SUMMARY

ASTRAZENECA PHARMACEUTICALS

FINISHED PRODUCT:

ACTIVE INGREDIENT: Rosuvastatin

Trial title (number): A 9-Week, Randomised, Double-Blind, Placebo-Controlled Trial to Investigate the Effect of Rosuvastatin on Endothelial Function in Patients with Moderate Hypercholesterolaemia (4522IL/0037).

Clinical phase: III	**First subject recruited:** 01 September 1999
	Last subject completed: 30 January 2002
	AstraZeneca approval date: 16 December 2002

Publications: None at the time of writing this report.

OBJECTIVES

The primary objectives were to:

- test the hypothesis that in dyslipidaemic subjects rosuvastatin induces an increase in the endothelium-dependent vasodilator function, as judged by forearm arterial blood flow (shortened to forearm blood flow; FBF) response to Substance P and acetylcholine (ACh); both are agonists of nitric acid (NO) generation

- assess to what degree the increase in FBF in response to ACh is blocked by co-administration of the NO synthase inhibitor L-mono-N-methyl-arginine (L-NMMA), and to infer the contribution of NO to that increase

- test the hypothesis that in dyslipidaemic subjects rosuvastatin has no effect on the endothelium-independent vasodilator response to sodium nitroprusside (SNP); this acts as a positive control.

The secondary objectives of this trial were to:

- investigate whether any changes in endothelium-dependent vasodilator function (first primary objective listed above) relate to changes in low-density lipoprotein cholesterol (LDL-C), to the pharmacokinetics of rosuvastatin, or to dose

- estimate the effect of rosuvastatin on basal NO tone by looking at the effects of L-NMMA in the absence of any agonists

- investigate other possible mediators of change in blood flow by comparing the time-course of changes in FBF with respect to infusion with Substance P, SNP, ACh and L-NMMA with changes in plasma concentration of total cholesterol (TC, high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), lipoprotein a (Lp(a)), von Willibrand's Factor (vWF), tissue plasminogen activator (tpA) activity and antigen levels, plasminogen activator inhibitor-1 (PAI-1) activity and antigen levels, and pulse wave analysis (PWA)

- assess changes in arterial wall compliance using PWA, and measure changes in pulse wave velocity (PWV).

It was also of interest to investigate the changes in rosuvastatin plasma concentration, lipid variables (LDL-C, TC, HDL-C, TG, Lp(a)) and lipid ratios (LDL-C/HDL-C, TC/HDL-C, non-HDL-C/HDL-C).

METHODS

Design: This was a randomised, double-blind, placebo-controlled, 3-arm, parallel-group, single-centre trial. After a 6-week dietary lead-in period, subjects were randomised to 1 of 3 treatment groups: rosuvastatin 10 mg, rosuvastatin 80 mg, or placebo; subjects were given their randomised dose once daily for 9 weeks.

Population: A total of 30 randomised and evaluable, non-diabetic, adult male and postmenopausal female (not on HRT) subjects with Type IIa/IIb hypercholesterolaemia were recruited. Seven subjects were required per treatment group to have at least 90% power of detecting a difference in mean FBF of 4 ml/min/100 ml tissue between the 2 groups, assuming a common standard deviation of 2 mL/min/100 ml tissue; however, 10 subjects per group were to be recruited to allow for the number of secondary endpoints of interest, the assessment of the 3 groups of subjects together and separately, and for dropouts during the trial.

Key inclusion criteria: Non-diabetic adult males aged 18 to 75 years and non-diabetic postmenopausal females (not on HRT) up to 75 years; discontinuation of all cholesterol-lowering drugs and dietary supplements; fasting LDL-C levels ≥3.50 and ≤7.76 mmol/L (≥135.5 and ≤300 mg/dL); fasting TG levels <4.52 mmol/L (<400 mg/dL); an Eating Pattern Assessment Tool (EPAT) score of ≤28 to demonstrate dietary compliance; normal physical examination; and ECG results that show no acute changes.

Key exclusion criteria: Known previous inability to tolerate forearm blood flow measurements; history of hypersensitivity reactions to HMG-CoA reductase inhibitors; various concomitant illnesses, including active liver disease or dysfunction, active arterial disease, history of malignancy, uncontrolled hypertension, and uncontrolled hypothyroidism; history or presence of

Type 1 or Type 2 diabetes mellitus; history of bleeding disorders; history of bowel resection or splenectomy; history of migraine associated with neurological symptoms; history of backache that would prevent taking efficacy measurements; known homozygous or heterozygous familial hypercholesterolaemia or known Type III hyperlipoproteinaemia; serum creatine kinase (CK) concentration >3 x Upper Limit Normal (ULN); serum creatinine >220 µmol/L (2.5 mg/dL) before randomisation; and usage of concomitant medications known to affect the lipid profile or present a potential safety concern (eg, through drug interaction).

