

Randomized clinical trial of mast cell inhibition in patients with a medium-sized abdominal aortic aneurysm

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Background: Abdominal aortic aneurysm (AAA) is thought to develop as a result of inflammatory processes in the aortic wall. In particular, mast cells are believed to play a central role. The AORTA trial was undertaken to investigate whether the mast cell inhibitor, pemirolast, could retard the growth of medium-sized AAAs. In preclinical and clinical trials, pemirolast has been shown to inhibit antigen-induced allergic reactions.

Methods: Inclusion criteria for the trial were patients with an AAA of 39–49 mm in diameter on ultrasound imaging. Among exclusion criteria were previous aortic surgery, diabetes mellitus, and severe concomitant disease with a life expectancy of less than 2 years. Included patients were treated with 10, 25 or 40 mg pemirolast, or matching placebo for 52 weeks. The primary endpoint was change in aortic diameter as measured from leading edge adventitia at the anterior wall to leading edge adventitia at the posterior wall in systole. All ultrasound scans were read in a central imaging laboratory.

Results: Some 326 patients (mean age 70.8 years; 88.0 per cent men) were included in the trial. The overall mean growth rate was 2.42 mm during the 12-month study. There was no statistically significant difference in growth between patients receiving placebo and those in the three dose groups of pemirolast. Similarly, there were no differences in adverse events.

Conclusion: Treatment with pemirolast did not retard the growth of medium-sized AAAs. Registration number: NCT01354184 (<https://www.clinicaltrials.gov>).

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Introduction

Abdominal aortic aneurysm (AAA) is a common disease among the elderly, and left untreated it is potentially fatal. Important risk factors for AAA are age, male sex, heredity, atherosclerosis and smoking¹. The aetiology of this potentially fatal disease is still partly unknown, but it is likely that inflammation in the media and adventitia wall of the aneurysm influences both development and growth.

Mast cells are accountable for much of the inflammatory response in AAA². Several studies have shown the

importance of mast cells in the development of AAA in experimental models^{3,4}. It is always uncertain whether findings from experimental animals can be translated to humans. In humans, a positive correlation between the number of mast cells in AAA tissue and aortic diameter has been reported². In addition, mast cells are increased in AAA tissue⁵. A role for mast cells in humans is suggested by the correlation between the number of mast cells in the outer aortic media and adventitia of patients with an AAA⁴. A likely consequence is that high levels of mast cell-derived proteases in plasma and aortic

tissue are associated with AAA⁶. Mast cells develop from bone marrow-derived progenitor cells and are present as two different types: MCt cells, which express tryptase, and MCtc cells, which in addition express chymase, carboxypeptidase and cathepsin G².

The growth of small AAAs is related to proteolysis in the aortic matrix. Macrophages are considered to be of importance, as they contain matrix metalloproteases (MMPs), of which MMP-2 and -9 may be particularly important⁷. The influence of mast cells on the release of MMPs is important, because chymase and tryptase released from mast cell granules initiate the release and activation of MMPs from macrophages^{8,9}. Activation of pro-MMP-9 by chymase has been described in human AAA¹⁰. Mast cells also contribute to the synthesis of MMP-9 in macrophages¹¹.

Important hallmarks of AAA growth include smooth muscle cell death, neovascularization and activation of the renin-angiotensin system¹². Smooth muscle cell death is caused by anoikis¹³, a process initiated by chymase from mast cells¹⁴. Neovascularization has been reported to be associated with inflammation and elastin degradation¹⁵, most prevalent in areas with large numbers of mast cells¹⁶. Cathepsin G is present in MCtc cells and neutrophil leucocytes found in the luminal layer of the intraluminal thrombus, which is present in most AAAs, and the adventitia of the aneurysmal aorta also stores cathepsin G¹⁷.

Many attempts have been made to find a growth-inhibiting agent for AAA, including statins, angiotensin-converting enzyme (ACE) inhibitors and doxycycline, based on initial successful animal models. So far no drug has been approved for retarding the growth of small AAA in order to prevent rupture^{1,18,19}.

The mast cell inhibiting properties of pemirolast have been demonstrated in clinical studies to reduce antigen-induced allergic reactions. Mast cells are associated with the pathophysiological processes of human AAA development, and inhibition of mast cells in experimental animals can reduce AAA development^{4,10,20,21}. The present study was undertaken to investigate whether pemirolast could inhibit the growth of medium-sized AAAs.

Methods

The Anti-inflammatory ORal Treatment of AAA (AORTA) trial was an international, multicentre, randomized, stratified, double-blinded, placebo-controlled, four-parallel group trial. Three different doses of pemirolast administered twice daily for 1 year were investigated with respect to the safety and efficacy in subjects with a medium-sized AAA in comparison with matching placebo tablets. The AORTA trial was registered with clinicaltrials.gov

(NCT01354184), and was approved by ethics committees in Sweden, Denmark and the UK.

