combination of high-fat diet and continuous infusion of angiotensin II, aortic plaque coverage and aneurysm formation substantially reduced after 4 weeks' treatment with A-002.[31] In the third study, the potential synergy between a statin (pravastatin) and A-002 on atherosclerosis progression was investigated in apolipoprotein-E-null mice that were fed a western diet (formulated to deliver the study drugs) for 3 months.[32] A-002 reduced the amount of atherosclerosis by 40% (low dose) and 74% (high dose) more than did placebo ($p<0·05$). Low-dose pravastatin alone resulted in a small (14%), but non-significant ($p=0·48$), reduction in atherosclerosis, whereas combined low doses of A-002 and pravastatin decreased atherosclerosis by 75% or to a greater extent than either drug when used alone, suggesting synergy of effect.

Our study has several limitations. We enrolled patients irrespective of entry LDL-cholesterol concentration, and the effects of A-002 on LDL cholesterol might underestimate the magnitude of LDL-cholesterol-lowering in hypercholesterolaemic patients. Baseline C-reactive protein concentrations were less than 39·8 nmol/L and the low level of systemic inflammation might have diminished the anti-inflammatory effect seen with A-002 treatment. We were unable to assess the effect of the drug on plasma $sPLA_2$ activity since baseline concentrations of the enzyme were five-fold lower than the lower limit of quantification of the assay.[24] Furthermore, we did not measure intravascular $sPLA_2$ activity because the $sPLA_2$-V and $sPLA_2$-X isoenzymes are mainly found in the vessel wall[33] and there are no specific assays for these isoenzymes. We also did not measure lipoprotein-associated PLA_2 activity.

We measured the effects of A-002 on circulating lipoproteins, whereas the atherogenic effects of $sPLA_2$ might largely take place in the vessel wall. Further investigations into the larger LDL-cholesterol-lowering effect of A-002 seen in statin-treated patients are needed. Even though the biomarkers that were reduced after treatment with A-002 have proved to predict atherosclerosis progression and to identify patients

with coronary heart disease at increased risk of cardiovascular events, the effects of the drug on atherosclerosis and cardiovascular events should also be established.

Clinically relevant findings associated with A-002 treatment are a result of a combination of effects on more than one of the pathways in atherogenesis. These effects include decreasing the adverse modification of lipoproteins by $sPLA_2$ that results in an overall reduction in LDL cholesterol, and reducing the inflammation and flux through the arachidonic-acid pathways. Arachidonic acid and its biologically active products serve to amplify inflammation and have a role in the pathogenesis of atherosclerosis within the vessel wall. The reduction in LDL cholesterol, C-reactive protein, arachidonic acid, and oxidised LDL cholesterol resulting from A-002 treatment over a background of statin therapy is potentially clinically relevant. Furthermore, combined use of A-002 with a statin may have complementary benefits.

Contributors

RSR, CH, and DW participated in the design and analysis of the study, in the interpretation of study results, and contributed to revising the manuscript. RSR drafted the manuscript. DM participated in the assay of samples, and in the review and interpretation of assay results. ME participated in the study management and coordination. NW participated in the statistical data analysis. All authors saw and approved the final manuscript.

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Conflict of interest statement

RSR received research grant support for the PLASMA trial, and these funds were provided directly to the University of Michigan. RSR was an expert witness for the US Food and Drug Administration's end of phase II meeting about varespladib. RSR has received honoraria and acted as a consultant to Anthera Pharmaceuticals for activities unrelated to PLASMA. DW has received honoraria and has acted as a consultant to Anthera Pharmaceuticals. CH, ME, YS, and DW have ownership interest in Anthera Pharmaceuticals. DM and NW declare that they have no conflict of interest.

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