Dosage: After a 6-week dietary lead-in period, subjects took oral doses of encapsulated trial treatment once daily, approximately 3 hours after the evening meal. Doses of treatments were as follows: rosuvastatin 10 mg, rosuvastatin 80 mg, and matching placebo. The same dose of trial treatment was taken for 9 weeks. Formulation and batch numbers were as follows: rosuvastatin 10 mg (F12572, 65731F99); rosuvastatin 80 mg (F12568, 70235I00); placebo (F12545, 67534H99).

Key assessments:

Efficacy: Because of the exploratory nature of this trial efficacy endpoints associated with the trial objectives could be derived a number of ways. Analyses of those endpoints considered most important (main endpoints) were used to draw conclusions on the primary and secondary objectives of the trial. Analyses of supplementary endpoints were considered as supportive to the main analyses. All main and supplementary endpoints and analyses were performed according the statistical analysis plan.

Endothelial function was assessed by parameters derived from FBF: endothelium-dependent vasodilation (EDV), endothelial function index (EFI) and the NO proportion of ACh response (NOPROP). EDV, EFI and NOPROP were the primary endpoints of the trial and were derived from FBF in the infused arm (FBF (infused)) and ratio infused:noninfused arm (FBF-R) at high dose and area under the curve (AUC) of infused ACh, Substance P, SNP, L-NMMA and L-NMMA plus ACh at Weeks 0, 2 and 9. The endpoints of greatest interest were those associated with the primary objectives, ie EDV, EFI and NOPROP; EDV was calculated for ACh, Substance P (NO agonists), SNP (NO-independent vasodilator) and L-NMMA (NO antagonist). EFI was calculated for ACh and Substance P. The main endpoints were derived from FBF infused, at high dose of infused substance at Week 9.

Other main endpoints of interest (associated with secondary objectives) were: tpA and PAI-1 activity and antigen levels (mean of infused and non-infused arm values, saline); and tpA and PAI activity / antigen net release levels; vWF levels; PWA comprising augmentation index (AI) and pulse wave velocity (PWV); and blood pressure (systolic and diastolic) at Substance P infusion (the first infused substance). Data for tpA and PAI-1 were collected and assessed at Weeks 0 and 9, data for the other parameters were collected and assessed at Weeks 0, 2 and 9.

Other endpoints of interest included: estimation of rosuvastatin levels at approximately 9 hours post-dose at Weeks 0, 2, and 9; and assessment of the fasting plasma levels (percentage change from Week 0 and absolute values) for LDL-C, TC, HDL-C, TG, Lp(a), and lipid ratios (LDL-C/HDL-C, TC/HDL-C, non-HDL-C/HDL-C) at Weeks 0, 2 and 9.

All efficacy measures were summarised by treatment group using descriptive statistics or frequency distributions, whichever was appropriate, for the raw data. All the main endpoints were subjected to formal statistics (main analysis), the results were presented and interpreted. Analyses were performed on the PP population using the observed data. Analysis of covariance

(ANCOVA) was used on each of the main endpoints and in each case, the ANCOVA model included a term for treatment group and the Week 0 value as covariate. Because of the investigational nature of this 'science' trial, no adjustments were made for multiple comparisons between the different analyses. However, to allow for multiple comparisons between treatment groups, a hypothesis testing procedure was carried out for each analysis and comparisons were made between each of the two rosuvastatin groups (rosuvastatin 10 mg and 80 mg) and placebo, and between the rosuvastatin 80 mg and rosuvastatin 10 mg groups. Where appropriate, the results of these comparisons were presented and interpreted. The same analyses were performed on supplementary endpoints.

The term 'Week 0' was used to describe all measurements (including FBF) that were carried out at the randomisation visit. The term 'saline' was used to describe FBF measurements that were obtained before administration of the challenge infusions at Weeks 0, 2, and 9.

Safety: Standard safety assessments included adverse event reports, clinical laboratory data (hepatic biochemistry, CK, renal biochemistry, haematology, urinalysis), vital signs (including heart rate and blood pressure), electrocardiograms (ECGs), and physical examination. All data were summarised.