Primary hypothesis

The primary objective was to investigate the optimal dose of pemirolast (10-, 25- and 40-mg tablets) in comparison with matching placebo tablets with respect to efficacy in terms of change in aortic diameter as assessed by ultrasound imaging using a standard measurement method (see method A below).

Patients

Subjects were identified through hospital outpatient clinics, national AAA screening and surveillance programmes. Inclusion criteria were: presence of an infrarenal AAA with a maximum diameter of 39–49 mm inclusive, age 50 years or more, and informed consent following verbal and written information about the trial.

Exclusion criteria were: previous infrarenal aortic surgery, planned major surgery, known aortic dissection, known diabetes, systemic treatment with corticosteroids or other systemic immunomodulatory therapy, known or suspected inherited connective tissue disorder, calculated creatinine clearance of less than 30 ml/min, known significant liver disease, known human immunodeficiency virus infection at the time of screening, serious concomitant illness associated with life expectancy of less than 2 years, and any other significant and unstable condition that could limit compliance with the trial protocol.

Study protocol

Patients were recruited from 15 sites in Sweden, Denmark and the UK (see list of Collaborators). All data were collected on an electronic case report form. At baseline, a standard ultrasound scan of the aorta was forwarded for review and diameter measurement checked in a central ultrasound laboratory.

The trial schedule comprised a total of eight visits, including the screening visit (*Fig. 1*). The treatment interval was 12 months from randomization; ultrasound examinations for evaluation of AAA diameter were performed at screening, 6 and 12 months.

Blood samples were taken at baseline, and again at 12 months to investigate a panel of biomarkers associated with inflammation that could potentially correlate with AAA growth.

Quintiles Laboratories (Livingston, UK) provided central laboratory services for the analysis of all safety laboratory parameters as well as biomarkers.

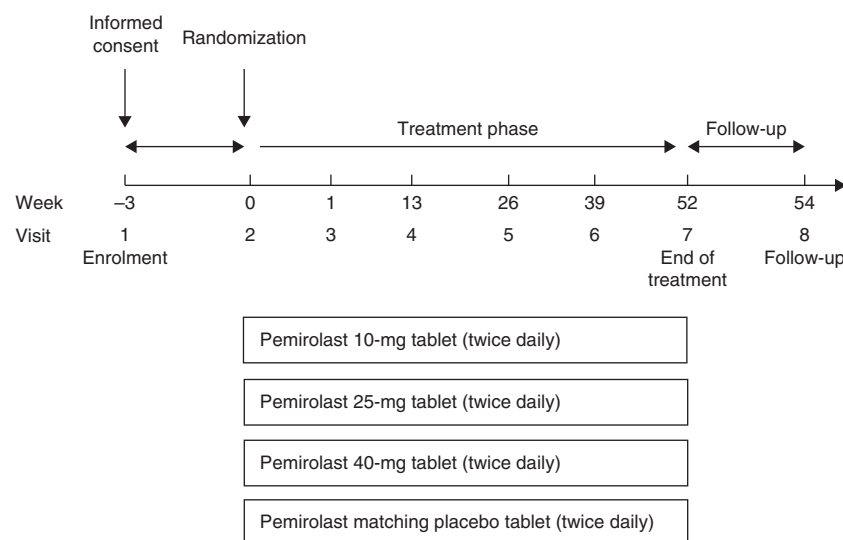


Fig. 1 Diagram showing the timeline of the AORTA trial

Ultrasound methods

All ultrasound scans were recorded with identical ultrasound hardware and software equipment: Philips® CX50 ultrasound system equipped with a C5-1 curved array transducer (Philips, Bothell, Washington, USA), using electrocardiographic (ECG) gating. Examinations were done by experienced sonographers trained in a standard acquisition protocol.

The ultrasound imaging procedure included a cross-sectional still image performed and assessed according to local procedures and protocols, in order to allow the investigators to monitor the progression of the patient's aneurysm following local practice guidelines. Diameter measurements were also performed on a 10-s ultrasound video recorded in the cross-sectional plane at the location of greatest infrarenal aortic diameter, using a 90° angle to the AAA centre line. This video was included in the clinical trial database and used in the statistical analysis. Recordings were performed with continuous ECG recording activated. Details of the scanning protocol have been published elsewhere²².

All ECG-gated recordings were transferred to the central ultrasound laboratory at Rigshospitalet, Copenhagen. An experienced ultrasound reader, blinded to treatment allocation, evaluated all recordings during the trial. This reader evaluated each scan consecutively, blinded to previous scans. Next, a second experienced reader adjudicated all scans performed by the first reader. The second reader, who was also blinded to treatment allocation, was allowed to adapt measurements based on image information obtained from simultaneous review of all three

scans in each patient. The measurements performed during the adjudication procedure were used for the primary analysis.

