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Different Effects of Commonly Prescribed Statins on Abdominal Aortic Aneurysm Wall Biology☆

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Abstract *Background:* Pharmaceutical stabilisation of the abdominal aortic aneurysm (AAA) wall can delay surgery and improve outcome. Observational studies indicate statins can be used to reduce AAA growth but mechanistic data are scarce. In this study, our aim was to determine the pleiotropic effects of different statins on AAA wall composition.
Methods: We included 216 patients undergoing open AAA repair, of which 60 used simvastatin, 52 atorvastatin and 23 pravastatin. The AAA wall histology and protein expression (IL 1β,2,4,5,6,8,10,12, interferon-gamma (IFNγ), tumour necrosis factor (TNF)α,β, matrix metalloproteinase (MMP)2 and 9 activities, total MMP8,9 and cathepsin A and B levels) between statin users and non-users were compared as also among the use of different statins.
Results: As far as histological inflammation goes, the AAA walls of statin users did not differ from those not using them. After multivariate adjustment for risk factors, pravastatin use was associated with tendencies of increased MMP8 ($p = 0.022$), active MMP9 ($p = 0.040$) and higher cathepsin B ($p = 0.056$) levels. The AAA walls of simvastatin and atorvastatin users showed no differences in proteases or cytokines in multivariate analyses.
Conclusions: The use of statins was not associated with a decrease in protease levels or inflammation. The trends of elevated protease levels associated with pravastatin use suggest pleiotropic differences among the various statins, supporting the need for further research to target pharmaceutical AAA treatment.

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Abdominal aortic aneurysm (AAA) formation is a degenerative disease with a prevalence of 5.32–8.02%.[1] Progressive expansion of AAA eventually leads to rupture; it caused 6800 deaths in the UK in 2000.[1–3] Preventive exclusion of the AAA from circulation is associated with a mortality of 9.6%.[4] As a consequence, pharmaceutical stabilisation of the AAA wall is a promising target to postpone intervention and thereby improve outcome.[5] Various medications have been suggested to inhibit AAA expansion of which some, including roxithromycin[6] and doxycycline,[7] have been tested in small randomised controlled trials. Observational studies indicate AAA growth reduction by hydroxymethylglutaryl-coenzyme A reductase inhibitors (statins),[8,9] but mechanistic data are scarce. In rodents, atorvastatin and simvastatin are shown to suppress AAA formation when compared to vehicle, independent of their lipid-lowering effects.[10–12] Moreover, statins are described to have pleiotropic effects on the arterial wall, mainly showing up in atherosclerotic plaques.[13]

In AAA pathophysiology, matrix metalloproteinases (MMPs) are key molecules for aortic wall degradation; MMP 9 especially has been studied extensively.[14–16] Previous relatively small studies suggested that statin use attenuates MMP activity, leading to a decreased AAA growth rate.[17,18] We aimed to confirm these findings and add both cytokine data and histology, while correcting for confounders to obtain more information on possible mechanisms. Besides this, other studies suggest dissimilar effects different types of statins have on serum lipid levels and atherosclerotic plaque composition.[19,20] Therefore, our aim also was to address possible differences between commonly prescribed statins on AAA wall composition, including proteases, cytokines and histology in a large patient cohort.

Methods

Patients and materials

A total of 216 consecutive patients from two centres scheduled for operative open AAA repair were included in the Aneurysm-express cohort study. The indication for surgery was based on international standards.[21] The ethical review boards of the participating hospitals approved the study and all patients signed a written informed consent. Baseline characteristics of the patients who participated in the Aneurysm-express study included medical history and medication use. These data were extracted from clinical patient records and a questionnaire based on the Rose cardiovascular survey was filled in by the patients.[22] In cases of doubt or inconsistencies, the patients' general practitioner or pharmacist was contacted for further details. The AAA diameter and morphology were assessed via computed tomographic angiography or magnetic resonance angiography. For all patients, protein was isolated from the AAA wall and used for measuring cytokine and protein levels. Blood samples were available for 100 patients and histology for 129. Total cholesterol, triglycerides, high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL) and high-sensitivity C-reactive protein (hsCRP) were determined before surgery.

Tissue processing

During surgery a part of the ventral AAA wall was harvested at the site of maximum diameter, segmented and immediately processed. One segment was fixed using 4% formaldehyde, decalcified using ethylenediaminetetraacetic acid (EDTA) and paraffin-embedded for histological stainings. Adjacent segments were snap-frozen using liquid nitrogen and stored at −80 °C. The following stainings were performed for AAA wall analysis: haematoxylin and eosin (overview), elastica-Van Giesson (elastin), picrosirius red (collagen), alpha smooth muscle actin (smooth muscle cells (SMCs)), CD68 (macrophages), CD3 (T-lymphocytes), CD20 (B-lymphocytes) and CD138 (plasma cells). All stained sections were scored semi-quantitatively on a two-value scale (no to minor vs. moderate to heavy staining). Extracellular matrix components (elastin, collagen) and cells (SMCs, macrophages, T-lymphocytes, B-lymphocytes and plasma cells) were scored for the total vessel wall.

Adjacent segments were used for protein isolation. Samples were crushed in liquid nitrogen and afterwards partly dissolved in 1.5 ml 40 mmol TrisHCl (Roche) and centrifuged at max rpm and stored at −80 °C. Another segment was isolated using Tripure Isolation Reagent (Boehringer Mannheim) according to the manufacturer's protocol. Protein concentrations were measured via the BCA Protein Assay Kit (Pierce Biotechnology, Rockford, USA). Total amounts of MMP 8, 9, cathepsin A and B were determined using the Bio-Plex system employing Luminex multianalyte profiling technology, as described and performed previously.[23,24] MMP activities were measured using the Amersham matrix metalloproteinase 2 and 9 biotrak activity assay system (GE Healthcare UK limited).

Levels of interleukin (IL) 1β, 2, 4, 5, 6, 8, 10, 12p70, interferon (IFN) γ and tumour necrosis factor (TNF) α and β were quantified in the aneurysmal wall by fluorescent bead immunoassay (Bendermed Systems).

Statistical analyses

Demographic and clinical characteristics with discrete variables were summarised as frequencies and percentages and normal distributed continuous variables as means and their standard deviation; non-normally distributed continuous variables were presented as median and interquartile range. Chi-square and Mann–Whitney tests were used for comparing discrete and continuous variables, respectively between groups. Multiple groups were compared using the Kruskal–Wallis test. The association between statin groups and AAA-wall characteristics was adjusted for cardiovascular risk factors and baseline parameters showing an association ($p < 0.200$) with statin use in the multivariate logistic regression model. For this purpose, continuous variables were dichotomised at the median. Dependent variable was the measured protease, cytokine or histological parameter. For each variable three models were run: one with statin use included in the independent variables, a second with the different statins and a third with statins categorised according to their pharmacokinetic profile.