RESULTS

Demography: A total of 193 subjects entered the dietary lead-in period of this single-centre trial, of whom 30 were eligible for entry into the randomised treatment period. Ten subjects were randomised to the placebo group, 10 to the rosuvastatin 10 mg group and 10 to the rosuvastatin 80 mg group. The three groups were similar with respect to age, sex, weight and BMI; all subjects were Caucasian. In the placebo and rosuvastatin 10 mg groups, 5 and 4 subjects were current smokers, respectively, compared with 1 subject in the rosuvastatin 80 mg group. The first subject entered the trial on 7 April 2000 and the last subject completed on 30 January 2002. There were three major protocol deviations (all non-compliant with treatment medication): 1 subject at Week 2 in the rosuvastatin 10 mg group, and 1 subject at Week 2 and 1 subject at Week 9 in the rosuvastatin 80 mg group. There were no major protocol violators. Four subjects withdrew: 1 subject in the placebo group at Week 9, 1 subject in the rosuvastatin 10 mg group at Week 2 and 2 subjects in the rosuvastatin 80 mg group at Week 2. Two of the 3 subjects who withdrew at Week 2 were the same subjects who were non-compliant with trial medication at Week 2 (1 in the rosuvastatin 10 mg and 1 in the rosuvastatin 80 mg groups). The subject in the rosuvastatin 80 mg group who withdrew at Week 2 and who was not a major protocol violator, had post-Week 0 efficacy assessments for vWF but did not have post-Week 0 FBF or PWA efficacy assessments. Therefore, this subject was included in the PP population, but was excluded for the PP analyses of FBF and FBF-derived parameters. The subject who withdrew from the placebo group had vWF and PWA assessments at weeks 0, 2 and 9 but had FBF assessment only at Week 0; therefore, this subject was included in the PP population. Thus, there were 10 subjects in the safety population for each of the treatment groups and, 10, 9 and 8 subjects, for the placebo, rosuvastatin 10 mg and 80 mg groups, respectively, in the PP population at Week 9 (7 subjects for the FBF-derived PP analyses in the rosuvastatin 80 mg group).

Efficacy: A summary of the key efficacy findings (main endpoints associated with primary objectives) is presented in Table I.

Table I   Summary of key efficacy findings from analyses of main endpoints associated with primary objectives at Week 9[a] (PP population)

Efficacy endpoint	Placebo (N = 10)	Rosuvastatin 10 mg (N = 9)	Rosuvastatin 80 mg (N = 8)[b]
Analysis of EDV (High dose infused substance):			
EDV(Substance P), %			
lsmean (SE)	188.93 (21.12)	146.67 (22.62)	192.13 (25.57)
Difference from placebo (SE)[c]	NA	-42.26 (31.04)	3.20 (33.07)
Difference from rosuvastatin 10 mg (SE)[d]	NA	NA	45.46 (34.63)
EDV(SNP), %			
lsmean (SE)	193.27 (35.52)	239.27 (37.48)	229.44 (42.72)
Difference from placebo (SE)[c]	NA	45.99 (51.57)	36.17 (55.68)
Difference from rosuvastatin 10 mg (SE)[d]	NA	NA	-9.82 (57.04)
EDV(ACh), %			
lsmean (SE)	207.63 (39.90)	172.74 (39.99)	220.00 (45.39)
Difference from placebo (SE)[c]	NA	-34.89 (56.48)	12.37 (60.44)
Difference from rosuvastatin 10 mg (SE)[d]	NA	NA	47.26 (60.66)
Analysis of EFI (High dose infused substance):			
EFI(Substance P), %			
lsmean (SE)	0.87 (0.08)	0.83 (0.08)	0.95 (0.09)
Difference from placebo (SE)[c]	NA	-0.04 (0.11)	0.08 (0.12)
Difference from rosuvastatin 10 mg (SE)[d]	NA	NA	0.13 (0.12)
EFI(ACh), %			
lsmean (SE)	0.95 (0.14)	0.99 (0.14)	1.01 (0.16)
Difference from placebo (SE)[c]	NA	0.04 (0.21)	0.06 (0.21)
Difference from rosuvastatin 10 mg (SE)[d]	NA	NA	0.02 (0.21)
Analysis of NOPROP (High dose infused substance):			
NOPROP, %			
lsmean (SE)	112.62 (41.59)	36.32 (41.57)	102.17 (50.45)
Difference from placebo (SE)[c]	NA	-76.29 (59.36)	-10.44 (65.40)
Difference from rosuvastatin 10 mg (SE)[d]	NA	NA	65.85 (65.35)