Measurement of AAA diameter was performed using QLab software version 9.1 (Philips), as described elsewhere²². In brief, AAA diameter was measured by two methods. Method A included measurement from leading edge adventitia at the anterior wall to leading edge adventitia at the posterior wall, and method B from leading edge adventitia at the anterior wall to the luminal intimal border at the posterior wall. The efficacy analysis was based on the arithmetic mean of five consecutive measurements (from 5 different cardiac cycles) performed during both systole and diastole. Reproducibility of this method has shown limits of agreement from -2.8–2.9 to 2.2–2.6, and the accuracy of rereading in the central ultrasound laboratory was from -0.8 to 1.3 mm for method A²².

Efficacy outcomes

The primary objective was to investigate the optimal dose of pemirolast (10-, 25- and 40-mg tablets) in comparison with matching placebo tablets with respect to efficacy in terms of change in aortic diameter, as assessed by ultrasound imaging using measurement method A.

Secondary efficacy parameters included measurement of aortic diameter in systole and diastole with methods A and B, for each dose of pemirolast. Other secondary outcomes included rates of AAA repair, AAA rupture or cardiovascular complications, and mortality. Changing levels

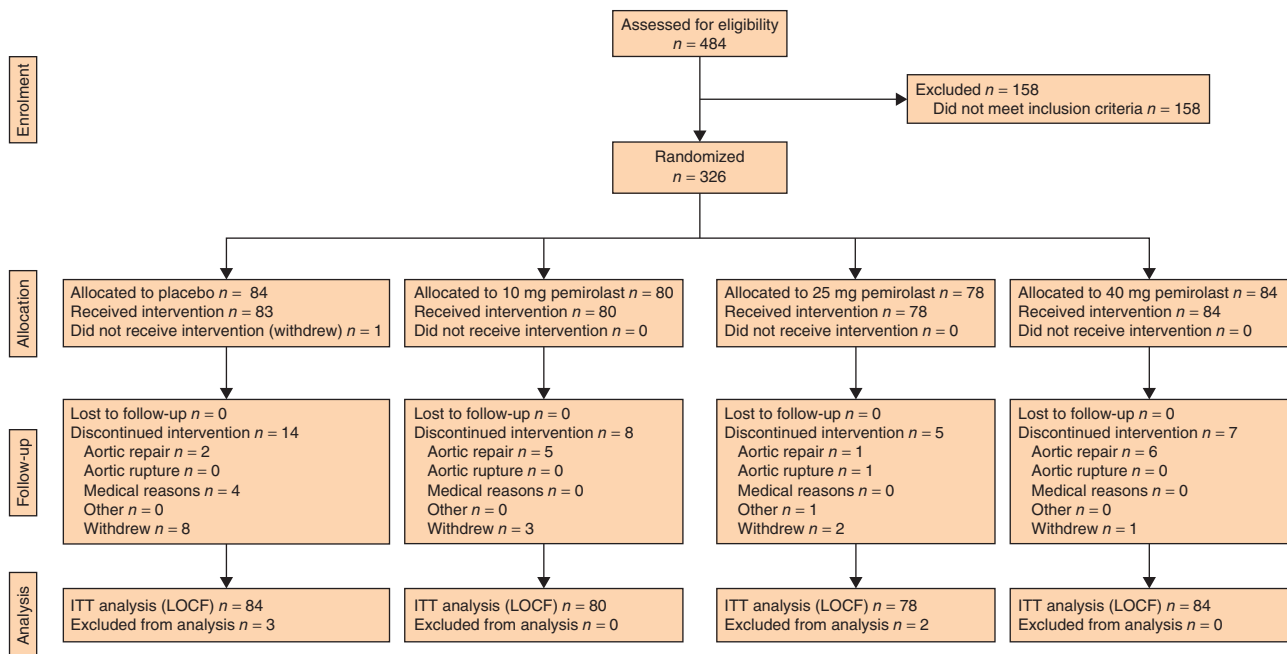


Fig. 2 CONSORT diagram for the AORTA trial. ITT, intention-to-treat; LOCF, last observation carried forward

of plasma biomarkers were related to AAA growth and dose of pemirolast.

Adverse events were monitored by an independent safety monitoring committee, which met regularly and reviewed trial data.

Statistical analysis

The primary efficacy endpoint, change in maximum aortic diameter from screening to 12 months of treatment measured according to measurement method A, was, as prespecified in the protocol, analysed using ANOVA adjusted for self-reported current smoking status (stratification factor). Missing data for the 12 months' endpoint were imputed using the most recent value after baseline (last observation carried forward). Type I error was controlled using the principle of closed testing by first testing the hypothesis of no difference between treatment arms and subsequently testing for no difference between each active dose *versus* placebo, starting with the highest dose. If the hypothesis of no difference was accepted, the test of differences *versus* placebo must be interpreted as exploratory. Biomarker levels were reported using the same analysis approach.

Based on an expected growth rate of 2.12 mm per year of AAAs with a diameter of 35–49 mm, and a reduction in growth rate of 40 per cent with active treatment, the number of subjects needed for the overall test was estimated

at 90 per cent treatment group under the assumptions of 80 per cent power and 5 per cent significance level. With a baseline aortic diameter restricted to 39–49 mm, the sample size was reduced by 33 per cent – to 60 subjects per treatment group. With an estimated 20 per cent drop-out rate, 300 subjects (75 in each treatment group) needed to be randomized.

Results

A total of 484 patients with a medium-sized AAA not requiring surgical treatment were screened, and 326 were randomized amongst the four cohorts (Fig. 2). Some 158 patients were excluded because they did not fulfil the aortic diameter criterion of the trial, had one or more exclusion criteria, or imaging was of inadequate quality. Of the 326 randomized patients, 291 completed the study. The other 35 patients were excluded for various reasons; however, there were no differences between treatment groups. Thirty of these 35 patients did have a second ultrasound scan, and thus only five had to be excluded from the intention-to-treat (ITT) analysis as they had no observations to be carried forward. A further eight patients did not fulfil the requirements for compliance or the time window for rescanning, resulting in 283 patients in the per-protocol analysis.

Among the 326 randomized patients the mean age was 70.8 years, 88.0 per cent were men, mean body mass index

Table 1 Baseline characteristics of patients with a medium-sized abdominal aortic aneurysm in the four treatment groups

	Placebo	Pemirolast 10 mg	Pemirolast 25 mg	Pemirolast 40 mg	Overall
Screened					484
Randomized	84	80	78	84	326
ITT analysis set	84	80	78	84	326
Safety analysis set	83 (99)	80 (100)	78 (100)	84 (100)	325 (99.7)
Full analysis set	81 (96)	80 (100)	76 (97)	84 (100)	321 (98.5)
Age (years)*	70.7(5.7) (53–84)	71.2(6.9) (55–87)	70.0(5.8) (53–82)	71.1(6.2) (59–89)	70.8(6.2) (53–89)
Sex					
F	7 (8)	12 (15)	12 (15)	8 (10)	39 (12.0)
M	77 (92)	68 (85)	66 (85)	76 (90)	287 (88.0)
Body mass index (kg/m ²)*	27.8(3.7) (20.7–41.3)	27.5(4.0) (18.7–35.2)	27.9(4.4) (19.9–39.1)	27.2(4.2) (17.5–37.8)	27.6(4.1) (17.5–41.3)
Current smoking status					
Smoker	34 (40)	32 (40)	29 (37)	35 (42)	130 (39.9)
Non-smoker	50 (60)	48 (60)	49 (63)	49 (58)	196 (60.1)
Patient source					
Screening programme	36 (43)	30 (38)	32 (41)	30 (36)	128 (39.3)
Incidental finding	48 (57)	50 (63)	46 (59)	54 (64)	198 (60.7)
Antithrombotic agents or platelet inhibitors	72 (86)	65 (81)	65 (83)	71 (85)	273 (83.7)
Lipid-lowering drugs	74 (88)	67 (84)	69 (88)	73 (87)	283 (86.8)

Values in parentheses are percentages unless indicated otherwise; *values are mean(s.d.) (range). ITT, intention to treat.

Table 2 Change in diameter from baseline to end of study for the four groups of patients with medium-sized abdominal aortic aneurysms using measurement methods A and B during systole and diastole

	AAA diameter (mm)				P‡
	Placebo (n = 81)	Pemirolast 10 mg (n = 80)	Pemirolast 25 mg (n = 76)	Pemirolast 40 mg (n = 84)	
Method A during systole					
At enrolment*	44.00(2.82)	44.38(2.91)	44.03(2.61)	44.11(2.79)	
At 12 months*	46.02(3.59)	46.93(3.77)	46.33(3.40)	46.79(3.40)	
Adjusted mean change†	2.04 (1.58, 2.50)	2.58 (2.12, 3.05)	2.34 (1.86, 2.81)	2.71 (2.25, 3.16)	0.189
Method A during diastole (LOCF)					
At enrolment*	43.19(2.88)	43.39(2.61)	43.25(2.53)	43.29(2.90)	
At 12 months*	45.28(3.69)	46.23(3.80)	45.54(3.40)	46.13(3.47)	
Adjusted mean change†	2.11 (1.60, 2.61)	2.85 (2.34, 3.36)	2.31 (1.79, 2.84)	2.86 (2.36, 3.35)	0.086
Method B during systole (LOCF)					
At enrolment*	41.97(2.81)	42.08(3.05)	41.72(3.01)	41.73(2.99)	
At 12 months*	43.99(3.58)	44.85(3.73)	44.02(3.45)	44.59(3.42)	
Adjusted mean change†	2.07 (1.57, 2.58)	2.82 (2.31, 3.34)	2.37 (1.85, 2.90)	2.91 (2.41, 3.41)	0.073
Method B during diastole (LOCF)					
At enrolment*	41.01(2.90)	41.13(3.12)	40.76(3.04)	40.72(3.16)	
At 12 months*	43.36(3.60)	44.18(3.69)	43.29(3.44)	43.96(3.48)	
Adjusted mean change†	2.40 (1.85, 2.94)	3.09 (2.55, 3.64)	2.60 (2.03, 3.16)	3.28 (2.74, 3.81)	0.080