[a] ANCOVA analysis at Week 9 of main endpoints using observed data from the PP population
[b] There are 7 subjects in the PP analyses for FBF-derived parameters as 1 subject had no post-Week 0 FBF efficacy assessments.
[c] Not statistically significant (p >0.05) for comparison of placebo versus rosuvastatin 10 mg and placebo versus rosuvastatin 80 mg
[d] Comparison rosuvastatin 10 mg versus rosuvastatin 80 mg only performed if p ≤ 0.05 for placebo vs. either of the rosuvastatin doses.
ANCOVA = analysis of covariance; ACh = acetylcholine; SNP = sodium nitroprusside; L-NMMA = L-mono-N-methyl-arginine; EFI = Endothelial function index; EDV = Endothelium-dependent vasodilation; lsmean = Least squares mean; NA = Not applicable; SE = Standard error; SD = Standard deviation.

At Week 9 for the analysis of the main endpoints (FBF in the infused arm at high dose of challenge substance) associated with the primary objectives, there were no statistically significant differences between placebo and the 2 rosuvastatin doses for EDV for Sustances P,

ACh, L-NMMA, EFI for Substance P or for ACh and NOPROP (assessing endothelium-dependent vasodilator function); and EDV for SNP (endothelium-independent vasodilation). The pattern of FBF response in the infused arm to challenge infusion was generally as predicted; namely, increase from saline following Substance P, generally larger increases following SNP and ACh, a decrease following L-NMMA, and a small increase following concurrent ACh plus L-NMMA. However, there was no apparent treatment or rosuvastatin dose-related pattern in magnitude of changes at Week 2 or 9. Analysis of supplementary endpoints (FBF-R and AUC dose of challenge infusion) supported analysis of the main endpoints. There was no confounding effect on FBF from BP as analysis of change in Saline 3 BP from baseline to Week 9 did not show any statistically significant differences between placebo and the 2 rosuvastatin doses. Analysis of clotting factors at Week 9 (main endpoints associated with secondary objectives) showed a statistically significant greater decrease for PAI antigen net-release for rosuvastatin 80 mg compared with rosuvastatin 10 mg and placebo. However, data were generally variable and there was no statistically significant differences between the 3 treatment groups for any other of the clotting factor parameters. Namely, PAI activity / net-release; tpA activity and antigen / net-release; and vWF. Analysis of the main PWA parameters, AI and PWV, at Week 9 (main endpoints associated with secondary objectives) did not show any statistically significant differences between placebo and the 2 rosuvastatin doses. There was no apparent relationship between changes in EDV and lipid variables and rosuvastatin plasma concentration, and between tpA activity and antigen net-release variables and lipid parameters. Overall, the change in atherogenic lipid profile of subjects was favourable for rosuvastatin 10 mg and rosuvastatin 80 mg.

Safety: Rosuvastatin 10 mg and 80 mg were well tolerated during the randomised treatment period. The incidence of treatment-emergent adverse events was similar in all 3 treatment groups: 10 subjects experienced 51 adverse events in the placebo group, 8 subjects experienced 52 events in the rosuvastatin 10 mg group and 10 subjects experienced 57 events in the rosuvastatin 80 mg group. The majority of adverse events were of mild or moderate intensity and there were generally no treatment or rosuvastatin dose-related patterns or trends. The most commonly reported adverse events were injection site reactions, which resulted from the forearm blood flow procedure used in this trial. There was 1 serious adverse event, which occurred on rosuvastatin 10 mg (corneal ulcer, unrelated to treatment) and led to withdrawal of the subject. There were no other withdrawals due to adverse events. No subjects had clinically significant elevation of ALT and / or AST (>3 x ULN). There were slightly higher numbers of subjects with mildly elevated (> 1x ULN) ALT and / or AST on rosuvastatin compared with placebo; however, for the majority of subjects changes in these enzymes were small and within normal range. In 2 subjects receiving rosuvastatin 80 mg, mildly elevated ALT and AST were reported as adverse events ("SGPT increased" and "SGOT increased"); both subjects were reported as "recovered". No subject had clinically elevated CK (>10 x ULN) and for the majority of subjects changes in this enzyme were small and within normal range. Other than back pain, which occurred in 2 subjects receiving rosuvastatin 10 mg and 3 subjects receiving placebo, there were no reports of myalgia, muscle pain, tenderness or weakness. Data from other laboratory parameters, ECG and vital signs were unremarkable and there were generally no treatment or rosuvastatin dose-related trends or patterns.