*Values are mean(s.d.) †values in parentheses are 95 per cent c.i. AAA, abdominal aortic aneurysm; LOCF, last observation carried forward. ‡ANOVA of change from baseline to 12 months, controlling for smoking status in the intention-to-treat population.

was 27.6 kg/m² and 39.9 per cent were smokers (*Table 1*). Co-morbidity included a history of cardiac disorders in 42, 39, 29 and 38 per cent, and musculoskeletal and connective tissue disorders in 42, 32, 28 and 36 per cent in those allocated to placebo, 10, 25 and 40 mg pemirolast respectively. History of metabolic, nutritional and gastrointestinal disorders among treatment groups was similar (data not shown). Statins were taken by 86.3 per cent of patients, 47.5 per cent were on ACE inhibitors, 32.2 per cent on calcium

blockers and 36.5 per cent on calcium channel blocking agents, again balanced among the groups (data not shown).

Growth rate

The overall mean growth rate (ITT) across the four cohorts was 2.42 mm during the 12-month study. For the primary endpoint, mean adjusted change in systolic diameter measured from leading edge adventitia at the anterior

wall to leading edge adventitia at the posterior wall (method A), growth was 2.04 (95 per cent c.i. 1.58 to 2.50) mm for the placebo group, and 2.58 (2.12 to 3.05), 2.34 (1.86 to 2.81) and 2.71 (2.25 to 3.16) mm for the 10-, 25- and 40-mg pemirolast groups respectively (Table 2). There was no significant difference in growth between treatment groups. Analysis of the per-protocol results did not change the results: growth was 2.24 (1.76 to 2.72) mm for the placebo group, and 2.62 (2.14 to 3.11), 2.35 (1.87 to 2.83) and 2.65 (2.19 to 3.12) mm for the respective pemirolast groups ($P = 0.556$). Similarly, growth rate as assessed by method A during diastole, and method B during systole and diastole did not differ between groups (Table 2).

Results for selected biomarkers revealed no statistically significant differences among treatment groups (Table S1, supporting information). Tryptase levels were reduced in all groups: $-7.1 \mu\text{g/l}$ for the placebo group, and -7.0 , -7.2 and $-10.3 \mu\text{g/l}$ in the 10-, 25- and 40-mg pemirolast groups respectively. The numerically greater reduction in the 40-mg group was not statistically significant.

There were no differences in serious adverse events or adverse events among treatment groups; serious events occurred in 15, 16, 14 and 15 patients, and adverse events in 68, 62, 67 and 67 patients in the placebo, 10-, 25- and 40-mg pemirolast groups respectively (Table S2, supporting information).

During the study 14 patients underwent AAA repair: two had received placebo, five had 10 mg pemirolast, one had 25 mg and six had 40 mg. The need for AAA repair, considered by the local principal investigator, did not differ between groups ($P = 0.182$).

Discussion

The AORTA trial did not show any inhibition of growth of medium-sized AAAs when different doses of pemirolast were compared with placebo, even though pemirolast was supposed to inhibit mast cells in these patients. Similarly, beta-blockers, statins and ACE inhibitors have been proven ineffective in other studies of AAA growth^{23,24}. Most recently, the Pharmaceutical Aneurysm Stabilisation Trial (PHAST)¹⁹, comparing doxycycline with placebo, had negative findings in a population similar to that of the AORTA trial.

In animal models mast cell inhibition prevents *de novo* AAA formation by various methods. In this trial, the AAA was already present when attempts to retard aneurysm growth with pemirolast were started. The lack of effect on the AAA growth rate in the AORTA trial does not preclude that inflammation, mediated by mast cells and other possible mechanisms, could play a role in the pathophysiology

of AAA. Pemirolast did not affect tryptase levels, which could suggest that the lack of benefit in the AORTA trial was due either to a dosing issue or to the short study interval. The plasma level of C-reactive protein is not specific for inflammatory response²⁵ and is not indicative of rapid AAA growth, which is better reflected by diameter measurements^{25,26}. Alternatively, treating medium-sized AAAs, as in the present trial (39–49 mm), may be too late to affect the inflammatory reactions that lead to expansion.

A number of inflammatory mechanisms other than those associated with mast cell inflammation could initiate AAA formation and growth^{27–31}.

As one of the major drawbacks of AAA growth rate investigations has been poor methodology, the AORTA trial used a new method for more accurate assessment of AAA diameter by two-dimensional ultrasonography²². During the cardiac cycle, even small AAAs may dilate up to 5 mm or more; however, there is great variation in the distensibility of aneurysms. In the study of Grøndal and colleagues³², the mean dilatation over the cardiac cycle was 1.94 (range 0–10) mm. In the AORTA trial the electrocardiogram was recorded simultaneously with ultrasound videos, allowing for ECG gating in the reading process. Many studies using ultrasound imaging do not define precisely how the diameter was measured³³. The method used was tested in a reproducibility study of participants in the AORTA trial and found to be accurate: limits of agreement for the primary endpoint were ± 2.5 – 3 mm²². Further, the readings taken locally at participating centres were compared with video recordings in the central ultrasound laboratory. Similar limits of agreement were found: -3 to $+2.8$ mm among data available for local readings in 315 of the 326 participants (data not shown). Thus, considering the negative results of the AORTA trial, it is not likely that variation in diameter measurements affected the outcome.

The overall AAA growth rate in the AORTA trial, 2.42 mm per year, was similar to or slightly less than that of previous studies. The RESCAN collaborators³⁴ reported an average growth ranging from 2.4 mm per year for AAAs 40 mm in diameter to 3.6 mm per year for AAAs reaching 50 mm; similar data were reported from the recent PHAST study¹⁹. Modification of risk factors and treatment with cardioprotective drugs may account for this difference; many studies in published reviews include patients treated more than 10 years ago³⁴. Men with a baseline aortic diameter of 30–49 mm were included in the RESCAN review; however, measurement could be either inner-to-inner or outer-to-outer, affecting the baseline diameter measurement. As baseline diameter is closely related to growth rate, this might be responsible for the differences observed.

Finally, the present trial population could have been inhomogeneous with respect to risk factors for AAA growth; however, this did not seem to be the case.

Collaborators

The AORTA Trial Investigators are: A. Wanhainen (Uppsala University Hospital, Uppsala), R. Hultgren (Karolinska University Hospital, Stockholm), I. Janson (Mälarsjukhuset, Eskilstuna), U. Wingren (Sahlgrenska Hospital, Gotheborg), A. Hellberg (Centrallazarettet, Västerås), T. Larzon (Universitetssjukhuset, Örebro), C. Drott (Södra Älvsborgs Sjukhus, Borås) and J. Holst (Skåne University Hospital, Malmö) in Sweden; H. Sillesen (Rigshospitalet, University of Copenhagen, Copenhagen), N. Eldrup (Århus University Hospital, Skejby), J. Jepsen (Sygehus Lillebælt, Kolding), J. Lindholdt (Regionshospitalet, Viborg) and M. L. Grønholdt (Ålborg University Hospital, Ålborg) in Denmark; and M. Thompson (St George's Hospital, London) and C. McCullum (University Hospital of South Manchester, Manchester) in the UK.

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Disclosure: The authors declare no other conflict of interest.

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Supporting information

Additional supporting information may be found in the online version of this article:

Fig. S1 Relative change in C-reactive protein concentration from baseline to end of study for the four treatment groups (Word document)

Table S1 Relative change in biomarkers from baseline to end of study for the four treatment groups (Word document)

Table S2 Adverse events in the four study groups (Word document)

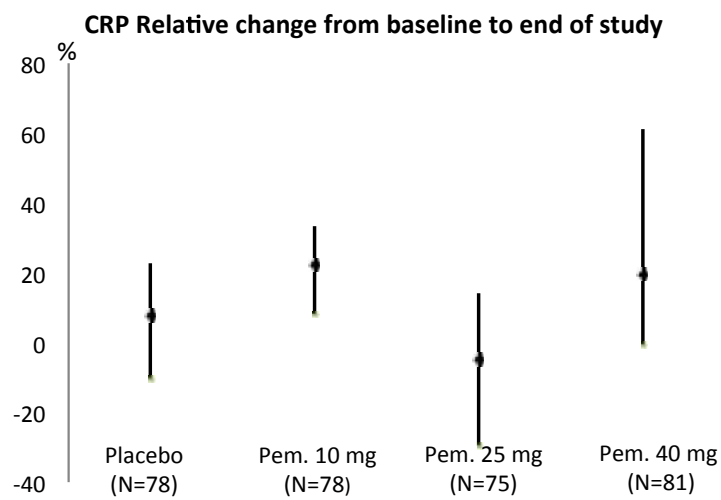


Fig. S1 Relative change in C-reactive protein (CRP) concentration from baseline to end of study for the four treatment groups. Pem, pemirolast

Table S1 Relative change in biomarkers from baseline to end of study for the four treatment groups

	Placebo	Pemirolast 10 mg	Pemirolast 25 mg	Pemirolast 40 mg	Overall	<i>P</i> §
High-sensitivity CRP (mg/l)*						
No. of subjects	48	45	42	48	183	
At enrolment†	2.00	2.05	2.17	1.96	2.04	
At 12 months†	2.15	2.47	2.34	2.52	2.36	
Relative change (%)‡	7.5 (-14.1, 34.5)	20.7 (-4.4, 52.3)	8.1 (-15.1, 37.6)	28.9 (3.0, 61.4)		0.623
HbA1c (%)						
No. of subjects	81	79	76	84	320	
At enrolment†	5.92	5.89	5.97	5.92	5.93	
At 12 months†	5.91	5.87	5.93	5.87	5.89	
Relative change (%)‡	-0.2 (-1.1, 0.7)	-0.4 (-1.2, 0.6)	-0.8 (-1.7, 0.1)	-1.0 (-1.8, -0.1)		0.581
Low-density lipoprotein (mmol/l)						
No. of subjects	80	79	74	83	316	
At enrolment†	2.34	2.24	2.41	2.43	2.35	
At 12 months†	2.25	2.25	2.46	2.47	2.36	
Relative change (%)‡	-3.6 (-9.1, 2.3)	0.6 (-5.2, 6.7)	1.9 (-4.2, 8.3)	2.0 (-3.8, 8.0)		0.510
High-density lipoprotein (mmol/l)						
No. of subjects	80	79	74	83	316	
At enrolment†	1.26	1.23	1.25	1.30	1.26	
At 12 months†	1.27	1.23	1.23	1.30	1.26	
Relative change (%)‡	0.8 (-2.4, 4.1)	0.7 (-2.5, 4.0)	-1.5 (-4.7, 1.9)	0.2 (-2.9, 3.5)		0.765
Triglycerides (mmol/l)						
No. of subjects	80	79	74	83	316	
At enrolment†	1.57	1.59	1.55	1.57	1.57	
At 12 months†	1.55	1.54	1.65	1.47	1.55	
Relative change (%)‡	-0.8 (-7.9, 6.7)	-3.2 (-10.1, 4.2)	6.1 (-1.7, 14.6)	-6.6 (-13.1, 0.4)		0.112
Cholesterol (mmol/l)						
No. of subjects	80	79	74	82	315	
At enrolment†	4.29	4.17	4.31	4.42	4.30	
At 12 months†	4.15	4.14	4.35	4.40	4.26	
Relative change (%)‡	-3.3 (-6.8, 0.3)	-0.9 (-4.5, 2.8)	0.9 (-2.9, 4.8)	-0.4 (-3.9, 3.3)		0.451

Galectin 3 (ng/ml)						
No. of subjects	81	79	75	83	318	
At enrolment†	13.78	14.29	14.19	14.08	14.08	
At 12 months†	13.96	14.27	14.15	14.19	14.14	
Relative change (%)‡	1.3 (−3.0, 5.8)	−0.2 (−4.5, 4.3)	−0.3 (−4.8, 4.3)	0.7 (−3.5, 5.2)		0.949
NT-proBNP (pmol/l)						
No. of subjects	81	79	75	82	317	
At enrolment†	10.48	12.60	15.06	13.84	12.84	
At 12 months†	11.84	13.64	15.28	14.63	13.76	
Relative change (%)‡	13.3 (−1.5, 30.3)	8.6 (−5.8, 25.1)	1.8 (−12.0, 17.8)	6.0 (−7.8, 21.8)		0.766
GDF15 (pg/ml)						
No. of subjects	81	79	75	83	318	
At enrolment†	1244.0	1274.3	1151.6	1281.2	1238.4	
At 12 months†	1084.5	1182.3	1083.6	1187.8	1134.4	
Relative change (%)‡	−12.7 (−16.8, −8.4)	−7.1 (−11.5, −2.4)	−5.7 (−10.3, −0.8)	−7.1 (−11.5, −2.6)		0.126
Tryptase (µg/l)						
No. of subjects	81	79	75	82	317	
At enrolment†	7.10	7.86	6.52	6.91	7.09	
At 12 months†	6.59	7.31	6.05	6.19	6.52	
Relative change (%)‡	−7.1 (−10.6, −3.4)	−7.0 (−10.6, −3.2)	−7.2 (−10.9, −3.4)	−10.3 (−13.7, −6.8)		0.492

*Baseline values 1–4 mg/l inclusive; change from baseline to last measurement (last observation carried forward). Values are †geometric means and ‡back-transformed estimated means from model estimating change from baseline using log of biomarker (95 per cent c.i.). CRP, C-reactive protein; HbA1c, haemoglobin A1c; NT-proBNP, N-terminal pro-brain natriuretic peptide; GDF, growth differentiation factor. §ANOVA of change from baseline to 12 months, controlling for smoking status in the intention-to-treat population.

Table S2 Adverse events in the four study groups

	Placebo (n = 83)	Pemirolast 10 mg (n = 80)	Pemirolast 25 mg (n = 78)	Pemirolast 40 mg (n = 84)	Overall (n = 325)
Adverse events					
Any adverse event	68 (82)	62 (78)	67 (86)	67 (80)	264 (81.2)
General disorders	9 (11)	11 (14)	12 (15)	11 (13)	43 (13.2)
Infections and infestations	19 (23)	16 (20)	25 (32)	23 (27)	83 (25.5)
Neoplasms (benign, malignant and unspecified, including cysts and polyps)	5 (6)	1 (1)	1 (1)	2 (2)	9 (2.8)
Blood and lymphatic system disorders	1 (1)	0 (0)	1 (1)	0 (0)	2 (0.6)
Immune system disorders	0 (0)	1 (1)	1 (1)	0 (0)	2 (0.6)
Metabolism and nutrition disorders	4 (5)	7 (9)	5 (6)	2 (2)	18 (5.5)
Psychiatric disorders	4 (5)	3 (4)	2 (3)	6 (7)	15 (4.6)
Nervous system disorders	15 (18)	11 (14)	14 (18)	13 (15)	53 (16.3)
Eye disorders	3 (4)	3 (4)	2 (3)	3 (4)	11 (3.4)
Ear and labyrinth disorders	2 (2)	0 (0)	0 (0)	3 (4)	5 (1.5)
Cardiac disorders	7 (8)	4 (5)	6 (8)	5 (6)	22 (6.8)
Vascular disorders	12 (14)	10 (13)	8 (10)	20 (24)	50 (15.4)
Respiratory, thoracic and mediastinal disorders	9 (11)	5 (6)	4 (5)	7 (8)	25 (7.7)
Gastrointestinal disorders	23 (28)	14 (18)	22 (28)	16 (19)	75 (23.1)
Hepatobiliary disorders	1 (1)	0 (0)	0 (0)	0 (0)	1 (0.3)
Skin and subcutaneous tissue disorders	7 (8)	6 (8)	6 (8)	9 (11)	28 (8.6)
Musculoskeletal and connective tissue disorders	16 (19)	13 (16)	18 (23)	13 (15)	60 (18.5)
Renal and urinary disorders	4 (5)	3 (4)	7 (9)	6 (7)	20 (6.2)
Reproductive system and breast disorders	2 (2)	1 (1)	0 (0)	1 (1)	4 (1.2)
Congenital, familial and genetic disorders	0 (0)	1 (1)	1 (1)	0 (0)	2 (0.6)
Serious adverse events					
Any serious adverse event	15 (18)	16 (20)	14 (18)	15 (18)	60 (18.5)
General disorders	0 (0)	1 (1)	1 (1)	0 (0)	2 (0.6)
Infections and infestations	2 (2)	1 (1)	0 (0)	1 (1)	4 (1.2)
Neoplasms (benign, malignant and unspecified, including cysts and polyps)	2 (2)	1 (1)	1 (1)	0 (0)	4 (1.2)
Psychiatric disorders	0 (0)	0 (0)	0 (0)	1 (1)	1 (0.3)
Nervous system disorders	0 (0)	1 (1)	2 (3)	1 (1)	4 (1.2)
Eye disorders	0 (0)	0 (0)	0 (0)	1 (1)	1 (0.3)
Cardiac disorders	5 (6)	1 (1)	4 (5)	4 (5)	14 (4.3)
Vascular disorders	4 (5)	6 (8)	2 (3)	8 (10)	20 (6.2)

AAA operations	2 (2)	5 (6)	1 (1)	6 (7)	14 (4.3)
AAA rupture		0 (0)	1 (1)	0 (0)	1 (0.3)
Respiratory, thoracic and mediastinal disorders	1 (1)	0 (0)	0 (0)	0 (0)	1 (0.3)
Gastrointestinal disorders	2 (2)	1 (1)	2 (3)	0 (0)	5 (1.5)
Musculoskeletal and connective tissue disorders	1 (1)	3 (4)	2 (3)	0 (0)	6 (1.8)
Renal and urinary disorders	0 (0)	1 (1)	0 (0)	1 (1)	2 (0.6)
Congenital, familial and genetic disorders	0 (0)	1 (1)	1 (1)	0 (0)	2 (0.6)

Values in parentheses are percentages. AAA, abdominal aortic aneurysm.